Research Article

Characterization of Pathogenic Bacterial Bioaerosols in Polluted Freshwater Lakes in Southern India: Implications for Public Health

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ABSTRACT

Background: Bioaerosols are airborne microbial particles that pose a threat to the environment and human health. **Materials and Methods:** Bacterial bioaerosol samples were collected following standard protocols with a respirable dust sampler with microfibre filters from two freshwater lakes in Madurai, India - Vandiyur Lake (urban, highly polluted) and Valandur Lake (rural, less polluted). Gram staining and culture-based techniques were used to assess the bacterial diversity, Concentration (CFU/m^3), and pathogenicity of the bacteria. **Results and Discussion:** Vandiyur Lake had higher bacterial concentrations (2.6×10^5 CFU/m^3), irregular, large colonies (57.14% Gram-negative, including *Vibrio* sp.), compared to Valandur Lake (1.13×10^5 CFU/m^3), which features smaller, circular colonies of bacteria that were balanced between Gram-positive (54.54%) and Gram-negative (45.45%). *Bacillus* (20%), *Staphylococcus* (4%), *Streptococcus* (8%), and *Vibrio* (28%), with polymicrobial mixtures, were among the taxa identified, indicating anthropogenic influence in Valandur Lake. The predominance of Gram-negative pathogens in Vandiyur Lake highlights pollution-driven microbial shifts, posing risks of respiratory and gastrointestinal infections. **Conclusion:** The study identified polluted water bodies as significant bioaerosol sources, with concentrations exceeding urban ambient levels. The presence of antibiotic-resistant genera (*Staphylococcus*, *Vibrio*) further emphasizes public health concerns, particularly in densely populated areas. This pivotal study in India highlights the need for pollution mitigation and focused monitoring of bioaerosols from contaminated water. Future research should employ molecular techniques to assess pathogenicity and seasonal variations, informing policymakers to reduce exposure risks. The results support integrated water-air quality management to protect public health in areas that are becoming rapidly urbanized.

Keywords: Antibiotic Resistance, Bioaerosol, Water pollution, Toxicity.

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INTRODUCTION

Water is a fundamental resource essential for the survival of all living organisms. Rapid population growth and industrialization have intensified water demand worldwide, resulting in increased contamination of aquatic systems.[1] Water pollution, arising from industrial effluents, agricultural runoff, domestic sewage, and medical waste, disrupts the natural balance of nutrients such as chloride, sulphate, nitrate, phosphate, calcium, magnesium, potassium and sodium,[2] threatening aquatic biodiversity and human health by altering the composition of airborne bacterial communities.[3]

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A consequence of water pollution is the generation of bioaerosols-airborne particles of biological origin, including bacteria, fungi, viruses, and endotoxins.[4] They originate from natural sources (soil, water bodies, vegetation) and anthropogenic activities (waste treatment, agriculture, industrial processes).[5] Bioaerosols consist of microorganisms and biological particles ranging from 0.02 to 100 μm.[6] In aquatic ecosystems, aerosols are primarily formed through bubble bursting at the water-air interface, a process that releases microorganisms and organic matter into the atmosphere.[7] When contaminated water, such as sewage-laden freshwater, enters marine or estuarine systems, pathogens can be aerosolised and transported over land via wind currents.[8] These bioaerosols can deposit through gravitational settling or surface interception, posing risks to both the environment and human health. [9]

Among these, bacterial bioaerosols are of particular concern due to their potential pathogenicity and role in respiratory and gastrointestinal illnesses.[10] Immunocompromised individuals are especially vulnerable to infections caused by airborne pathogens.[11] Recent studies highlight that microbial communities in bioaerosols vary depending on environmental conditions, including temperature, humidity, and air pollution levels.[12] For instance, high humidity promotes bacterial survival, while UV radiation can reduce viability.[13]

Exposure to pathogenic bacterial aerosols has been linked to respiratory diseases, allergies, and chronic inflammatory conditions.[14] Common pathogenic genera found in bioaerosols include *Pseudomonas*, *Bacillus*, *Staphylococcus*, and *Streptococcus*, some of which carry antibiotic resistance genes.[15] The spread of antimicrobial-resistant bacteria via aerosols is still an emerging public health concern, particularly in regions with poor wastewater management.[16]

While bioaerosol research has advanced in developed nations, studies in India are scarce, particularly concerning pathogenic bacterial emissions from polluted aquatic ecosystems. In India, most studies have focused on indoor settings[17,18] or rural vs. urban air quality comparisons.[19] No study has investigated the diversity and pathogenicity of bacterial aerosols from sewage-polluted water bodies, the influence of seasonal variations on bioaerosol composition and dispersion, the potential long-range transport of waterborne pathogens via aerosols and the role of bioaerosols in antibiotic resistance dissemination in the environment.

Hence, this pivotal study was undertaken with the following objectives: 1. To characterize the bacterial diversity in aerosols generated from polluted waterbodies. 2. To identify pathogenic and antibiotic-resistant bacterial strains in bioaerosols. This study will help develop strategies to mitigate bioaerosol emissions from contaminated water sources and enhance public health policies, which will lead to future studies on environmental bioaerosols in India.

MATERIALS AND METHODS

Description of the study area

Bioaerosol sampling was done from two freshwater bodies (one urban and one rural) in Madurai district, Tamil Nadu, India. Site selection was guided by a pre-survey questionnaire assessing pollution sources, land use, and human activity levels to contrast aerosolised bacterial communities under differing anthropogenic influences.[13] The study was conducted in September and October 2022. Temperature (ºC), relative humidity (%), and solar radiation (W/m²) were recorded during sampling. Sampling avoided rainy days to preclude particle scavenging. The temperature was 32°C, the relative humidity was 59%, with the wind speed of 5km/hr.

Vandiyur Lake (9.9171° N, 78.1586° E), situated in Madurai city (~1.5 million population) (Figure 1). This site experiences high pollution loads from municipal runoff, vehicular emissions ($PM_{2.5}$/PM_{10}), and dense urban activity.

Valandur Lake (9.9484° N, 77.8884° E) is a rural site located in a peripheral village area with negligible industrial activity (Figure 1). Primary pollution sources were sporadic vehicular traffic (cars/motorcycles) and minor construction.

Sampling techniques

Aerosol sample collection was done using a Respirable Dust Sampler (Envirotech APM 460 BL) equipped with sterile glass microfibre filters (pore size: 0.45-2 µm;*Whatman GF/A or equivalent*) following standard protocols. Samplers were deployed 500 m from the shoreline at 1.5 m above ground (to approximate human inhalation height). Sampling duration was 6 hr/site (10:00-16:00) to standardize solar radiation effects.[20] Filters were aseptically transferred to sterile bags and stored at 4ºC until processing (<24 hr). Luria-Bertani (LB) agar medium for bacterial culture and isolation was selected for its efficacy in cultivating diverse aerosolised bacteria.[21] Filters were vortexed in 10 mL sterile Phosphate Buffered Saline (PBS) (pH 7.4) for 30 min to dislodge particles. Serial dilutions (10^{-1}-10^{-5}) were plated via the spread plate technique (triplicate per dilution). Plates were incubated at 21±1ºC for 72 hr for aerobic conditions. Colonies were then categorised by morphology (color, form, margin, elevation) and enumerated as Colony Forming Units (CFU/m³).

Distinct isolates were subcultured (patch plating) and Gram-stained with Hucker's modification[22] to differentiate cellular morphology (*Cocci, Bacillus*, or *Vibrios*) and wall structure (Gram-positive vs. Gram-negative). Pure isolates were preserved and stored at-80ºC in LB broth with 20% glycerol.

RESULTS

The results revealed significant differences in bacterial colony morphology, concentration, and diversity between Vandiyur Lake (highly polluted) and Valandur Lake (less polluted).

Bacterial colony morphology and Gram staining

The current study found that the size, shape, colour, margin, consistency, and surface of colonies varied depending on the sampling locations. We isolated and classified 25 different bioaerosol bacteria according to their shapes. The bacteria identified were *Bacillus, Coccus, Staphylococcus, Streptococcus*, and *Vibrio*. Few bacteria identified were *Polymicrobial*.

Cultured bacterial colonies from both lakes exhibited diverse morphological characteristics (Table 1). The higher bacterial diversity and concentration in Vandiyur Lake align with its polluted status, featuring high frequency of Gram-negative pathogens like *Vibrio*, and colonies were predominantly irregular (irregular margins, large size) and circular (medium to small size), with raised elevations and lobate or undulate margins. In contrast, Valandur Lake colonies displayed a higher proportion of circular and punctiform shapes, often with flat elevations and entire margins. Gram staining indicated the presence of both

Gram-positive (e.g., *Staphylococcus*, *Streptococcus*, *Bacillus*) and Gram-negative (*Vibrio*, polymicrobial mixtures) bacteria, indicating a more balanced distribution of Gram-positive *Cocci* and *Bacilli* (Table 2).

Bacterial Concentration (CFU/m³)

The concentration of bacteria varied from 1.13×10^5 to 2.6×10^5 from both study sites. Quantitative analysis demonstrated that Vandiyur Lake had a substantially higher bacterial load (2.6×10^5 CFU/m³) compared to Valandur Lake (1.13×10^5 CFU/m³) (Table 3). These values exceed those reported for urban and residential environments in France (791 ± 598 CFU/m³)[23] and Canada (17.2 CFU/m³),[24] but were lower than those from green waste composting facilities (3.7×10^7 CFU/m).[25]

Bacterial characterisation

Among the total, the bacteria identified were *Bacillus* (20%), *Coccus* (28%), *Staphylococcus* (4%), *Streptococcus* (8%), and *Vibrio* (28%), and a few were *Polymicrobial* (Figure 2). Post culture, the bacterial bioaerosol colonies from Vandiyur were higher as compared to those culturable bacterial aerosols from Valandur by 12%.

In the samples cultured from Vandiyur bioaerosol, 14 positive isolates were obtained, of which 8 were gram-negative bacteria (57.14%) and 6 were gram-positive (42.85%). In the samples cultured from Valandur bioaerosol, 11 positive isolates were obtained, of which 6 were gram-positive (54.54%) and 5 were gram-negative (45.45%) bacteria. Overall, the gram-negative bacteria dominated the gram-positive bacteria by 12%.

DISCUSSION

The results of the present study showed that contaminated aquatic habitats raise the bacterial level and increase their diversity in the air, which could be harmful to public health.

Bacterial diversity and morphological variations

The morphological and Gram-staining analyses revealed distinct bacterial populations between the two lakes. Vandiyur Lake exhibited a higher proportion of Gram-negative bacteria (57.14%), including pathogenic *Vibrio* sp., raising concerns as these bacteria are associated with waterborne diseases such as cholera and wound infections, whereas Valandur Lake had a more balanced distribution of Gram-positive (54.54%) and Gram-negative (45.45%) bacteria. The dominance of Gram-negative bacteria in polluted environments aligns with previous studies suggested

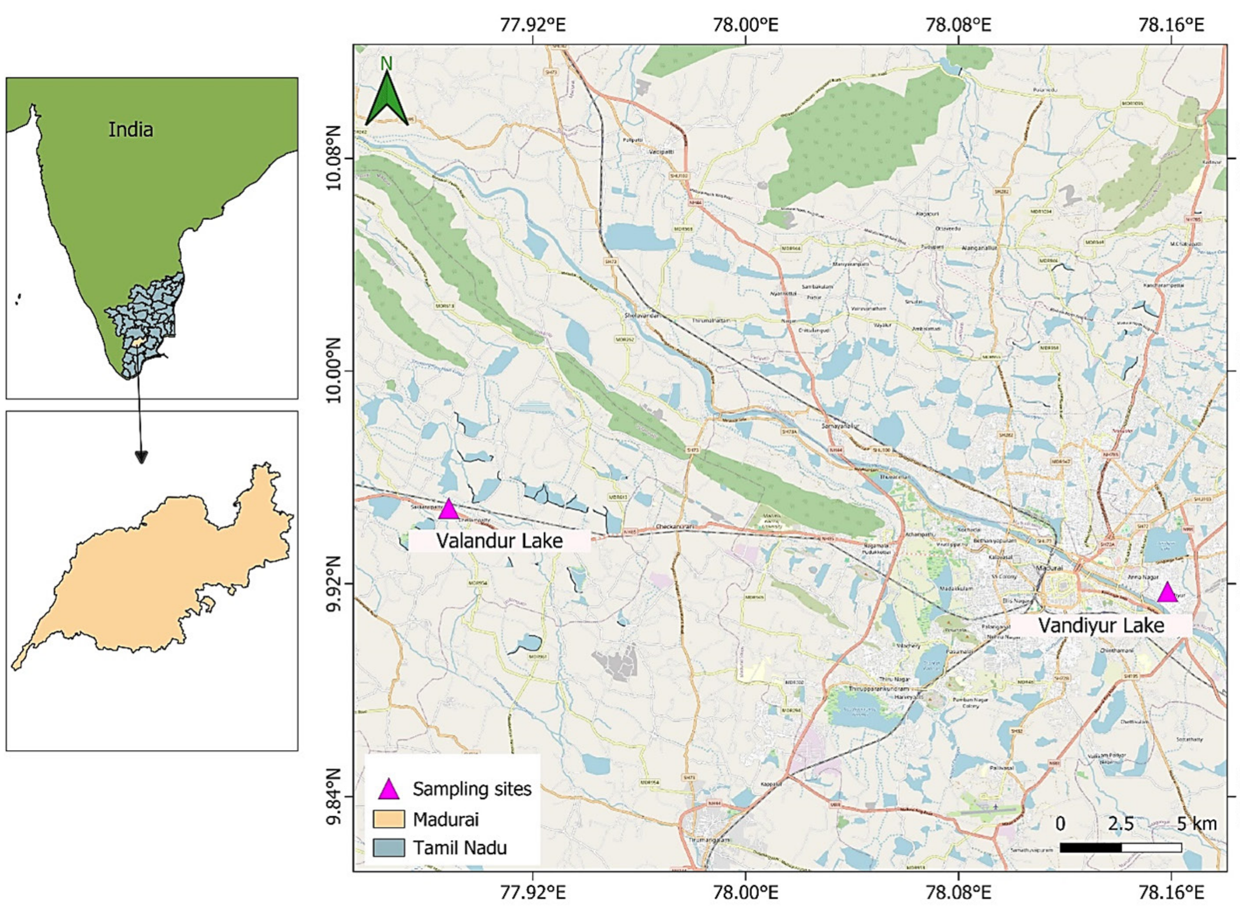

Figure 1: Study sites in Madurai - Vandiyur and Valandur Lakes.

Table 1: Morphological characteristics of colonies obtained at each dilution factor.

Study site	Dilution factor	No. of colonies obtained	Shape of the colonies	Size of colonies	Elevation of colonies	Margin of colonies
Vandiyur Lake	10^{-1}	3	Irregular	Large	Raised	Entire
			Irregular	Large	Raised	Entire
			Irregular	Large	Raised	Lobate
	10^{-2}	3	Circular	Medium	Raised	Undulate
			Circular	Small	Raised	Undulate
			Irregular	Medium	Raised	Undulate
	10^{-3}	3	Irregular	Large	Raised	Lobate
			Irregular	Large	Raised	Lobate
			Circular	Medium	Raised	Filamento us
	10^{-4}	3	Circular	Small	Flat	Entire
			Circular	Small	Flat	Entire
			Circular	Small	Flat	Entire
	10^{-5}	2	Circular	Medium	Raised	Entire
			Spindle	Small	Flat	Entire
Valandur Lake	10^{-1}	4	Circular	Medium	Pulvinate	Entire
			Circular	Small	Raised	Entire
			Circular	Small	Raised	Entire
			Punctiform	Small	Flat	Entire
	10^{-2}	3	Irregular	Medium	Raised	Lobate
			Irregular	Medium	Raised	Lobate
			Irregular	Small	Raised	Lobate
	10^{-3}	1	Punctiform	Large	Flat	Entire
	10^{-4}	1	Rhizoid	Large	Raised	Lobate
	10^{-5}	2	Irregular	Large	Raised	Lobate
			Rhizoid	Medium	Flat	Lobate

that anthropogenic pollution promotes the survival and dispersal of opportunistic pathogens.[26,27]

The irregular and larger colony morphologies, as well as higher bioaerosol bacterial colonies observed in Vandiyur Lake may indicate stress adaptations in bacteria due to pollution, temperature and humidity fluctuations or other anthropogenic activities as microbial communities in contaminated environments often develop unique survival strategies.[28] Although the airborne bacteria at both sampling points differed in their concentration and size, these variations might be due to differences in the environmental characteristics of the sampling sites, sampling times, and other anthropogenic activities. In contrast, the punctiform and circular colonies in Valandur Lake suggest a more stable, less disturbed ecosystem. The meteorological conditions can influence bioaerosols[29] as the small difference in temperature (4°C) and in relative humidity (3%) may or may not have influenced the growth of bacterial bioaerosols in our study other than sampling time and human activities that shape the characteristics of airborne bacteria.[29]

The colour of the glass microfibre filter paper differed substantially, indicating the difference in concentration of particles attached to the filter paper during the present study. The change in colour of the filter paper might be due to the pollution present in the air at the sampling site. The majority of the pollution in the sampling site could be due to anthropogenic activities. The microfibre filter paper had accumulated a greater number of dust particles and was darker in colour as compared to the filter paper from a less polluted area. This indicates that Vandiyur was subject to a higher rate of pollution as compared to the other study site.

Elevated Bacterial Concentrations in Polluted Sites

The bacterial load in Vandiyur Lake (2.6×10^5 CFU/m³) was significantly higher than in Valandur Lake (1.13×10^5 CFU/m³), reinforcing the link between water pollution and increased bioaerosol emissions. The elevated CFU levels in both lakes suggest that water bodies in tropical regions act as significant bioaerosol sources, potentially impacting nearby residential and recreational areas.[26]

Public Health Implications

The dominance of Gram-negative bacteria in Vandiyur Lake raises public health concerns, as many Gram-negative pathogens (e.g.,*Vibrio*,*Pseudomonas*) are associated with respiratory and gastrointestinal infections.[28] High concentrations of *Bacillus* in the bioaerosol samples can cause infections. In our study, the concentration of *Bacillus* was only 20% while that of *Vibrio* and *Cocci* were 28% which can cause infections. Even though the association of a polluted environment does not imply that a bacterium is fundamentally a concern to public health, it recommends that the effect of polluting water and soil isn't limited to these surfaces and instead also influences the kinds of bacteria found in nearby air masses. Likewise, the presence of sewage-associated bacteria in surface waters demonstrates a high probability of the pathogens in both the water and air. The potential of public waterbodies to incubate bacteria or to act as a repository for pathogenic microbes that can then be transmitted to the air has significant implications for public health, especially in crowded urban environments. The presence of *Staphylococcus* and *Streptococcus* further indicates potential exposure risks, particularly for immunocompromised individuals.[27]

The presence of gram-negative bacteria in the environment is harmful, and these bacteria need to be identified and studied further to create awareness programs about anthropogenic activities in that area. These findings underscore the need for stricter pollution control measures and public awareness

Table 2: Results of Gram staining with bacteria strain and shape of the bacteria observed at each dilution factor and in each colony.

Study site	Dilution factor	No. of colonies obtained	Gram staining result	Shape of the bacteria	Bacterial stain
Vandiyur Lake	10^{-1}	3	Positive	Circular	*Staphylococcus*
			Negative	Comma	*Vibrio*
			Positive	Circular	*Coccus*
	10^{-2}	3	Negative	Rod	*Bacillus*
			Negative	Comma	*Vibrio*
			Negative	Comma and Circular	*Polymicrobial*
	10^{-3}	3	Negative	Comma and Circular	*Polymicrobial*
			Positive	Circular	*Coccus*
			Positive	Circular	*Coccus*
	10^{-4}	3	Negative	Comma	*Vibrio*
			Positive	Rod	*Bacillus*
			Negative	Circular	*Coccus*
	$_{10}$-5	2	Positive	Rod	*Bacillus*
			Negative	Comma	*Vibrio*
Valandur Lake	10^{-1}	4	Negative	Comma	*Vibrio*
			Negative	Circular	*Coccus*
			Negative	Comma	*Vibrio*
			Positive	Circular	*Streptococcus*
	10^{-2}	3	Positive	Rod	*Bacillus*
			Positive	Circular	*Coccus*
			Negative	Comma	*Vibrio*
	10^{-3}	1	Negative	Rod and Circular	*Polymicrobial*
	10^{-4}	1	Positive	Circular	*Streptococcus*
	10^{-5}	2	Positive	Circular	*Coccus*
			Positive	Rod	*Bacillus*

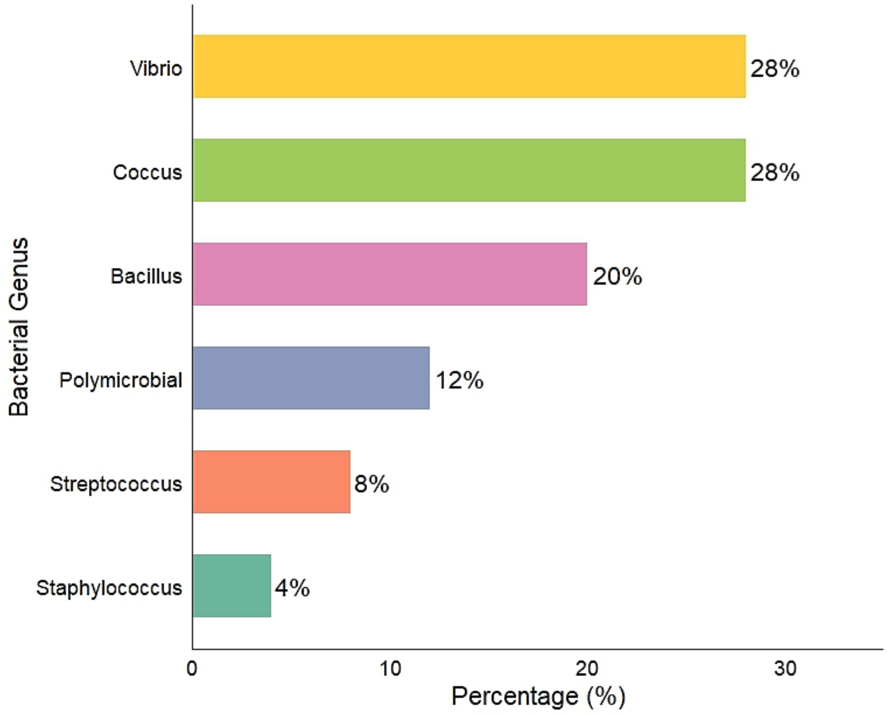

Figure 2: Percentages of various bacterial stains isolated in the study.

Table 3: Concentration of bacteria (CFU/m³) in Vandiyur and Valandur lakes in comparison with a few earlier studies in the world.

Location	Environment	CFU/m³	Source
Vandiyur Lake (India)	Highly polluted lake	2.6×10^5	Present study
Valundur Lake (India)	Less polluted lake	1.13×10^5	Present study
South East England (UK)	Green waste compost facility	$3.7 \text{ x}10^7$	[25]
Marseilles (France)	City	791 ± 598	[23]
Laval. Quebeck (Canada)	Apartment building	17.2	[24]

programs to mitigate health risks associated with bioaerosols from polluted water bodies.

CONCLUSION

This pioneering study in India provides a preliminary overview of the harmful effects of certain bacteria. Water pollution in Vandiyur Lake and its secondary effects, such as the generation of pathogenic bioaerosols, pose significant risks to both environmental and human health. This study bridges the gap by examining the diversity, pathogenicity, and health implications of bacterial bioaerosols. Future studies should focus on pathogen-specific risk assessments and mitigation measures to safeguard public health.

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ABBREVIATIONS

CFU: Colony Forming Units; **UGC-STRIDE:** University Grants Commission scheme for trans-disciplinary research for India's developing economy; **LB:** Luria-Bertani; **PBS:** Phosphate Buffered Saline; **$PM_{2.5}/PM_{10}$:** Particulate Matter 2.5/Particulate Matter 10.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

Jerusha Ann Thomas-experimental studies, data acquisition; Byju H-manuscript preparation, manuscript editing and manuscript review; Maitreyi H-data analysis, statistical analysis, manuscript editing.

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