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ORIGINAL RESEARCH

Airborne Bacteria and Fungi in the Urban Area of Kathmandu

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ABSTRACT

Objectives: The study was conducted to enumerate and identify the airborne bacteria and fungi in the urban area of Kathmandu using the settle plate method and to determine the antibiotic susceptibility pattern of the identified bacteria.

Methods: The cross-sectional study was done from March to May 2025. A total of 39 air samples were collected from 3 distinct sites: Asan, Kamaladi Ganesh Mandir, and Tri-Chandra Multiple Campus, with 13 samples from each site, using the gravity settle plate method in Nutrient Agar and Potato dextrose agar media at 37˚C for 24 hours for bacteria and 28˚C for 3-5 days for fungi and the research was carried out at the Department of Microbiology, Tri-Chandra Multiple Campus.

Results: The bacterial load ranged from 640 to $4x10^4$ CFU/m^3, while fungal load ranged from $8.4x10^2$ to $4.9x10^3$ CFU/m^3. The dominant bacterial isolates were *Micrococcus* spp (25.17%), followed by *Bacillus* spp (21.79%), *S. aureus* (19.05%), *E. coli* (18.37%), and *Klebsiella* spp (15.65%), whereas *Aspergillus* spp (21.4%) was the most dominant fungi followed by *Fusarium* spp (18.25%), *Penicillium* spp (15.9%), *Cladosporium* spp (15.1%), *Mucor* spp (14.3%), *Rhizopus* spp (8.7%), and *Alternaria* spp (6.4%).

Conclusion: The present study shows that air contains various bacteria and fungi, which can be harmful to human health. It highlights the need to reduce air pollution and raise public awareness.

Keywords: Airborne, Bacteria, Fungi, Kathmandu, Gravity settle plate method

INTRODUCTION

Air is made up of various gases, dust, and droplets of aerosol. Approximately 78% of the various gas types are nitrogen, 21% are oxygen, and 0.04% are carbon dioxide (Manandhar & Sharma, 2018). There are many microscopic organisms in the air, ranging in size from 50nm to 10µm.. Humans and all other living things have survived by developing the ability to effectively manage harmful bioaerosols (Lee, 2011). Humans typically breathe in about 1.5L of air, which means they are consuming roughly 10^6 microbial pieces and cells per day. Hence, bioaerosols are a class of airborne pollutants that include bacteria, fungi, viruses, pollen, and allergens, as well as some secondary metabolites that are mostly linked to particulate matter, including mycotoxins, endotoxins, etc. (Ghosh et al., 2022).

Airborne microorganisms can originate from natural sources such as soil, dust particles, and water droplets, and anthropogenic sources such as human activities, industrial wastes, sewage, overpopulation, and activities of organisms such as birds, animals, and insects. These sources play a significant role in spreading airborne microorganisms and also play a role in environmental and public health (Manandhar & Sharma, 2018). In the atmosphere, microorganisms are common and have a great dispersal range. It is still unclear, therefore, how these airborne microbes differ and what variables affect the microbial dispersal in various areas of anthropogenic activity (Liu et al., 2019). Temperature, relative humidity, light intensity, and wind speed are the four environmental elements that have an impact on outdoor bioaerosol concentrations (Zhu et al., 2003).

Numerous illnesses, including cancer, neurological

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conditions, and infectious and allergic diseases, can result from exposure to these substances. As a result, bio aerosol detection and identification are essential (Rastmanesh et al., 2024). Bioaerosol sampling is a growing but difficult field of study since bioaerosols vary greatly in terms of their sizes, species, biological characteristics, and the conditions needed to detect and measure them (Mainelis, 2019). Even though bioaerosol sampling and analysis techniques have advanced significantly since the late 1800s, the field of bioaerosols is still understudied in comparison to atmospheric chemistry (Xu et al., 2011). We now know very little about the biogeography of the air, despite the potential significance of knowing how life is distributed in the atmosphere. The absence of precise and thorough estimations of numerous crucial aspects of airborne life is one of the research gaps (Womack et al., 2010). Sampling bioaerosols is an interesting and difficult field. Although much progress has been made in recent decades, there is still much to be done, such as creating and modifying tools that will help address the field's challenges (Mainelis, 2019).

In the context of Nepal, many studies were carried out on pathogenic bacteria in indoor areas, dumping sites, and hospital areas, but only a handful of studies were done in outdoor air. Hence, the objective of this study was to examine airborne bacteria and fungi in three different environments of Kathmandu, identify predominant pathogenic bacteria and fungi, and perform an antibiotic susceptibility test of bacterial isolates.

METHODS

This cross-sectional study was conducted in the core areas of Kathmandu during the spring season March-May, 2025. Air samples were collected from three urban areas [Asan area, Kamaladi Ganesh temple, and Tri-Chandra Multiple Campus] of Kathmandu. A total of 39 samples were collected during the course of the study: 13 from the crowded Asan area, 13 from the religious Kamaladi Ganesh temple area and 13 from an academic Tri-Chandra Multiple Campus area. Airborne bacterial and fungal samples were collected using the gravity settle plate method. Here, Petridishes containing culture media such as Nutrient Agar (NA), MacConkey Agar (MA) for bacteria and Potato Dextrose Agar (PDA) for fungi were used as sampling surfaces. Three different culture media were exposed in each of the 3 sampling sites for 15 minutes maintaining the sampling height, i.e., 1m above the ground, to eliminate possible contamination from the surface of the ground to eliminate possible contamination from the surface of the ground. The sampling was conducted twice a week in the afternoon over two months. After collection of samples, the exposed Petridishes were immediately transported to the Microbiology laboratory of Tri-Chandra Multiple Campus inside the ice box maintaining temperature (4°C) and incubated the plates at different temperatures, NA and MA plates at 37°C and PDA plates at 28°C till the bacterial and fungal colonies developed respectively. The number of colonies was counted and converted into CFU/m^3 using Omeliansky's formula. Then, isolated colonies of bacteria and fungi were maintained as pure cultures for further study. Bacteria were identified from colony morphology, Gram staining, Catalase test, Oxidase test, IMVIC test, TSIA, Urease test, and Oxidative/ fermentative tests. Similarly, fungi were identified from colony morphology, Lactophenol cotton blue staining, and microscopic features using reference from Ibrahim et al., 2014.

The Omeliansky formula was used to quantify the airborne bacteria and fungi found on each plate to determine the number of CFU/m^3 (Andriana et al., 2023). Omeliansky's formula is CFU/m^3= $5a\times10^4$ $(bt)^{-1}$

Where,
a= number of colonies on a plate
b= square centimeters of plate size
t= minutes of exposure time

Antibiotic susceptibility test was done by modified kirby bauer method. The antibiotics used were Amikacin (AK 30 mcg), Chloramphenicol (C30), Amoxicillin (AMC 30mcg), Co-trimoxazole (COT 25mcg), Ciprofloxacin (CIP 5 mcg), Cefoxitin (CX 30 mcg), Imipenem (IPM 10mcg), and Ceftriaxone (CTR 30 mcg) (CLSI, 2021).

RESULTS

A total of 39 air samples were collected from 3 different sites in Kathmandu. The highest bacterial load was found in Asan (2.3×10^3-4×10^4 CFU/m^3) with a mean of 2.15×10^4 CFU/m^3, and the lowest was in Tri-Chandra Multiple Campus (6.4×10^2-5.6×10^3 CFU/m^3) with a mean of 2.49×10^3 CFU/m^3. (Table 1). Five genera of bacteria were identified, i.e., *Bacillus* spp, *S. aureus*, *Micrococcus* spp, *E. coli*, and *Klebsiella* spp Among them, *Micrococcus* spp (25.17%) was found dominant, whereas *Klebsiella* spp (15.65%) was least prevalent. (Table 2).

As for fungi, a total of 30 samples were collected from 3 different sites in Kathmandu. Asan has the highest fungal load of 1.4×10^3-4.9×10^3 CFU/m^3 with a mean of 2.6×10^3 CFU/m^3 and the lowest was in Kamaladi Ganesh Mandir (8.9×10^2-2.6×10^3 CFU/m^3) with a mean of 1.9×10^3 CFU/m^3. (Table 3). Seven genera of fungi were identified, i.e., *Penicillium, Aspergillus, Cladosporium, Fusarium, Alternaria, Rhizopus,* and *Mucor*. Among them, *Aspergillus* (21.4%) was found dominant, whereas *Alternaria* (6.4%) was less prevalent. (Table 4).

Table 1: Bacterial load in three different sites

S.N.	Location	Bacterial load	
		Range (CFU/m^3)	Mean (CFU/m^3)
1.	Asan	2.3×10^3 - 4×10^4	2.15×10^4
2.	Kamaladi Ganesh Mandir	7.60×10^2 - 1.2×10^4	3.65×10^3
3.	Tri-Chandra Multiple Campus	6.4×10^2 - 5.6×10^3	2.49×10^3

Table 2: Distribution of total identified bacteria in three sites

S.N.	Bacteria	Kamaladi Ganesh Mandir	Asan	Tri-Chandra Multiple Campus
		Number(%)	Number(%)	Number(%)
1.	*Bacillus* spp	13 (27.66%)	13 (20.97%)	6 (15.79%)
2.	*Staphylococcus aureus*	7 (14.89%)	12 (19.35%)	9 (23.68%)
3.	*Micrococcus* spp	11 (23.40%)	13 (20.97%)	13 (34.21%)
4.	*Escherichia coli*	10 (21.28%)	13 (20.97%)	4 (10.53%)
5.	*Klebsiella* spp	6 (12.77%)	11 (17.74%)	6 (15.79%)
	Identified isolates	**47**	**62**	**38**

Table 3: Fungal load in three different sites

S.N.	Location	Fungal load	
		Range (CFU/m^3)	Mean (CFU/m^3)
1.	Asan	1.4×10^3-4.9×10^3	2.6×10^3
2.	Kamaladi Ganesh Mandir	8.9×10^2-2.6×10^3	1.9×10^3
3.	Tri-Chandra Multiple Campus	8.4×10^2-4.7×10^3	1.96×10^3

Table 4: Distribution of total identified fungi from three sites

S.N.	Fungal genera	Kamaladi Ganesh Mandir	Asan	Tri-Chandra Multiple Campus
1.	*Penicillium*	6(15.38%)	6(13.95%)	8(18.18%)
2.	*Aspergillus*	9(23.08%)	8(18.60%)	10(22.73%)
3.	*Cladosporium*	6(15.38%)	7(16.28%)	6(13.64%)
4.	*Fusarium*	7(17.95%)	8(18.60%)	8(18.18%)
5.	*Alternaria*	3(7.69%)	3(6.98%)	2(4.55%)
6.	*Rhizopus*	2(5.13%)	6(13.95%)	3(6.82%)
7.	*Mucor*	6(15.38%)	5(11.63%)	7(15.91%)
	Identified isolates	**39**	**43**	**44**

Antibiotic Susceptibility Test

Antibiotic susceptibility test was done for *Staphylococcus aureus, E. coli* and *Klebsiella* spp All 9(100 %) *S. aureus* tested was found to be resistant to Cefoxitin (100%). followed by Co-trimoxazole (66.67%), Amoxicillin (45.45%), Chloramphenicol (28.57%), and Amikacin (20%). (Table 5).

Out of total 5 *E. coli*, 60% of *E. coli* were found to be resistance to Ceftiraxone followed by Chloramphenicol (40%). Similarly, 2(50%) isolates of *Klebsiella* were found to be resistant to Ceftriaxone.

Table 5: Antibiotic Susceptibility Test of *Staphylococcus aureus*

Antibiotic discs	Total number of organisms	Sensitive	Intermediate	Resistant
Amikacin (AK30 mcg)	15	11 (73.33%)	1 (6.67)	3(20%)
Chloramphenicol (C 30 mcg)	14	8 (57.14%)	2 (14.29%)	4 (28.57%)
Amoxicillin (AMC 30mcg)	11	3 (27.27%)	3 (27.27%)	5 (45.45%)
Co-trimoxazole (COT 25mcg)	6	2 (33.33%)	-	4 (66.67%)
Ciprofloxacin (CIP5)	9	8 (88.89%)	1 (11.11%)	-
Cefoxitin (CX30)	9	-	-	9 (100%)

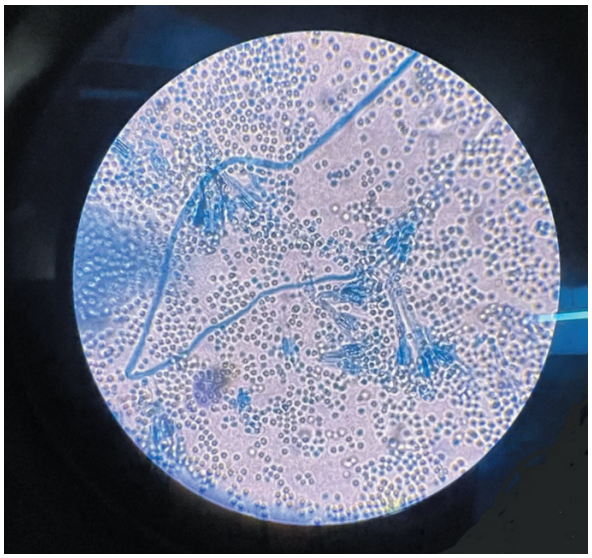

Figure 1: *Penicillium* spp isolated from Kamaldi Ganesh Temple

Figure 2: Fungal load on PDA obtained from Asan

DISCUSSION

For air sampling, three different environments, i.e., crowded Asan, religious Kamaladi Ganesh Mandir, and academic Tri-Chandra Multiple Campus, were chosen and samples were collected from the outdoor air of each site by the gravity settle plate method. The environmental factors, such as temperature and relative humidity, were taken during the sampling period. The temperature ranged from 20-26 °C while the humidity ranged from 32-72%. The lowest bacterial and fungal concentration was found in Tri-Chandra Multiple Campus i.e. ($6.4x10^2$CFU/m^3), ($2.6x10^3$ CFU/m^3) respectively. Similarly, the highest bacterial and fungal concentration ($4×10^4$ CFU/m^3 and $4.9×10^3$ CFU/m^3 respectively) was found from the crowded market Asan site. This is probably due to the market places, frequent movement of people, vehicles, all of which disrupt biological matter and dust. According to a study by Ogah et al., (2023), among the bacterial population in densely populated areas, the marketplaces were found to have a comparatively greater bacterial population. Bariga market had the highest bacterial population, which ranged from 140000 to 440000 CFU/m^3 and the lowest in a garage which ranged from 24600 to 28300 CFU/m^3 indicating that market areas have comparatively higher bacterial count among the public places.

Bacillus spp, *Staphylococcus aureus, Micrococcus* spp, *E. coli,* and *Klebsiella* spp were commonly found bacteria from the outdoor air of Kathmandu during the spring season. According to a study in an urban environment, the most frequently isolated bacteria were *Micrococcus* (41%), *Staphylococcus* (11%), and *Aerococcus* (8%) among the other 19 different genera (Mancinelli & Shulls, 1978). The presence of coliforms like *E. coli* and *Klebsiella* spp is an indicator of faecal contamination (Khan & Gupta, 2019). Here, the high concentration of *E. coli* (21%) and *Klebsiella* spp (13%) were found in religious sites. This suggests the contamination of faeces around religious sites. So, proper sanitation procedures should be implemented to reduce faeces contamination in the religious sites.

S. aureus was found to be 88.89% sensitive to

Ciprofloxacin and 100% resistant to Cefoxitin. Hence, urgent action needs to be taken to improve the quality of the air. According to the study by Kabir et al., (2016), *Staphylococcus aureus* isolates were subjected to antibiotic susceptibility tests and among the isolates, 18.75% showed resistance to Amoxicillin. Whereas none of the isolates showed resistance to Amikacin and Ciprofloxacin. The findings of this study were found to be similar to our study. According to a study in Saudi Arabia, the *E. coli* isolates isolated from outdoor air were 100% sensitive to Imipenem, Amikacin, and resistant to Ceftriaxone (Aabed et al., 2021). These findings are similar to the results of this project.

Median numbers of culturable fungi in Austria varied across environments and ranged from 3.5×10^2 to 4.7×10^3 CFU/m^3, and were usually higher in metropolitan areas than in rural and hilly areas (Haas et al., 2023). Moreover, Sabariego-Ruiz et al., (2000) reported moderate urban atmospheres with greater human activity often exhibited increased spore counts even in the city of southern Spain. In a study by Nageen et al., (2023), airborne fungal diversity was live-tracked over a full year across several urban regions in Tianjin. *Alternaria* (35%) and *Cladosporium* (18%) were the most abundant, and *Penicillium* and *Aspergillus* had low abundances of 5.6% and 2.8%, respectively.

It is common to find *Aspergillus* and *Penicillium* fungal genera in the control of fungal communities through the air because of their resistant and high sporulation nature within the metropolitan regions, as exhibited in various studies done in different parts across the globe. The most frequently found genera in Tianjin, China, proved to be *Alternaria, Cladosporium, Penicillium,* and *Aspergillus.* It was found that *Penicillium* is extensively distributed in most cities and can be hazardous to the respiratory system, such as allergies and asthma (Al-Shaarani et al., 2024).

CONCLUSION

Bacillus spp was the most common bacteria in the air, whereas *Aspergillus* spp was the most common fungi from the three sites we studied. The market's air had a notably higher concentration of microorganisms than the religious and academic sites.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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