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Microbial content of bioaerosols in outdoor urban recreation areas of an Atlantic coastal city (Fortaleza-CE, Brazil)

Ítalo Magno Pereira Cajazeiras, Fátima Cristiane Teles de Carvalho, Jade Oliveira Abreu, Carlos Mattoso Cattony, Kamila Vieira de Mendonça, Marcus Vinícius Chagas da Silva, Rivelino Martins Cavalcante, Oscarina Viana de Sousa*

Marine Sciences Institute, Federal University of Ceará (UFC), Fortaleza, Brazil

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CORRESPONDING AUTHOR:

oscarinavs@ufc.br
Tel: (+55) 85 3366-7000
Fax: (+55) 85 3366-7000

ABSTRACT

Introduction: Regular physical activity and outdoor leisure provide significant health benefits. In urban environments, issues related to the air microbiological quality have become a priority due to the pandemic situation we are experiencing. This study analyzed the aerial microbiota of outdoor public spaces, using a qualitative and quantitative approach in Brazilian coastal town.

Materials and methods: Three intra-urban areas were analyzed and characterizing according the thermo-hydrometric characteristics and vegetal cover. Bioaerosols were collected during the wet and dry seasons using the passive sampling technique with selective growth media for fungi and bacteria. Microbial groups were quantified on agar plates; colonies were randomly selected, purified and classified. The antibiotic resistance was evaluated against 6 antibiotics belonging to 6 classes.

Results: Bacteria were relatively more frequent than fungi in the three areas. Among isolates, bacteria represented from 76% (P1) to 90% (P3) of the suspended microbiota in the rainy season; in dry season, the percentages varied from 87% (P1) to 91% (P2 and P3). Genus *Bacillus* was the main representative of Gram positive and Enterobacter genus the most frequently identified among Gram-negative bacteria. *Aspergillus* and *Penicillium* genera were the dominant among fungi. Fifty per cent from bacterial strains analyzed were resistant to at least one of the tested antimicrobials.

Conclusion: Bacteria proved more abundant than fungi and more susceptible to climate and environmental changes in the leisure areas of the city. The monitoring of biological agents in the air is important for environmental management and population health.

Introduction

The aerial dispersion of microorganisms occurs through the force of the wind, water vaporization and human activities that spread biological particles from terrestrial surfaces (soil, water bodies, forests, cities, etc.) [1]. In the atmosphere, microorganisms are aerosolized as cells or single spores. These bioaerosols are small particles formed by biological material (bacteria, fungi,

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algae, protozoa and pollen) attached or not to particulate matter. Considerable amounts of bioaerosols are emitted by different sources including anthropogenic and natural processes [2]. Therefore, the composition of the particles is specific for each area and influenced by the environmental characteristics and surrounding activities.

The air microbiota in outdoor areas can have significant effects on human health and natural ecosystems. The guidelines for physical activities that improve health aimed at different subgroups of the population usually refer to the principles of training: mode, duration, intensity and frequency of activities. In recent years, however, the quality of the environment where happens the physical activity has become a determinant variable on the health benefits [3]. It is important to remember that a healthy human being inhales an average of 14m³ of air on a daily basis, but during physical activities, the demand for oxygen increases with the intensification of air inhaling and also biotic and abiotic pollutants, especially inhalable particulate matter (P_{10} e $P_{2.5}$) [4].

Many studies have documented the negative effects of air pollution on human health. Pathogenic microorganisms and excreted substances, suspended in air, may cause from allergies to severe infections on humans as well as sicknesses in plants and animals [5-7]. The amount of inhaled pathogens is one of the determining variables for the occurrence of airborne infections along with the emission rate (amount of pathogen released per unit of time), meteorological effects, inactivation (function of time or meteorological conditions) and the host's health response as a function of inhaled dose [8].

In urban centers, the relationship between exercise and quality of life and health leads a significant part of the population to exercise outdoors, especially in parks and squares with green areas. However, depending on the levels of air pollution, the areas used for leisure and sports can also represent hazards to public health. There is a consensus that physical activity is beneficial to human health, while long-term exposure to air pollution is harmful [9]. Thus, monitoring the chemical and biological air quality at points used for physical activity, especially in urban areas, is an important element in ensuring the health and safety of users. This research aimed to monitor the aerial microbiota of urban outdoor public areas for leisure and exercise in a coastal urban city (Fortaleza, Brazil).

Materials and methods

Area description

The city of Fortaleza is the capital of the state of Ceará, found in the northeastern region of Brazil. It is located on the Atlantic coast, at an average altitude of sixteen meters. According to statistics provided by The Brazilian Institute of Geography and Statistics [10], it is a county with an area of 314,930 (km²) and a population of approximately 2.591.188 people. The city has a well-defined rain season (from January to July) and drought season (from August to December), with an average temperature of 25°C to 28°C, average wind speed of 3,53 m/s and average rainfall of 1600 mm with very sparse rain distributed throughout the year.

Environmental and climatic variables

Three interurban recreational spaces (squares) used by the population for leisure and physical exercises were monitored: (P1): (-3.727941, -38.545396), (P2): (-3.7254728, -38.4920045)

and (P3): (-3.7392737, -38.498728).

The samples were taken in June and July (rainy season) and September and October (dry season), between 16:00 and 17:00, period with great flow of people.

Meteorological station (Vantage Vueda of Davis Instruments) was used to monitoring temperature and relative air humidity. The vegetal cover area datawas gathered using satellite images (Quickbird satellite) and vector data obtained through planimetric digital chart of Fortaleza city in a scale of 1:5.000. ArcMap™ software was used alongside Google™ Earth.

Air microbiota sampling

To sample the atmospheric air, Petri dishes containing selective growth mediums for bacteria (Plate Count Agar+2% Propionic Acid) and for fungi (Potato Dextrose Agar+10 µg/mL Ampicillin). Samples were collected using the passive sampling technique and exposing the open dishes for 30 min. After samples were collected, Petri dishes were taken to laboratory and incubated at 35°C for 48 h (bacteria) and at 28°C for 7 days (fungi). The number of CFU in each cubic meter of air was calculated using Eq. 1 [11].

$$\frac{CFU}{m^3} number = CFU \frac{counting}{plate\ area\ (m^2)}\ x \frac{1}{23} \qquad (1)$$

where, plate area = 0,006362 m²

Bacterial strains isolating and identification

Ten bacterial colonies, from each sampling, were randomly selected on the surface of Plate Count Agar (PCA) medium. They were picked and then purified. Culture morphology and purity was assessed by Gram staining.

The pure cultures were submitted to phenotypic tests based on dichotomic identification keys according to the instructions of Holt and Krieg [12].

Antibiogram

Each isolated and identified culture was submitted to an antibiogram test carried out by the disk diffusion method, as recommended by the Clinical and Laboratory Standards Institute [13]. The tested pharmacological principles were Macrolides (Erythromycin – ERI, 15 µg), Penicillins (Amoxicillin – AMX, 30 µg), Quinolones (Nalidixic Acid – N/A, 30 µg), Aminoglycosides (Streptomycin – EST, 10 µg), Cephalosporins (Cefotaxime – CTX, 30 µg) and Nitrofurans (Nitrofurantoin – F/M, 300 µg). The antimicrobials were chosen from data about use frequency of antimicrobials in respiratory tract infection treatment. The antimicrobial disks were of commercial grade (Laborclin®).

Fungi strains isolating and identification

Ten fungal colonies, from each sampling, were randomly selected on the surface of Potato Dextrose Agar (PDA) medium. Selection was done based on characteristic diversity of colonies, such as color and shape.After purification, colonies were identified according to macro and microscopic morphologic characteristics using the Methylene Blue growth technique, following the instructions on the manual by Brazilian Health Regulatory Agency (ANVISA) [14].

Statistical analysis

Data was submitted to descriptive statistical analysis and non-parametric Mann-Whitney and Kruskal-Wallis tests to verify if average numbers were statistically different [15]. The level of significance was established on 5%.

Average count of aerial fungi and bacteria were analyzed divided on season and sampling area.

Results and discussion

Outdoor public spaces for recreation are important environments for leisure and physical activity for the population in large cities. The results of this effort serve to highlight the importance of monitoring respirable biological particles and the variables that can influence their abundance and diversity.

Vegetation cover is an important factor for the creation of microclimates areas. The space with larger vegetation cover (P3) showed lower mean temperature and higher relative humidity in the two seasons (Table1). Trees in urban environment help to improve air quality by facilitating widespread deposition of gases and particles through the provision of large surface areas as well as through their influence on microclimate [16].

The abundance of cultivable microorganisms in the air microbiota was determined (Fig. 1). A larger number of bacteria can be noticed when compared to the fungi numbers at all areas. Bacteria were also more abundant in bioaerosols at the seasonal station with less occurrence of rain.

Analyzing the areas, P1 had the highest amounts of suspended microorganisms and P2 the lowest. The highest bacterial counts were recorded during the dry season, although there were no significant differences between areas and seasons: P1, 4223 CFU/m³; P2, 1433 CFU/m³; P3, 2431 CFU/m³. In the city of Fortaleza, the dry season is also identified by strong winds that contribute to the resuspension of matter deposited in the soil and its permanence in the atmosphere. Other researches analyzing bacterial communities added to inhalable particulate matter in urban environments found that environmental factors influence the quantity and diversity of microorganisms in the air [17]. Bacteria were in larger quantities in the samples during both seasons and all areas even though fungi are considered more competent in atmospheric environment. Data reveals that bacteria represented from 76% (P1) to 90% (P3) of the suspended microbiota in the rainy season, and from 87% (P1) to 91% (P2 and P3) in dry season.

Table 1. Thermo-hydrometric characteristics and vegetal cover from outdoor urban leisure areas in the city of Fortaleza (Ceará, Brazil)

Sampling areas	Vegetal cover (%)	Temperature°C		Humidity %	
		Rainy	Dry	Rainy	Dry
P1	2,45	28,2 ± 0,2	27,8 ± 0,3	71 ± 1,1	72 ± 2,0
P2	8,84	28,7 ± 0,2	27,7 ± 0,1	74 ± 1,1	72 ± 0,7
P3	9,15	27,7 ± 0,2	27,0 ± 0,5	74 ± 1,0	76 ± 1,9

Similarly, in a study that analyzed microorganisms attached to particulate matter (PM_{10} and $PM_{2.5}$) in urban areas of Saudi Arabia, the authors found a higher number of bacteria compared to fungi [18].

The survival of aerosolized vegetative bacteria has been shown to be a function of size, shape, environmental variables and air pollutants. First, microbial particles are carried by air currents. They may be present only as microbial particles, they may form aggregates, or they may be attached to larger particles. The sizes of the formed structures condition the aerodynamic behavior influencing their half-life [19, 20]. The presence of these bacteria can also be influenced by the characteristics related to the movement and turbulence of terrestrial sources.

The average total of fungi in the samples varied very little between both seasons, making both statistically similar. Values in the rainy season were: 815/ m^3 CFU (P1); 116 CFU/m^3 (P2) and 159 CFU/m^3 (P3); and in the dry season these values were 615 CFU/m^3(P1); 138 CFU/m^3 (P2) and 231 CFU/m^3 (P3). Seasons were not determinant factors for fungi dispersion and these were detected in smaller numbers than suspended bacteria in sampled areas. Other researches show a significantly smaller number of fungi compared to bacteria in open areas such as recreational parks [21] and urban areas [22, 23].

Microbial identification

On the qualitative analysis of the air microbiota, Gram positive bacteria were more frequent among all isolates. The most abundant genus was *Bacillus* spp, representing over 60% of all isolates. The most frequently identified fungal genera in the air microbiota were the sporogenic *Aspergillus* and *Penicillium* and non-sporogenic *Cladosporium* and *Mycelia sterilia* fungi (Fig. 2).

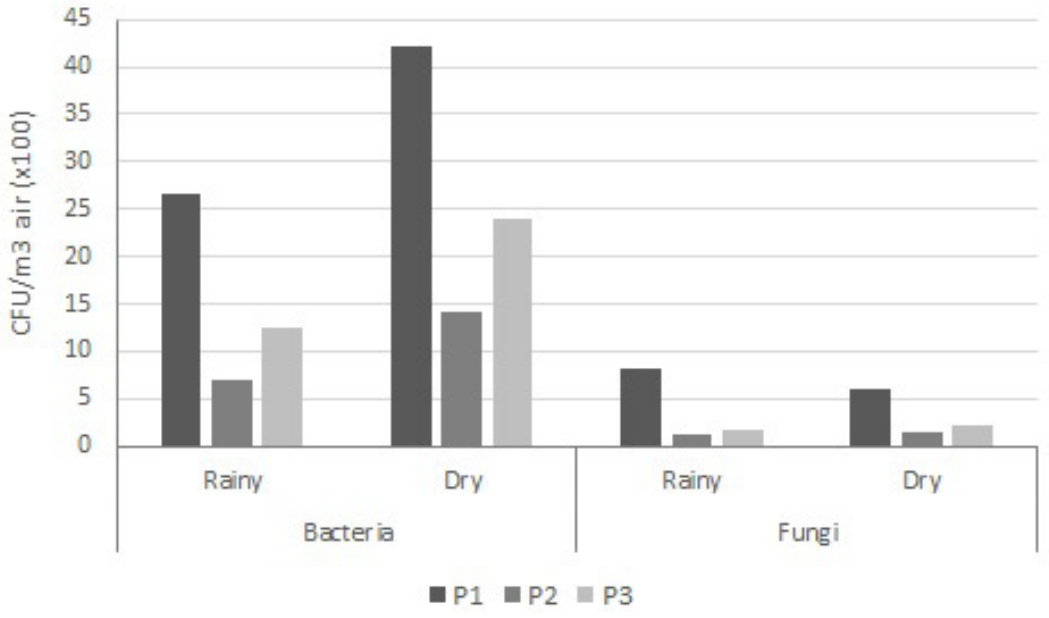

Fig. 1. Average count of sampled colony forming units of microorganisms per cubic meter of air from outdoor urban leisure areas in the city of Fortaleza (Ceará, Brazil)

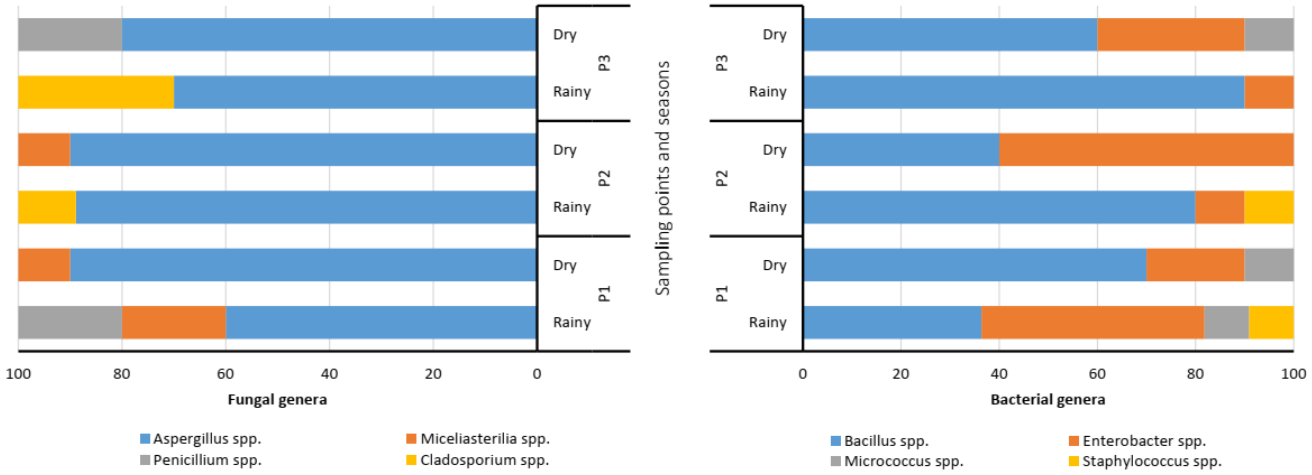

Fig. 2. Frequency (%) of fungal and bacterial genera isolated from the air in urban areas of Fortaleza city (Ceará, Brazil)

Gram-positive bacteria were more frequent among the isolates and have a greater ability to survive pressure factors in the atmosphere such as UV radiation, temperature, oxidizing reactions, etc. The presence of the *Bacillus* genus is distinguishable; and it is related to sporulation capacity of some strains, which makes them resistant to unfavorable atmospheric conditions. Other Gram positive genera were *Micrococcus* and *Staphylococcus*. A single genus was detected among all Gram negative isolates. The largest number (14) of isolates of the *Enterobacter* genus were obtained in areas P1 and P2. These bacteria are members of the Enterobacteriaceae family and the genus has bacteria that are pathogenic to humans and other animals. It can be isolated from various environmental sources and sewers. Its presence in the aerial microbiota is clinically associated with respiratory symptoms, allergies, asthma and immune responses depending on the microbial agent and the health of the host. Some species of Gram-negative bacteria are more dangerous when present in indoor air because of the production of endotoxins that can cause respiratory problems, including non-allergenic asthma.

Other researches that addressed the aerial microbiota in open environments showed similar results, with the genera *Bacillus*, *Micrococcus* and *Staphylococcus* among the most frequently isolated ones, with variable abundances according to their origin, indicating an environmental influence on the referred microbiota [24-27]. Some of the species belonging to these genera may be potentially hazardous to human health and its abundance was also registered in indoor environments [27, 28]. *Staphylococcus* genera isolates were detected only during on the rainy season sampling.

Variations in the abundance of fungi in the air microbiota may be related to the characteristics of the geographical area with the absence of biotic sources, arid environments and, additionally, high temperatures [29]. Considering the

characteristics of each analyzed area, P1 represented the lowest wooded area percentage, highest average temperature and lowest relative air humidity. P2 is located in the coastal region of the city and is under the influence of marine winds characteristic of the region with strong aerosol dispersion action.

Authors state that there is a disparity in the mycobiota between urban and countryside environments. Spores of *Cladosporium*, *Aspergillus* and *Penicillium* genera are usually found in both environments [30].

The anemophile mycobiota is typical to the region from origin and the structures of aerial dispersion found in the air, the spores, are known as aeroallergens. When inhaled, they may be responsible for allergenic respiratory reactions, such as asthma and rhinitis [31, 32]. Beyond allergy cases, many opportunist fungi such as *Penicillium* spp., *Aspergillus* spp. and *Cladosporium* spp. are responsible for diseases such as otitis, mycotoxicoses, urinary infections, onychomycoses, ocular infections and even fungemia [33].

In the latest decades, the importance of bioaerosols or biological contaminants have been emphasized for their relation to human health.

The most frequently detected fungal genus was *Aspergillus* spp., representing over 80% of the isolates. This genus is ubiquitous in outdoor environments, growing in soil, water and air, with simple growth and spreading, which may explain its abundance in the isolates. Because of the small size of the spores, these are very possible of entering the respiratory system and the human paranasal sinuses, causing infections in susceptible patients.

Cladosporium genus isolates were detected only in samples collected during the rainy season. It is important to point that the *Cladosporium* genus is also widely known as an allergenic and is related to phaeohypho mycoses in human beings and detected in several in the air microbiota from several cities in the world [34-36].

The microorganisms represent a relevant fraction of the suspended particles in the atmosphere; however, there is still very little information about abundance and diversity or environmental factors's influence over these communities. As a vital part of the suspended particulate matter, the microbial aerosols may have a definitive role over public health and maintain biogeochemical connection between oceans, atmosphere and land environments [37]. Several dispersal mechanisms have been described, but transport of both microbes and antibiotic resistance genes (ARGs) via atmospheric particles has received little attention as a pathway for global dissemination [38].

The antimicrobial resistance profiles in the bacterial strains can also be considered an additional risk factor towards human health. In the present study, resistance profiles were registered towards the following: Nitrofurantoin (F/M), Erythromycin (ERI), Nalidixic Acid (N/A), Amoxicillin (AMX), Streptomycin (EST) and Cefotaxime (CTX). Considering all 60 analyzed bacteria, fifty percent (30 strains) have shown (intermediate or full) resistance to at least one of the tested antimicrobials (Table 2).

Table 2. Relative frequency (%,n=10) of resistance to antibiotics by aerolized bacteria from air of urban parks in the city of Fortaleza (Ceará, Brazil) in two seasons

Antibiotic	Rainy						Dry					
	P1		P2		P3		P1		P2		P3	
	I	R	I	R	I	R	I	R	I	R	I	R
Nitrofurantoin	0	0	0	10	0	0	0	0	0	10	0	20
Erythromycin	0	0	0	0	0	0	0	0	10	0	0	0
Nalidixic Acid	10	10	0	10	10	0	0	10	0	10	0	10
Amoxicillin	0	0	10	0	0	10	0	0	0	0	0	0
Streptomycin	0	10	10	0	0	0	0	0	0	0	0	0
Cefotaxime	40	0	0	10	10	10	10	0	30	50	10	50

R: resistant; I: intermediate

The biggest resistance percentual from bacterial isolates was registered in Cefotaxime (CTX), of the Cephalosporin class. The resistance frequency for said antibiotic varied from 10% (dry season) to 50% (rainy season). Other researches detected the abundance of genes in the air of urban centers giving resistance to β-lactams [39]. The increased resistance as well as the environmental dispersion of the resistance genes imply serious health problems in a global scale.

There have also been intermediate resistance profiles that might be indication of selective pressure on the bacterial community. Spaces in environments can become genetic "hotspots", where resistance genes can thrive and be laterally transferred for clinically relevant pathogens [40].

Conclusion

Bacteria proved more abundant than fungi and more susceptible to climate and environmental changes in the leisure areas of the city. Even with their importance for health and welfare, they are very negligenced components of the air microbiota. It is very advisable to include these components in the measuring and modeling of atmospheric pollutants and measurement of potential risks to public health.

Even with the knowledge generated by the influence of urbanization over respiratory diseases, there are no legislative parameters that establish limits or a risk level to being exposed to microorganisms in outdoor environments. The elaboration of air quality control plans comes through the understanding of environmental variables and their epidemiological correlation including aerial microbiota in urban environments.

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Competing interests

The authors declare no potential conflict of interest affecting this work.

Authors' contributions

Ítalo Magno Pereira Cajazeiras, Fátima Cristiane Teles de Carvalho and Jade Oliveira Abreu: acquisition of data, microbial analysis and interpretation of data; Rivelino Martins Cavalcante, Oscarina Viana de Sousa: conception, design, drafted the manuscript; Kamila Vieira de Mendonça: statistical analysis; Marcus Vinícius Chagas da Silva, Carlos Mattoso Cattony: enviromental data analysis and interpretation

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Ethical considerations

Ethical issues (Including plagiarism, Informed Consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc) have been completely observed by the authors.

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