AIRBORNE BACTERIA AND FUNGI IN URBAN AREA OF KATHMANDU

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ABSTRACT

Air is essential for life, yet its quality is declining due to rapid urbanization, population growth and poor sanitation practices. This deterioration increases various serious skin and respiratory diseases due to the presence of pathogenic airborne bacteria and fungi. The present study aimed to assess and compare the bacterial and fungal load in three different sites Asan, Kamaladi Ganesh Mandir, and Tri-Chandra Multiple campus. Air samples were collected during March to May 2025 by the gravity settle plate method using Nutrient agar, MacConkey agar and Potato dextrose agar plates were exposing for 15 minutes each. Nutrient agar bacteria were identified by conventional morphological and biochemical tests. A total of 147 bacterial and 126 fungal isolates were identified. The bacterial concentration ranged from 640 to $4x10^4$ CFU/m^3, while fungal concentrations ranged from $8.4x10^2$ to $4.9x10^3$ CFU/m^3. The dominant bacterial isolates were *Bacillus spp.* and *Micrococcus spp.* whereas *Aspergillus* (21.4%) was the most dominant fungi and followed by *Fusarium* (18.25%), *Penicillium* (15.9%), *Cladosporium* (15.1%), *Mucor* (14.3%), *Rhizopus* (8.7%) and *Alternaria* (6.4%). The highest fungal load $2.6x10^4$ CFU/m^3 was recorded in Asan with mean bacterial load $2.15x10^4$ CFU/m^3 followed by $1.9x10^4$ CFU/m^3 fungal load in Kamaladi Ganesh Mandir with $3.6x10^3$ CFU/m^3 mean bacterial load and academic sites, Tri-Chandra Multiple Campus had a fungal load $1.96x10^4$ CFU/m^3 and $2.4x10^3$ CFU/m^3 mean bacterial load. From the above findings, it was clear that airborne bacteria and fungi were higher in improper urbanization, reduced air movement and poor sanitation practices. So, the policy of proper urbanization, planting green vegetation, allowing better air flow and sanitation practices should be implemented to reduce the microbial load in the surrounding air.

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 that range in size from 50nm to 10μm. we refer to these inhabitants as bio aerosols or airborne biological particles or airborne microorganism. We breathe in bio

aerosol through our noses every day, even at this very now. Human and all other living things have survived by developing the ability to effectively manage harmful bio aerosol (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 a number of secondary metabolites that are mostly linked to particulate matter including mycotoxins, endotoxins etc (Ghosh et al., 2022).

Airborne microorganism can be originated from natural sources such as soil, dust particles and water droplets and anthropogenic sources such as human activities, industrial wastes, sewages, over population, activities of organism such as birds, animal and insects. These sources play a significant role in spreading the airborne microorganism and also plays 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 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).Bio aerosol sampling is a growing but difficult field of study since bio aerosol vary greatly in terms of their sizes, species, biological characteristics and the conditions needed to detect and measure them (Mainelis, 2019). Even though bio aerosol sampling and analysis techniques have advanced significantly since the late 1800s, the field of bio aerosol 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 research gaps (Womack et al., 2010). Sampling bio aerosols 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 context of Nepal, many studies were carried out on pathogenic bacteria in indoor area, dumpling site, hospital area, but only a handful of study was done in outdoor air. Hence, the objective of this study is to examine airborne bacteria and fungi in three different environments of Kathmandu, identify predominant pathogenic bacteria and fungi and perform antibiotic susceptibility test.

Methods

This cross-sectional study was conducted in core areas of Kathmandu during spring season (March–May). Air samples were collected from three urban area [Asan area, Kamladi Ganesh temple and Tri Chandra Multiple Campus] of Kathmandu. A total of 39 samples were collected during the course of study time. 13 from crowded Asan area, 13 from religious Kamladi 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, Petri dishes containing culture media such as NA, MA for bacteria and PDA for fungi were used as sampling surfaces. Three different culture media were exposed in each of 3 sampling sites for 15 minutes. The sampling height i.e. 1m above the ground was maintained to eliminate possible contamination from the surface of ground. The sampling was conducted twice a week in the afternoon over a two month period. After collection of samples, the exposed Petri dishes were immediately transported to the Microbiology laboratory of Tri Chandra Multiple Campus inside the ice box maintaining temperature (4°C) and incubated at plates at different temperature, NA and MA plate at 37°C and PDA plate at 28°C till the bacterial and fungal colonies developed respectively. The number of colonies were counted and converted into CFU/m^3 using Omeliansky formula. Then, isolated colonies of bacteria and fungi were maintained as pure culture 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 feature using reference from Ibrahim et al., 2014.

The Omeliansky formula is used to quantify of airborne bacteria and fungi found on each plate in order to determine the number of CFU/m^3 (Andriana et al., 2023).Omeliansky formula is

$$CFU/m^3 = 5a*10^4 (bt)^{-1}$$

Where,

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

Results

Bacterial load in three different sites

Table 1

Bacterial load in three different sites

Range of bacterial load in different media	Religious site (Kamaladi Ganesh Mandir)	Mean count	Market place (Asan)	Mean count	Tri-Chandra Multiple Campus	Mean count
In NA	760-12000 CFU/m^3	3658.46	2300-40000 CFU/m^3	21576.92	640-5600 CFU/ m^3	2489.23

The marketplace was found to have the heaviest load which ranged from 2300-40000 CFU/m^3 and the campus area the least heavy which ranged from 640-5600 CFU/ m^3 as shown in table1. Whereas the religious site was somewhere in between (760-12000 CFU/m^3).

Bacterial genera identified

Table 2

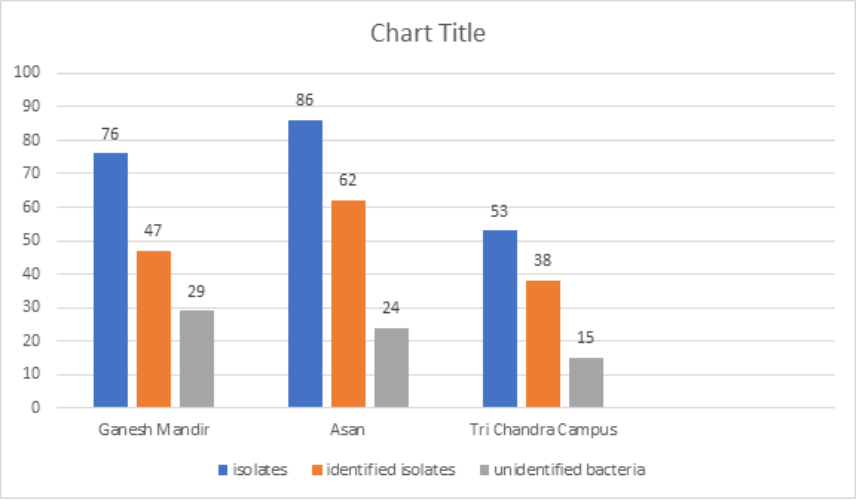
Distribution of total identified bacteria from three sites

S.N.	Bacteria	Kamaladi Ganesh Mandir	Asan	Tri-Chandra Multiple Campus
1.	*Bacillus spp.*	13 (28%)	13 (21%)	6 (16%)
2.	*Staphylococcus aureus*	7 (15%)	12 (19%)	9 (24%)
3.	*Micrococcus spp.*	11 (23%)	13 (21%)	13 (36%)
4.	*Escherichia coli*	10 (21%)	13 (21%)	4 (10%)
5.	*Klebsiella spp.*	6 (21%)	11 (18%)	6 (16%)
	Identified isolates	47	62	38

As per the results of table 2, 47 isolates from Ganesh mandir, 62 from Asan and 38 from Campus were identified. And, according to the distribution of bacteria identified, *Bacillus spp.* was found to be the predominant bacteria in Ganesh mandir (28%) and Asan (21%). In Asan, *Bacillus spp., E. coli* and *Micrococcus spp.* were equally predominant while *Micrococcus spp.* was predominant in camps area (36%). The least dominant bacteria in all the sites were different genera such as, in Ganesh mandir the least dominant was *Staphylococcus aureus* (15%), *Klebsiella spp.* in Asan (18%) and *Escherichia coli* in Campus (10%).

Figure 1

A bar graph of identified and unidentified bacteria from isolates



From 76 isolates from Kamaladi Ganesh Mandir, 47 were identified while among 86 isolates from Asan, 62 were identified and from 53 isolates in Campus, 38 were identified [Figure1]. Therefore, from a total of 215 isolates, 147 were identified and 68 remained unidentified.

Antibiotic Susceptibility Test

Altogether 8 antibiotics were used for Antibiotic Susceptibility test. A Gram-positive bacterium (*Staphylococcus aureus*) and two Gram-negative bacteria (*E. coli)* and *Klebsiella spp.)* were subjected to the test.

Table 3

Antibiotic Susceptibility Test of Staphylococcus aureus

S.N.	Antibiotic discs	Total number of organisms	Sensitive	Intermediate	Resistant
1.	Amikacin (AK30)	15	11 (73.33%)	1 (6.67)	3(20%)
2.	Chloramphenicol (C30)	14	8 (57.14%)	2 (14.29%)	4 (28.57%)
3.	Amoxicillin (AMC30)	11	3 (27.27%)	3 (27.27%)	5 (45.45%)
4.	Co-trimoxazole (COT25)	6	2 (33.33%)	-	4 (66.67%)
5.	Ciprofloxacin (CIP5)	9	8 (88.89%)	1 (11.11%)	-
6.	Cefoxitin (CX30)	9	-	-	9 (100%)

As per Table 8, 88.89% of isolates of *Staphylococcus aureus* were found to be sensitive to ciprofloxacin, followed by amikacin (73.33%), chloramphenicol (57.14%) and co- trimoxazole (33.33%). However, none were found to be sensitive to cefoxitin. The isolates showed varied antibiotic susceptibility results.

Table 4

Antibiotic Susceptibility Test of E. coli

S.N.	Antibiotic discs	Total number of organisms	Sensitive	Intermediate	Resistant
1.	Imipenem (IPM10)	5	3 (60%)	1 (20%)	1(20%)
2.	Amikacin (Ak30)	5	5 (100%)	-	-
3.	Chloramphenicol (C30)	5	2 (40%)	1 (20%)	2 (40%)
4.	Ceftriaxone (CTR30)	5	1 (20%)	1 (20%)	3 (60%)

As per Table 4, 5 isolates of *E. coli* were subjected to AST against four antibiotics (imipenem, amikacin, chloramphenicol and ceftriaxone). 100% of isolates of *E. coli* were found to be sensitive to amikacin, followed by imipenem (60%), chloramphenicol (40%) and lastly ceftriaxone (20%). High amount of resistance of isolates was found towards ceftriaxone (60%).

Table 5

Antibiotic Susceptibility Test of Klebsiella spp.

S.N.	Antibiotic discs	Total number of organisms	Sensitive	Intermediate	Resistant
1.	Imipenem (IPM10)	4	3 (75%)	1 (25%)	-
2.	Amikacin (Ak30)	4	4 (100%)	-	-
3.	Chloramphenicol (C30)	4	3 (75%)	-	1 (25%)
4.	Ceftriaxone (CTR30)	4	2 (50%)	-	2 (50%)

As per Table 5, 4 isolates of *Klebsiella spp.* were subjected to AST against imipenem, amikacin, chloramphenicol and ceftriaxone. 4(100%), 3(75%) and 3(75%) isolates were found to be sensitive to amikacin, imipenem and chloramphenicol respectively. Comparatively, least sensitivity was shown by isolates towards ceftriaxone (50%). In conclusion, *Klebsiella spp.* resulted in being highly sensitive to imipenem, amikacin and chloramphenicol.

Fungal load in three different sites

Table 6

Fungal load in three different sites

Range of fungal load in different media	Religious site (Kamaladi Ganesh Mandir)	Mean count	Colony count	Market place (Asan)	Mean count	Colony count	Tri-Chandra Multiple Campus	Mean count	Colon count
In PDA	8.9×10^{2}-2.6×10^{3} CFU/m^3	1.9×10^{3}	367	1.4×10^{3}-4.9×10^{3} CFU/m^3	2.6×10^{3}	500	8.4×10^{2}-4.7×10^{3} CFU/m^3	1.96×10^{3}	371

Asan Bazar had the highest fungal load across three sites, with a range of 1.4×10^3 to 4.9×10^3 CFU/m^3 and a mean of 2.6×10^3 CFU/m^3. Kamaladi Ganesh Mandir has a fungal load of 8.9×10^2 to 2.6×10^3 CFU/m^3, with a mean of 1.9×10^3 CFU/m^3. At

Tri-Chandra Multiple Campus, fungal loads ranged from 8.4×10^2 to 4.7×10^3 CFU/m³, with a mean of 1.96×10^3 CFU/m³ as shown in Table 6 above. The statistics show that Asan Bazar has the highest fungal contamination, indicating a higher risk of exposure than the other sites.

Fungal genera identified

Table 7

Distribution of total identified fungi from three sites

S.N.	Fungi	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

According to the results in Table 7, a total of 39 fungal isolates were identified from Kamaladi Ganesh Mandir, 43 from Asan, and 44 from Tri-Chandra Multiple Campus. Seven different types of fungi were identified from three different locations. *Aspergillus* was the most dominant fungus across all sites, with the highest at Tri-Chandra Campus (22.73%), followed by Kamaladi Ganesh Mandir (23.08%) and Asan (18.60%). *Fusarium* and *Penicillium* were also common, while *Cladosporium* and *Mucor* showed moderate presence. *Alternaria* and *Rhizopus* were the least frequent.

Figure 2

A bar graph showing total, identified and unidentified isolates of fungi

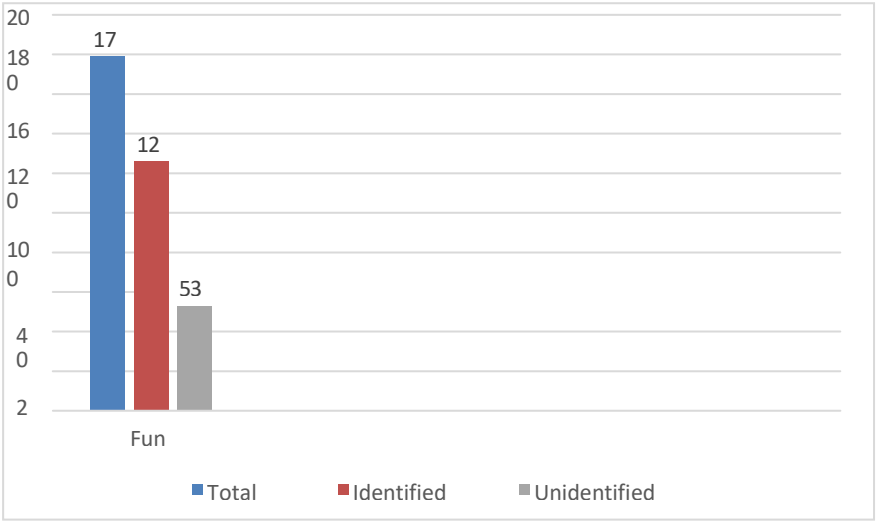


Figure 2 indicates that out of the total number of 179 fungal isolates, 126 (70%) were successfully identified, and 53 (30%) were not identified. It means that there is a high identification rate, but still, some more sophisticated ways of methods could be required to identify the unidentified fungi properly.

Discussion

For environmental factors, temperature and humidity were considered. During sampling, both the factors were recorded. The temperature and humidity recorded during sampling in both the places were same in both sites. The temperature ranged from 20-26℃ while humidity ranged from 32-72 %. The bacterial load in market place was found to be the heaviest among other sites. According to a study by Ogah et al. (2023), among the bacterial population in densely populated areas, the market places were found to have comparatively greater bacterial population. Bariga market had the highest bacterial population which ranged from 140000 to 440000 CFU/m³ and lowest in a garage which ranged from 24600 to 28300 CFU/m³. these results or findings do suggest that market areas have comparatively higher bacterial count among the public places.

According to a review, the presence of coliforms like *E. coli* and *Klebsiella spp.* is an indicator of faecal contamination (Khan & Gupta, 2019). Thus, such high concentration and presence of *E. coli* (21%) and *Klebsiella spp.* (13%) in the religious site suggests the contamination of faeces around the area. According to a study in urban environment, the most frequently isolated bacteria were *Micrococcus* (41%), *Staphylococcus* (11%), and *Aerococcus* (8%) among the 19 different genera (Mancinelli & Shulls, 1978).

According to the study by Kabir et al. (2016), *Staphylococcus aureus* isolates were subjected to AST 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 project are found to be similar to this study. Another study by Leta et al. (2014) also found *S. aureus* to be resistant to amoxicillin (53.9%) which is similar to the result of this project. According to the study, majority 26 (66.67%) of the *S. aureus* isolates were found to be multiple drug resistant as well. Thus, the results of this project are in agreement with the findings of these studies. 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 according to various environments and were between 3.5×10^2 and 4.7×10^3 CFU/m^3, and were usually higher in metropolitan areas compared to 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.

Conclusion

This study was aimed to perform the analysis of airborne bacteria of outdoor surroundings in Kathmandu city. A total of 39 samples were collected from 3 different places. Based on the results obtained after performing different tests, 5 different types of bacterial genera were identified. They were *Staphylococcus aureus, Micrococcus spp., Bacillus spp., Klebsiella spp. and E. coli. Bacillus spp.* was the predominant bacteria in the religious site while *E. coli* and *Micrococcus spp.* along with *Bacillus spp.* were dominant in the market place. Whereas, the campus area was dominated by *Micrococcus spp.* Along with that, a drastic difference in terms of bacterial load in between the market place, religious site and campus area was observed. There was much higher bacterial load in the market place. On determining the Antibiotic Susceptibility test, isolates of *Staphylococcus aureus* were found to be sensitive to ciprofloxacin, amikacin and chloramphenicol. They were found to be resistant to ceftriaxone, amoxicillin and co- trimoxazole which is a thing of concern. Isolates of *E. coli* and *Klebsiella spp.* were found to be more sensitive towards imipenem, amikacin and chloramphenicol than ceftriaxone. Since the bacteria were resistant to the antibiotics used, more research work should be done. Future research can be done to observe the seasonal variations in bacterial load and diversity. The market places, religious sites, public places etc should focus

more towards cleanliness and spread awareness towards general people and people in charge of these sites.

The experiment determined the fungal load and diversity in three places: Kamaladi Ganesh Mandir, Asan Bazar, and Tri-Chandra Multiple Campus. Asan Bazar possessed the largest fungal load with a range of 1.4×10^3-4.9×10^3 CFU/m^3, where the average was 2.6 x 10^3 CFU/m^3, which is the most exposed to the risk of exposure. Kamaladi Ganesh Mandir and Tri-Chandra Campus were less contaminated with fungi, with the mean of 1.9 x 10^3 CFU/m^3 and 1.96 x 10^3 CFU/m^3, respectively. Among 179 fungal isolates from all locations, 126 (70%) were identified, which belonged to seven genera. *Aspergillus* was the most dominant one, especially at Tri-Chandra Campus (22.73%) and Kamaladi Ganesh Mandir (23.08%). *Fusarium* and *Penicillium* were prevalent and *Alternaria* and *Rhizopus* were the least common. The other 30% of the unidentified isolates indicate that further sophisticated methods of identification are required.

Overall, the research points out location-specific differences in fungal loads and community structure, but the site of the maximum contamination is Asan Bazar. The prevalence of genera that may be of health-relevant interest, such as *Aspergillus* and *Fusarium*, highlights the necessity of tracking the airborne fungi in the urban and religious areas, and the findings stress the necessity to strive to improve the methods of identification to get a better insight into fungal diversity.

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