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Urban Aerobiome and Effects on Human Health: A Systematic Review and Missing Evidence

Elena Franchitti *,†, Chiara Caredda †, Elisa Anedda and Deborah Traversi

Department of Public Health and Pediatrics, University of Turin, Piazza Polonia 92, 10126 Torino, Italy;
chiara.caredda635@edu.unito.it (C.C.); anedda.elisa@gmail.com (E.A.); deborah.traversi@unito.it (D.T.)
* Correspondence: elena.franchitti@unito.it; Tel.: +39-6705703; Fax: +39-2365703
† These authors contributed equally to this work.

Abstract: Urban air pollutants are a major public health concern and include biological matters which composes about 25% of the atmospheric aerosol particles. Airborne microorganisms were traditionally characterized by culture-based methods recognizing just 1.5–15.3% of the total bacterial diversity that was evaluable by genome signature in the air environment (aerobiome). Despite the large number of exposed people, urban aerobiomes are still weakly described even if recently advanced literature has been published. This paper aims to systematically review the state of knowledge on the urban aerobiome and human health effects. A total of 24 papers that used next generation sequencing (NGS) techniques for characterization and comprised a seasonal analysis have been included. A core of Proteobacteria, Actinobacteria, Firmicutes, and Bacteroides and various factors that influenced the community structure were detected. Heterogenic methods and results were reported, for both sampling and aerobiome diversity analysis, highlighting the necessity of in-depth and homogenized assessment thus reducing the risk of bias. The aerobiome can include threats for human health, such as pathogens and resistome spreading; however, its diversity seems to be protective for human health and reduced by high levels of air pollution. Evidence of the urban aerobiome effects on human health need to be filled up quickly for urban public health purposes.

Keywords: aerobiome; urban environment; air microbiota; NGS; health; particulate matter

1. Introduction

Ambient air pollution is a major public health concern that is associated with the increase of worldwide morbidity and mortality. It is the result of air contamination by anthropogenic (especially in urban environments) or natural sources. The parameters today that are engaged in the air quality monitoring are mainly black carbon (BC), particulate matter (PM_{10} and $PM_{2.5}$), ground level ozone (O_3), sulfur dioxide (SO_2), nitrogen dioxides (NO_2), and volatile organic compounds (VOCs). Such criteria pollutants are present in the air at critical concentrations that are above the updated guidelines [1], for the large part of the year and in wide urban territories [2]. In addition to chemical compounds, the population is exposed to biological entities that occupy the urban atmosphere such as viruses, bacteria, fungi, spores, endotoxins, archaea, pollen, and others [3]. To date, the biological component of the urban outdoor environment has not been properly studied, while a wider range of data are present for the indoor setting where the bioaerosol is more concentrated and more easily detected [4–7]. Instead, researchers are now focusing on the detection of potentially harmful microorganisms and their transmission in the outdoor environment. A high population density is an ideal situation for direct human–human airborne transmission [8]. Of course, with the COVID-19 pandemic the interest in SARS-CoV-2 and outdoor pathogens diffusion is increasing. Outdoor risk still needs to be evaluated because PM can be a carrier of such viruses and of other pathogens favoring their dissemination [9].

The atmosphere can be considered an extreme environment due to its chemical and physical characteristics, such as high solar radiation, low moisture and nutrients, and its large dispersing capability [10]. Despite these limitations, microorganisms can indeed survive and be metabolically active [11,12].

The term "urban aerobiome" can overlap with the more common term "urban bioaerosol" (complex mixture of small airborne particles, 0.001–100 μm, with a biological origin). The bioaerosol is generally detected by culture-based methods while, typically, aerobiome studies include a biomolecular detection, such as next generation sequencing method and it considers the qualitative and quantitative relationships between aerobiota and the environmental conditions.

Traditionally in the extant literature, most airborne microorganism's characterization depended on culture-based methods [13–15], leading to an underestimation of the total bacterial diversity. The cultural method only recognizes 1.5–15.3% of all the species that are able to form a colony and the microorganisms that are not viable or unable to grow are not identified [16]. On the contrary, high throughput sequencing can offer further insights by revealing the diversity of previously undetected or uncultured bacteria [17] and NGS methods have been recently applied in the characterization of temporal and spatial variability of microbial communities in different environments [18].

The detection approaches that have been adopted in recent years are commonly integrated. Often a cultural enrichment step is included for specific target detection when the absence certification is necessary (such as for pathogens) [19].

The aero-dispersed microbiome can contain pathogenic and/or non-pathogenic microorganisms, dead or alive, as well as parts of those microorganisms (e.g., endotoxins) [20]. Aero-dispersed microbes and their fragments are emitted from different sources, such as soil, freshwater, ocean, plants, animal feces, human skin, oral cavity, wastewater treatment plants, and composting facilities [10,21]. It was suggested that about 25% of atmospheric particles is from biological sources [21] and, therefore, bioaerosol is typically part of the particulate matter, which is a key indicator of urban air pollution. Particulate matter works has a support for microorganism survival in the air and particle size is a crucial factor for the degree of penetration into the human respiratory tract: coarse particles ($PM_{2.5-10}$) are mainly deposited in the extra thoracic area, whereas the fine particles ($PM_{2.5}$) are deposited in the trachea, bronchial, and alveolar region [22]. The finest particles are able to overlap the epithelium barriers showing a tight junction disruption [23].

About 3 to 11% of $PM_{2.5}$ mass is composed of biological particles [6]. Therefore, it could have a role in infective and chronic degenerative pathological outcomes. Cell-culture-based studies have suggested that PM toxicity can be due to, or modified by, the bioaerosol modulating, for example, the oxidative potential of toxic chemicals that are present in PM [24,25]. In addition, air pollution influences the microbial interaction in the air and experimental evidence showed that urban $PM_{2.5}$ exposure seems to significantly reduce (−44%) the bacteriophage infectivity compared to an unexposed control [25].

PM carries a large number of microorganisms that cause oropharyngeal [26] and nasal dysbiosis [27]. Rylance et al. (2016) reported the presence of Petrobacter in the lung lavage of Malawian people using biomass for lightening and cooking, suggesting that PM can transfer environmental microorganisms inside the human body [28,29]. A dysbiosis state causes the reduction of immune-related commensal species and the increase of pathogens and pathobionts (commensal microorganisms which can become pathogens when genetic and environmental conditions change) [29,30]. Microorganisms that composed bioaerosol are also a reservoir for the resistome, the complex of the antimicrobial resistance genes (ARGs), further decreasing antibiotic's efficacy [15]. Exposure to PM-associated bioaerosol has been markedly associated with various health effects, mainly with infectious diseases but also respiratory diseases, allergies, and lung cancer [13,14,20,26,27,29].

The airborne microorganisms that are inhaled can modulate the composition of the nasal and respiratory microbiota. This was observed clearly in occupational settings where work-related microbial and non-microbial exposures may modify the worker microbiome (even if such modification was not permanent for pig farmer's microbiota composition) [31].

Such influence can modulate health effects not only at the respiratory tract level, recent data showed that the exposure to gaseous nitrogen dioxide could contribute to skin dysbiosis, which would affect skin homeostasis; the response of the microbiota to the nitrosative stress could involve pathologies such as atopic dermatitis [32].

In urban areas, a person could inhale one hundred million bacteria each day [3] and recent studies are focused on the characterization of airborne bacterial communities in urban areas [25,26]. However, published manuscripts that are focused on the effects on human health and the interaction with urban environment factors are lacking.

Only a previous systematic review was conducted on this emerging research field focusing on the comparison between rural and urban aerobiomes [14].

This paper aims to review the state of knowledge on the urban aerobiome by reviewing the updated literature that characterize the urban airborne communities utilizing NGS techniques. The sampling methods, the environmental determinants, the presence of pathogens, as well as other human health risks were also included when they were present in the selected articles.

2. Materials and Methods

We conducted a systematic search of peer-reviewed articles which started in August 2020 and ended in February 2022 using Scopus, PubMed, and Web of Science as search engines. We used the following string: TITLE-ABS-KEY (bioaerosol OR airborne OR aerobiome) AND (bacteria* OR microb* OR communit*) AND (urban) AND (metagenomic OR sequencing) and a data interval 2011–2021, finding 206 articles (67 papers on Scopus, 41 on PubMed, and 98 on Web of Science) (Figure 1). Following the updated PRISMA 2020 statement, a 27-item checklist (reporting recommendations for each item) [33], the titles and abstracts were screened to assess eligibility. A document was deemed relevant if (a) it was a peer-reviewed journal publication and (b) it presented microbial characterization of airborne urban microbiota using next-generation sequencing methodologies.
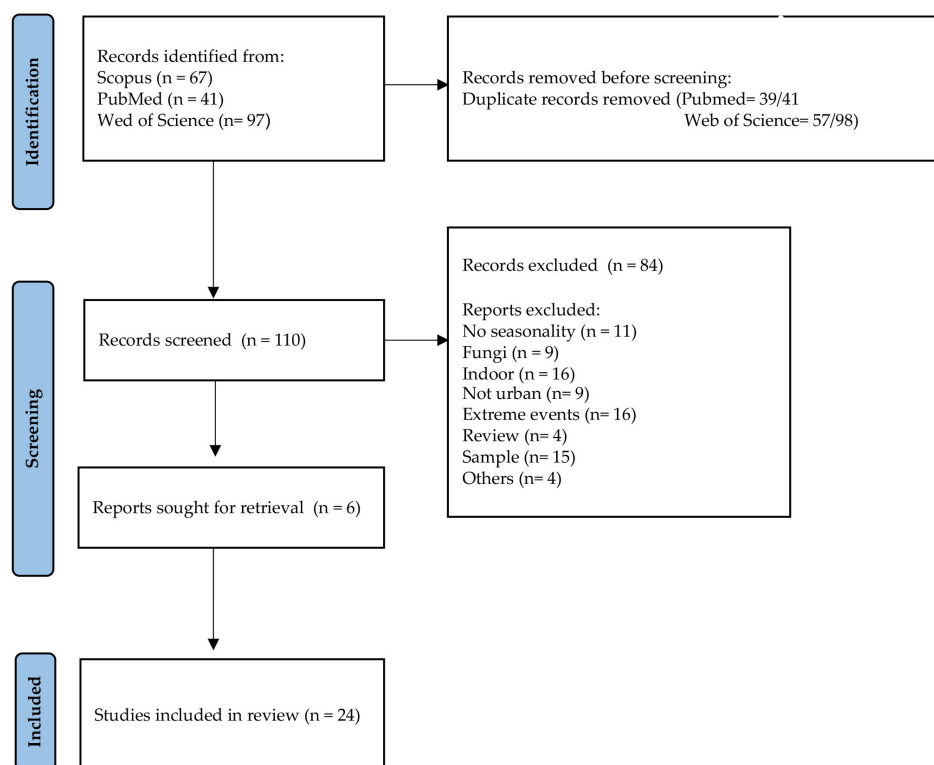

Figure 1. Flow diagram of the systematic review steps following the PRISMA 2020 statement [33].

To narrow the subject's review, we decided to add a few more exclusion criteria such as extreme events as hazy days, rainfall or heat events, and papers with exclusive fungal characterization. We also excluded research that conducted monitoring that lasted less than two seasons. There were 6 papers that were references of the selected papers and that met the inclusion criteria: they were included. Eventually we obtained 24 papers to include in this review. At least 2 authors working independently screened each record and then collected the data. Data on urban aerobiome alpha and beta diversity were collected as well as data on the significant affecting factors (such as space, time, season, and meteorological conditions).

The risk of bias due to aerobiome sampling methods and to the methods of analysis was evaluated for each record.

3. Results

From evaluating the microbial composition in the selected papers, the urban aerobiome around the world presents 10^3 cells/m^3 on average and a common core of bacterial phyla: Proteobacteria (reported in 88% of the selected papers), Actinobacteria (79%), Firmicutes (83%), Bacteroidetes (44%), and Cyanobacteria (40%) (Table 1). The abundance of the main phyla ranged widely in relation to the sampling urban site. For example, the Proteobacteria make up from the 75% to the 17% of the total urban aerobiome while the minor phyla can be accountable for the 1–20% [34]. At the genus level, several bacteria are frequently reported such as *Bacillus, Ralstonia, Enterobacter, Klebsiella, Chloroplast, Staphylococcus, Streptococcus, Sphingomonas, Methylobacterium,* and *Acinetobacter* (Table 1). Species level is commonly defined when pathogen presence is analyzed. Often, pathogens were present in the aerobiome samples and their seasonal modulation was also reported. Commonly reported harmful bacteria are *Pseudomonas aeruginosa, Corynebacterium, Enterobacter, Salmonella, Streptococcus,* and *Staphylococcus*.

The major sources of bacteria are soil (79% of the selected papers), plants (58%), water (50%), human and animals feces (17%), and human skin (13%) (Table 2). The influence of each source differs during the year, with a prevalence of leaf-associated microorganisms during hot seasons and soil-associated ones during winter. Bowers et al. reported the prevalence of Actinobacteria, which are soil bacteria, during the winter-spring period, and Pseudomonadales, a leaves-related microorganism, during the late-spring and summer [16]. In specific insular locations, the airborne bacterial community composition was strictly influenced by the origin of the air masses and by the altitude of the urban site [35].

Bacterial communities' structure is defined by two parameters, which are species richness (count of the species in the community) and evenness (the relative abundance of species in a community). Species richness, which is the count of the number of species or operational taxonomic units (OTUs) that are present in an area, is the simplest measure of alpha diversity. However, there are many other metrics or indices, which consider the abundance or frequencies of the OTUs (for example Shannon, Simpson, and Chao1) [36].

Species relative abundance (beta-diversity) is usually compared using Bray–Curtis or UniFrac and it is visually clustered with principal component analysis (PCoA). A total of 13 of the selected papers reported seasonal variabilities in the bacteria community. The microbial abundance and diversity during the year were compared and higher indexes are more often observed in the hot seasons (spring, summer) (52% of the selected papers, 36% summer and 16% spring). Samples analysis showed a season-based cluster where samples that were collected during the same season were separated from the other samples [17,18,37–40]. Bertolini et al. (2013) reported the presence of a common core of bacterial species that causes a visual overlap of the summer and winter samples with spring/fall ones [41].

Environmental factors and atmospheric conditions commonly influence the abundance and the diversity of the urban microbial communities. Among those variables there are wind speed, humidity, temperature, air quality levels (AQLs), and less frequently UV light and precipitations (Table 2). Temperature is the main atmospheric condition, and it is reported as positively correlated in 58% of the selected papers. In 7 of the 13 (54%) papers

that evaluate the effect of the environmental factors on the bacterial community structure, air pollution is analyzed. Of these, two of them reported a negative correlation between the air quality index (AQI) and species richness [42].

Table 1. Geographic localization, sequencing methods, bacteria community structure, and pathogens for each selected paper are reported.

Reference	Location	Sequencing Method	Phyla More Abundant	Class and Order More Abundant	Genera and Species More Abundant	Pathogens
(Be et al., 2014) [17]	USA	Illumina	Bacteroidetes, Firmicutes, Fusobacteria		*Bacillus cereus, B. clausii, B. coagulans, B. megaterium, B. psychrosaccharolyticus, B. pumilus, B. smithii, B. subtilis, B. thuringiensis, Klebsiella pneumoniae, Geobacillus caldoxylosilyticus, Ralstonia pickettii, R. solanacearum, Cupriavidus necator, C. basilensis, Staphylococcus epidermidis, S. hyicus, Stenotrophomonas maltophila*	*Bacillus cereus, Staphylococcus*
(Becsei et al., 2021) [15]	Hungary	Ion Torrent	Proteobacteria, Firmicutes		*Bacillus, Acinetobacter, Paenibacillus, Atlantibacter, Citrobacter, Enterobacter, Klebsiella pneumoniae, Leclercia, Pseudoescherichia*	
(Bertolini et al., 2013) [41]	Italy	Illumina	Actinobacteria	Actinobacteriales, Chloroplast, Burkholderiales, Sphingobacteriales, Clostridiales, Rhizobiales, Sphingomonadales, Pseudomonadales, Lactobacillales, Bacillales, Rhodospirillales, Rhodobacterales, Flavobacteriales, Enterobacterales		
(Bowers et al., 2013) [16]	USA	Pyrosequencing Illumina	Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria	Pseudomonadales, Sphingobacteriales, Rhizobiales, Rhodospirillales, Burkholderiales, Actinobacteriales, Bacteroidales, Lactobacillales, Clostridiales		

Table 1. *Cont.*

Reference	Location	Sequencing Method	Phyla More Abundant	Class and Order More Abundant	Genera and Species More Abundant	Pathogens
(Calderón-Ezquerro et al., 2021) [43]	Mexico	Illumina	Actinobacteria, Proteobacteria, Firmicutes, Bacteroidetes, Cyanobacteria		*Microbispora, Kocuria, Paracoccus, Corynebacterium, Friedmanniella, Propionicimonas, Aeromicrobium, Nocardioides, Modestobacter, Geodermatophillus, Arsenicoccus, Phycicoccus, Janibacter, Roseomonas, Bacillus, Staphylococcus, Jeotgalicoccus, Salinicoccus, Pseudomonas, Sphingomonas, Streptococcus, Rheinheimera, Janhtinobacterium, Enterobacteraceae*	*Corynebacterium diphteriae, Mycobacterium tuberculosis, Bacillus antracis, Clostridium botulinum, Clostridium tetani, Kocuria rhizophila, Staphylococcus* spp., *Acinetobacter* spp., *Psychrobacter sanguinis, Mycobacterium arupense, Rhodococcus fascians, Enterococcus cecorum, Pseudomonas viridiflava, Erwinia* sp.
(Chen et al., 2021) [37]	China	Illumina	Proteobacteria, Actinobacteria, Firmicutes	Myxobacteriales	*Summer: Melittangium, Winter: Arthrobacter, Kocuria, Romboutsia, Clostridium sensu stricto 1, Bacillus, Fall: Sphingomonas, Methylobacterium, Paracoccus, Planomicrobium*	*Streptococcus, Acinetobacter lwoffii, Bacillus anthracis, Prevotella, Clostridium perfringens, C. novyi, Erysipelothrix, Corynebacterium minutissimum, Serratia marcescens, Pseudomonas aeruginosa, Nocardia carnea, N. asteroides, Bacteroides fragilis, Campylobacter jejuni, Legionella pneumophila, Fusobacterium necrophorum*
(Du et al., 2018) [13]	China	Illumina	Actinobacteria, Proteobacteria, Firmicutes, Cyanobacteria, Ascomycota, Bacteroidetes, Gemmatimonadetes, Acidobacteria, Chloroflexi	Alphaproteobacteria, Clostridiales	*Streptophyta, Pseudolabrys, Bacillus, Clostridium, Sphingomonas, Blastococcus, Segetibacter, Methylobacterium, Kocuria, Staphylococcus*	
(Fan et al., 2019) [38]	China	Illumina	Proteobacteria, Actinobacteria, Firmicutes, Cyanobacteria	Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria	*Ralstonia, Kocuria, Blastococcus, Planococcus, Arthrobacter, Paracocccus, Rubellimicrobium, Sphingomonas, Rhizobium, Propionibacterium, Bacillus, Pseudomonas, Acinetobacter Burkholderia, Bacteroides, Flavisolibacter, Halomonas, Psychrobacter, Candidatus jettenia, Nitrosomonas, Nitrosospria, Nitrosococcus*	Spring: *Streptococcus, Pseudomonas,* Winter: *Clostridium, Enterococcus,* Air pollution: *Escherichia, Shigella, Burkholderia,* Others: *Staphylococcus, Mycobacterium, Aeromonas, Neisseria, Haemophilus, Vibrio, Campylobacter, Corynebacterium, Mycoplasma, Rickettsia*

Table 1. *Cont.*

Reference	Location	Sequencing Method	Phyla More Abundant	Class and Order More Abundant	Genera and Species More Abundant	Pathogens
(Franzetti et al., 2011) [44]	Italy	Pyrosequencing	Proteobacteria, Firmicutes, Actinobacteria	Winter: Actinomycetales; summer: Bacillales, Clostridiales; air pollution: Sphingomonadales, Rhizobiales, Pseudomonadales, Sphingobacteriales, Burkholderiales, Clostridiales, Bacteroidales	*Chloroplast*	
(Gandolfi et al., 2015) [45]	Italy	Illumina		Burkholderiales, Actinomycetales Enterobacterales, Rhodobacterales, Sphingobacteriales, Rhizobiales, Flavobacteriales, Pseudomonadales, Xanthomonadales, Bacillales, Clostridiales	*Chloroplast*	
(Genitsaris et al., 2017) [46]	Greece	Pyrosequencing	Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes, Cyanobacteria, Deinococcus-Thermus	Alphaproteobacteria, Betaproteobacteria, Hot: Gammaproteobacteria	*Bacillus aquimaris, B. oceanisediminis, Pseudomonas, Synechococcus* sp.	*Pseudomonas, Propionibacterium, Staphylococcus, Streptococcus, Corynebacterium*
(González-Martín et al., 2021) [35]	Spain	Illumina	Proteobacteria, Bacteroidetes, Actinobacteria, Firmicutes	Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Deltaproteobacteria, Lachnospiraceae	*Cellvibrio, Blastomonas, Limnobacter, Sediminibacterium, Cloacibacterium, Enterococcus, Thermicanus, Terrisporobacter, Kocuria, Delftia, Mesorhizobium, Methylobacterium, Tepidisphaera, Alycobacillus, Acinetobacter, Bacillus, Brucella, Neisseria, Staphylococcus, Streptococcus, Pseudomonas, Brevundimonas, Corynebacterium, Sphingomonas, Blastomonas*	*Acinetobacter, Enterococcus, Pseudomonas, Bacillus, Brucella, Neisseria, Staphylococcus, Streptococcus*
(Lee et al., 2017) [47]	China, South Korea and Japan	Illumina	Proteobacteria, Firmicutes, Actinobacteria, Deinococcus-Thermus		*Rubellimicrobium, Streptomyces, Kaistobacter, Bacillus, Kocuria, Brevibacillus*	
(Li et al., 2019) [39]	China	Ion Torrent	Proteobacteria, Firmicutes, Actinobacteria, Cyanobacteria		Winter: *Chloroplast* Spring/summer: *Lactobacillus* Fall: *Pseudomonas*	

Table 1. *Cont.*

Reference	Location	Sequencing Method	Phyla More Abundant	Class and Order More Abundant	Genera and Species More Abundant	Pathogens
(Hu Li et al., 2019) [48]	China	Illumina	Firmicutes, Proteobacteria, Acidobacteria		*Bacillus, Acinetobacter, Brevibacillus, Sphingomonas, Pseudomonas, Kocuria, Paracoccus, Hymenobacter, Corynebacterium*	
(Mhuiereach et al., 2019) [49]	USA	Illumina	Proteobacteria, Actinobacteria, Firmicutes, Bacteroidetes, Planctomyces	Deinococci, Thermomicrobia, Anaerolineae, Alphaproteobacteria, Gammaproteobacteria, Flavobacteria	*Gluconobacter, Granulibacter, Erwinia billingiae, Rubritepida, Acidicaldus, Citrobacter, Arsenophonus*	
(Mu et al., 2020) [50]	China	Illumina	Proteobacteria		*Cupriavidus, Lactobacillus, Bifidobacterium, Paraburkholderia, Burkholderia*	
(Núñez et al., 2019) [40]	Spain	Illumina	Actinobacteria, Proteobacteria	Micrococcales, Alphaproteobacteria, Sphingomonadales, Rhodobacterales, Rhizobiales, Acetobacterales, Bacillales, Clostridiales	*Acinetobacter, Actinomyces, Aerococcus, Aeromonas, Arcobacter, Bacillus, Bacteroides, Campylobacter, Clostridium sensu stricto, Corynebacterium, Enterobacter, Enterococcus, Erysipelothrix, Escherichia, Shigella, Fusobacterium, Geodermatophilus, Gordonia, Haemophilus, Helicobacter, Klebsiella, Lactococcus, Legionella, Micrococcus, Micromonospora, Mycobacterium, Mycoplasma, Neisseria, Nocardia, Porphyromonas, Prevotella, Pseudomonas, Roseomonas, Serratia, Staphylococcus, Stenotrophomonas, Streptococcus, Thermoactinomyces, Thermomonospora, Vibrio*	*Roseomonas, Corynebacterium, Staphylococcus* spp., *Streptococcus* spp., *Enterococcus, Bacteroides, Prevotella, Fusobacterium, Enterobacteriaceae*
(Stewart et al., 2020) [51]	USA	Ion Torrent	Proteobacteria, Firmicutes, Cyanobacteria, Deinococcus-Thermus, Bacteroidetes, Actinobacteria, Tenericutes, Fusobacteria	Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria		*Klebsiella, Escherichia coli, Streptococcus, Salmonella*
(Tanaka et al., 2020) [52]	Japan	Illumina	Proteobacteria, Actinobacteria, Firmicutes	Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria	*Bacillus, Staphylococcus, Propionibacterium, Corynebacterium, Sphingomonas, Methylobacterium, Streptococcus*	

Table 1. *Cont.*

Reference	Location	Sequencing Method	Phyla More Abundant	Class and Order More Abundant	Genera and Species More Abundant	Pathogens
(Uetake et al., 2019) [53]	Japan	Illumina	Proteobacteria, Firmicutes, Cyanobacteria, Actinobacteria, Bacteroidetes, Acidobacteria, Parcubacteria			
(Wang et al., 2021) [54]	China	Illumina	Proteobacteria, Actinobacteria, Firmicutes, Bacteroidetes		*Halomonas, Aliihoeflea, Acinetobacter, Sphingomonas, Pelagibacterium, Methylobacterium, Clostridium_sensu_stricto, Prevotella_9, Halorubrum, Rhodococcus, Corynebacterium_1, Rubellimicrobium, Nocardioides, Paracoccus, Bacillus*	
(Woo et al., 2013) [55]	Hong Kong	Illumina	Cyanobacteria, Proteobacteria	Betaproteobacteria: Burkholderiales Gammaproteobacteria: Pseudonomadales, Chromatiales, Xanthomonadales		*Legionella, Pseudomonas, Shigella, Salmonella, Staphylococcus, Streptococcus, E. coli O157:H7, Clostridium perfringens*
(Xu et al., 2020) [34]	China	Illumina	Proteobacteria, Actinobacteria, Cyanobacteria, Firmicutes, Bacteroidetes		*Sphingomonas, Curvibacter, Acinetobacter, Bradyrhizobium, Methylobacterium, Halomonas, Aliihoeflea, Phyllobacterium*	

Table 2. Parameters that affect the microbial community: microorganisms' emission sources, environmental and meteorological factors, and the season in which the alpha diversity was higher reported by each selected paper. Strength (indicated with ↑) and criticalities (↓) for each paper are reported.

Reference	Location	Sampling Method	Microbes' Origin	Environmental and Meteorological Factors	Seasons (Highest Diversity →-> Lowest)	Strength and Weaknesses
(Be et al., 2014) [17]	USA	Bioaerosol	Soil, skin microflora		Summer (*Bacillus thuringiensis, Staphylococcus* spp.) → spring → winter (Firmicutes, Fusobacteria, Bacteroides) → fall Constant through the year: *Ralstonia, Cupriavidus, Bacillus*	↓ 1 week sampling for each season ↑ Use whole genome sequencing Consider effects on human health
(Becsei et al., 2021) [15]	Hungary	PM$_{10}$				↓ Few samples ↑ Consider ARGs
(Bertolini et al., 2013) [41]	Italy	Total suspended particles (TSP)	Plant, soil	Temperature (<+6.32 °C *Actinobacteridae*), PM fraction	Summer and Spring (*Sphingomonadales* and *Rhizobiales*), winter: lower (*Actinobacteriales, Pseudomonadales* e *Burkholderiales*)	↓ No data about effects on human health ↑ Sampling 10 days for each season

Table 2. *Cont.*

Reference	Location	Sampling Method	Microbes' Origin	Environmental and Meteorological Factors	Seasons (Highest Diversity →-> Lowest)	Strength and Weaknesses
(Bowers et al., 2013) [16]	USA	Bioaerosol	Soil, plants, cow feces	Air pollution, temperature	Summer → fall → winter (*Actinobacteridae*) → spring	▼ No data about effects on human health Comparison of urban and rural aerobiome ▲ Samples collected every 6 days for 14 months
(Calderón-Ezquerro et al., 2021) [43]	Mexico	Bioaerosol	Plant, soil, human skin, water	Meteorological conditions, land-use type, anthropogenic activities, temperature	Dry season (Actinobacteria) → Rainy season (Bacteroidetes)	▼ Comparison of urban and suburban aerobiome Sampling with Hirst-type spore trap ▲ Sampling weekly for a year Evaluation presence of pathogens
(Chen et al., 2021) [37]	China	PM$_{2.5}$	Soil, water	T, humidity, UV, air pollution (decrease in the diversity)	winter (*Streptomyces*) → spring (*Arthrobacter, Chlostridium sensu stricto 1, Corinebacterium*)/fall (*Sphingomonas, Methilobacterium, Melittangium*) → summer (*Bacillus*)	▼ Suburban area Samples taken at 30 m height ▲ Evaluate health effects Sampling 8–10 days for each season Comparison of 4 years (winter)
(Du et al., 2018) [13]	China	PM$_{2.5}$			Spring (Cyanobacteria: *Streptophyta; Blastococcus, Kocuria, Sphingomonas, Rubellimicrobium*) → winter (Actinobacteria, *Pseudolabrys, Kocuria, Blastococcus, Staphylococcus*) → fall (Firmicutes: *Chlostridium; Bacillus, Sphingomonas*) → summer (Actinobacteria; *Pseudolabrys, Bacillus, Blastococcus, Streptophyta, Segetibacter*)	▼ Suburban area Samples taken at 30 m height ▲ More than 10 samples for each season Evaluation presence of pathogens
(Fan et al., 2019) [38]	China	PM$_{2.5}$	Plant, soil	Air quality index, NO$_2$, SO$_2$, T, RH: positively correlated; O$_3$: negatively correlated		▼ None ▲ Sampling weekly for each season Air samples taken at 1.5 m Evaluation presence of pathogens
(Franzetti et al., 2011) [44]	Italy	PM$_{10}$-PM$_{2.5}$	Soil, plant	T: negatively correlation	Summer (α-proteobacteria: *Sphingomonadales*) → winter (Actinomycetales, Firmicutes)	▼ Sampling 2 seasons No data about effects on human health ▲ Sampling daily for a month for each season

Table 2. *Cont.*

Reference	Location	Sampling Method	Microbes' Origin	Environmental and Meteorological Factors	Seasons (Highest Diversity →-> Lowest)	Strength and Weaknesses
(Gandolfi et al., 2015) [45]	Italy	PM$_{10}$	Soil, plants, water, feces	T: positively correlated to plant-associated mo; wind speed and humidity	Diversity between sites is higher in spring (*Rhodobacterales*) and winter (*Flavobacteriales, Pseudomonales, Burkholderiales, Xhantomonadales*). In autumn there are *Sphingobacteriales* and *Actinomycetales*, and summer *Chloroplast*	↓ Comparison of urban and suburban aerobiome No data about effects on human health ↑ Sampling two days per week for a month for each season
(Genitsaris et al., 2017) [46]	Greece	Bioaerosol	Soil, wastewater, plants	Air pollution, T: positively correlated humidity: negatively correlated, precipitation	Summer → fall/winter → spring	↓ Samples taken at 30 m height ↑ Sampling weekly for one month for each season Describe presence of pathogens
(González-Martín et al., 2021) [35]	Spain	Bioaerosol	Soil, water	Wind direction, temperature	Summer (*Blastomonas*) → spring (*Cellvibrio*) → fall (*Sphingomonas, Sediminibacterium*) → winter (*Cellvibrio*)	↓ Comparison of Urban area of Tenerife (little island town) and rural area ↑ Sampling 3 times a week for 1 year Describe presence of pathogens
(Lee et al., 2017) [47]	China, South Korea, Japan	Bioaerosol	Water, soil, plant	Humidity, T: negative correlation; wind speed: positive correlation	Winter → fall/spring → summer	↓ Samples taken above 10 m of height No data about effects on human health ↑ Comparison of 3 urban areas Sampling the same day in 3 city
(Li et al., 2019) [39]	China	PM$_{2.5}$	Plant, soil, feces	Wind speed, O$_3$, PM$_{2.5}$: negatively correlated T: positively correlated, NO$_2$	Richness = spring (Proteobacteria, Actinobacteria, Firmicutes) Diversity = summer (Proteobacteria, Firmicutes (*Lactobacillus*), Actinobacteria)	↓ Samples taken at 25 m of height 3 samples for each season ↑ Evaluation presence of pathogens
(Hu Li et al., 2019) [48]	China	Bioaerosol				↓ Comparison of urban, suburban and rural aerobiomes Evaluates 2 seasons ↑ Evaluation presence of pathogens Samples taken at 1.5 m of height
(Mhuireach et al., 2019) [49]	USA	Bioaerosol	Soil, plant, water	Anthropogenic activities, geography, biotic processes, wind speed		↓ Few samples ↑ Focus on vegetation effects for human health

Table 2. *Cont.*

Reference	Location	Sampling Method	Microbes' Origin	Environmental and Meteorological Factors	Seasons (Highest Diversity →-> Lowest)	Strength and Weaknesses
(Mu et al., 2020) [50]	China	Bioaerosol	Plant, soil	Humidity		▼ 2 seasons sampled Comparison of urban and rural aerobiomes No data about effects on human health ▲ Samples taken at 1.5 m of height
(Núñez et al., 2019) [40]	Spain	Bioaerosol	Soil, water, plants	T, precipitation	Summer (*Bacillales, Clostridiales, Corynebacteriales,*) → fall/spring (*Betaproteobacteriales, Sphingomonadales, Pseudomonadales, Frankiales*) → winter (*Micrococcales, Sphingomonadales, Rhodobacteriales, Acetobacteriales*)	▼ Sampling 7 days for each season Used a sampling method validated by them: Hirst-type Spore Trap ▲ Evaluation presence of pathogens
(Stewart et al., 2020) [51]	USA	PM$_{2.5}$-TSP				▼ Few samples Comparison of urban and suburban aerobiome ▲ Evaluation presence of pathogens
(Tanaka et al., 2020) [52]	Japan	Bioaerosol	Human skin, soil, water			▼ 2 seasons ▲ Evaluation presence of pathogens
(Uetake et al., 2019) [53]	Japan	Bioaerosol	Soil, water	Humidity, wind speed		▼ Collecting samples for 48–72 h Comparison of urban and suburban aerobiome No data on effects on human health ▲ Regular Sampling for 3 seasons
(Wang et al., 2021) [54]	China	PM$_{2.5}$	Plant, soil, water	PM, CO, NO$_2$, SO$_2$, O$_3$, AQI, T	Fall (*Clostridium_sensu_stricto_1, Corynebacterium_1, Rubellimicrobium, Nocardioides, Paracoccus*) → Spring → Summer (*Bacillus*)/ Winter (*Halomonas, Aliihoeflea, Pelagibacterium, Halorubrum, Rhodococcus*)	▼ Samples taken at 36–39 m of height ▲ Evaluation presence of pathogens Sampling 1 or 2 weeks for each season
(Woo et al., 2013) [55]	Hong Kong	Bioaerosol		T, wind speed, humidity and precipitation, CO$_2$ affect species richness	Summer (*Bacillariophyta* and *Chlorophyta*)	▼ None ▲ Samples taken at 2 m of height Sampling weekly for 2 months for each season Detection of pathogens

Table 2. *Cont.*

Reference	Location	Sampling Method	Microbes' Origin	Environmental and Meteorological Factors	Seasons (Highest Diversity →-> Lowest)	Strength and Weaknesses
(Xu et al., 2020) [34]	China	PM$_{2.5}$		Anthropogenic sources, atmospheric changes, air pollution		↓ Comparison of urban and suburban aerobiome Urban samples taken at 21 m of height ↑ Detection of pathogens

4. Discussion

In the last ten years, several research groups have collected PM$_{10}$ and PM$_{2.5}$ air samples in urban areas and characterized the aerobiome using NGS techniques. Comparing urban bioaerosol from different cities in Asia (13 studies: China, Japan, Hong Kong); America (6: USA, Mexico); and Europe (7: Italy, Spain, Greece, Hungary), it was possible to define a unique core of bacteria. This core mainly contains four phyla: Proteobacteria, Firmicutes, Bacteroidetes, and Actinobacteria (Table 1). Differences in the core composition are primarily related to the geographic localization of the city and seasonality.

Lee et al. (2017) compared three Asian cities reporting a greater influence of the season compared to the localization. Seoul, which is located between Beijing and Nagasaki, presents a similar bacterial community to both the communities of the Chinese and the Japanese cities [47].

Regarding the influence of the season on the microbial abundance and diversity in the urban areas, there are several discordant results. Few studies reported that hot seasons (summer and spring) are associated with higher bacterial abundance [13,16,17,35,39,40,44–46,55]. The seasonal effect is determined by bioaerosol emission source and atmospheric conditions [41]. Plants, for example, contribute more in spring and soil in winter, while microorganisms living on human skin are more abundant in the summer than in the other seasons [17]. Bowers et al. (2011) explained that in winter, there are limited sources; plants, for example, are leafless and the water is frozen. In the spring and summer, highest temperature are reached supporting the positive correlation between temperature and abundance [18]. Others, such as Lee at al. (2017), reported a higher abundance in winter, when the wind speed is higher causing the suspension of soil-derived bacteria, and the relative humidity is lower allowing the microbial cells to remain suspended because dry particles are lighter [47]. Mu et al. (2020) speculated that low humidity permits an increase in dust rising and bacteria could adhere easily to the particulate matter [50]. Wang et al. (2021) reported that the diversity is higher in the autumn due to the extreme conditions that are present in summer (UV ray) and winter (higher air pollution) that create a difficult environment for the microorganisms [54]. Various evidence showed that air dampness microbiota in indoor environments is a relevant issue for human health, inducing, for example, central or peripheral nervous system and/or respiratory symptoms [56].

Furthermore, the microorganisms' characteristics are also relevant. Spore-forming bacteria as Actinomycetales or Firmicutes are more abundant in winter [44]. Microorganisms in the bioaerosol could be present in different forms and this determines a size distribution in the atmospheric environment. Franzetti et al. (2011) also reported a higher presence of Actinomycetales in the fine fraction, maybe as spores [44]. Various studies showed as the space factor expressed as sampling site explained bacterial community similarity better than any other variable, followed by time expressed as sampling date [49].

Air pollutants affect the aerobiome, decreasing both the species richness and the relative abundance. Chen et al. (2021) reported the presence of a bacterial core that is present in all air quality conditions, but whose amount decreases with the increase of the air pollution [37]. Li et al. (2019), also reported a negative correlation with the ozone (O$_3$) [39]. Few papers disagree with the previous study, reporting a not significant correla-

tion between microbial community composition and air pollution factors [48,55]. Fan et al. reported a positive correlation between sulfur dioxide (SO_2) and some bacteria genera that are involved in the sulfur cycle, especially pathogens (*Burkholderia*, *Pseudomonas*, *Escherichia*, *Shigella*, *Clostridium*, and *Staphylococcus*) [38]. Others observed a positive correlation between microbial communities' abundance and diversity and PM_{10} [40], as microorganisms could easily attach to inorganic surfaces and remain suspended in the air for a long period.

However, recent international analysis on the SARS-CoV-2 transmission and environmental factors showed that changes in the weather (such as warmer temperature) or pollution reduction (due to outdoor ultraviolet exposure) alone are not enough to contain the spread of SARS-CoV-2, with other factors having greater effects [57].

Air pollution is part of the environmental factors. Du et al. (2018) and Want et al. (2021) reported a decrease of the species richness with the increase of the AQI [13,54]. Chen et al. (2021) identified a bacterial core that was more present when the AQI was higher, confirming a decrease in the species richness [37].

Several atmospheric conditions present a positive or negative correlation with microbial community structure. Temperature and wind speed are usually positively correlated with the alpha diversity (Table 2) and microbial abundance, and wind speed permits the diffusion over long distances of the bioaerosol [53].

Urban aerobiome characterization reveals the presence of different pathogen microorganisms. Li et al. (2019) observed an increase of pathogens that are positively correlated with the level of urbanization, speculating that it is caused by higher anthropogenic activities [48]. Liu et al. (2018) described a higher relative abundance of pathogenic bacteria during heavily air polluted days [58], since microorganisms have a greater availability of surfaces to adhere to and a selective pressure due to air pollution composition promoting the survival of pathogenic bacteria, whose growth is promoted by the presence of nutrients in the particulate matter (e.g., iron) [59]. Other proposed mechanisms by which PM promotes human diseases, especially respiratory infections, are the ability of PM to decrease the mucociliary mobility and to decrease antimicrobial peptide expression, but also inhibiting macrophages' phagocytic activity [60]. Not only the presence of pathogens in the aerobiome and the increase in abundance of those microorganisms that are correlated with air pollution represent a health threat for humans, but also the decrease in the diversity of the microbial communities in the urban area is associated with an increase of respiratory syndromes such as asthma and allergies [8]. The biodiversity theory suggests that the exposure in early life to many different microorganisms favors immune system development and prevents auto-immune disease onset [61]. Air pollution, especially PM, is involved in the dysbiosis onset in the airways. A decrease of commensals is observed, Bacteroidetes, which are involved in the polycyclic aromatic hydrocarbons (PAH) degradation, are lost. Immune-regulator commensals decrease and an inflammatory state is established. This causes an increase of ROS production, which are genotoxic, creating a carcinogenic microenvironment [29,62].

Finally, PM carries antimicrobial resistance genes (ARGs) that are emitted from numerous sources that are present in the urban area and nearby. Several anthropogenic activities increase the frequency of this process. The misuse of antibiotics is one of the main causes of the diffusion of antimicrobial-resistant pathogens and genes, that lead to the development of infectious diseases that are harder to threat [63]. Selective pressures favoring ARGs presence and spread are present mainly in hospitals, pharmaceutical implants, and wastewater treatment plants (WWTPs) [64,65]. He et al. (2020) reported the presence of the same ARGs that are present in a hospital at a distance of 5 km from the hospital [66].

Alteration that is induced by human exposure to urban aerobiome is a risk factor for several human pathological conditions. Different actions can be taken by the government to decrease this risk, among them an urban health policy, which increases and restores urban green areas favoring a mitigation of aerobiome diversity loss and stimulates citizens to actively use those spaces [49,67].

Monitoring of the aerobiome composition and understanding its potential, both in the human health effect modulation and the air pollution mitigation, is one of the most crucial points for future urban environment development. Further studies on aerobiome and human health effects are required to take more specific and effective actions also focusing on the air mycobiome and its products [68].

One previous systematic review was published in 2020 which not focused the discussion on the environmental factors such as seasonality and sampling methods, which was crucial points as previously discussed [14].

A comparison of the urban aerobiome in different parts of the world is limited by the prevalence in the literature of publications relating to Asia. Only few studies were conducted in Africa or South America and the majority of these were conducted with traditional culture-based methods, limiting the informational potential of the results. International collaboration, as well as the reduction of the cost for the application of dedicated sampling and biomolecular analysis, will improve the knowledge level in this specific field.

5. Conclusions

The aerobiome is composed of a core bacterial community that are mainly represented by Proteobacteria, Actinobacteria, Firmicutes, and Bacteroidetes. The community structure differs in distinct urban contexts due to several factors: seasonality, meteorological and environmental factors, and prevalent microorganisms' emission sources.

Particulate matter that was sampled in the urban environments can carry different pathogens that are associated with different human diseases (such as *Pseudomonas aeruginosa, Corynebacterium, Bacillus anthracis, Salmonella,* and *Legionella)*. Furthermore, a decrease of the bacterial diversity that was observed in the urban areas compared to the rural ones is considered a risk factor for increased asthma and allergies diagnosis. PM also carries ARGs, which are generated mainly in the hospitals that are located inside the cities.

Several studies, on urban aerobiome, have already been conducted using NGS techniques, but their results are still limited and various critical points can be identified: (1) standardization of the ad hoc sampling procedure and analytical methods; (2) better understanding of the urban aerobiome structure and seasonal variations; (3) evaluation of the real impact on human health of the aerobiome in all its complexity, including bacteria but also fungi and viruses; and (4) the effect of climate change, that affects the different meteorological events, should be evaluated. It is currently not clear how the increasing temperature, the development of urban heat islands, and the absence of precipitation can affect urban microbial composition and how these changes affect human health.

The urban aerobiome composition is described here considering an in-depth comparative analysis, especially of the bacteria fraction in terms of phyla, genera, and species when possible. Moreover, environmental determinants influencing the composition, such as sampling methods, were discussed.

The human health effects of the urban aerobiome modulation are still not well known and today not distinguishable by the effects of the exposure due to the indoor air microbiome. This is likely due to a lack of monitoring and surveillance data, rather than to the absence of considerable health effects.

It is estimated that 68% of the population will live in an urban environment by 2050 [69]. Consequently, all the previous questions have a relevant public health concern and quick answers are needed to protect the health of the majority of the world population.

Original data and evidence on human health effects remain very limited. The biotechnology development and the higher attention to biological risk of the last few years give us a unique opportunity to improve urban aerobiome knowledge and to assess the real impact for human health.

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