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Where environmental microbiome meets its host: Subway and passenger microbiome relationships

Mariana Peimbert[1] | Luis D. Alcaraz[2]

[1]Departamento de Ciencias Naturales, Unidad Cuajimalpa, Universidad Autónoma Metropolitana, Ciudad de México, Mexico

[2]Departamento de Biología Celular, Facultad de Ciencias, Universidad Nacional Autónoma de México, Ciudad de México, Mexico

Correspondence
Mariana Peimbert, Departamento de Ciencias Naturales, Unidad Cuajimalpa, Universidad Autónoma Metropolitana, Av. Vasco de Quiroga 4871. Col. Santa Fe, Cuajimalpa, Ciudad de México, Mexico.
Email: mpeimbert@cua.uam.mx

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Abstract

Subways are urban transport systems with high capacity. Every day around the world, there are more than 150 million subway passengers. Since 2013, thousands of microbiome samples from various subways worldwide have been sequenced. Skin bacteria and environmental organisms dominate the subway microbiomes. The literature has revealed common bacterial groups in subway systems; even so, it is possible to identify cities by their microbiome. Low frequency bacteria are responsible for specific bacterial fingerprints of each subway system. Furthermore, daily subway commuters leave their microbial clouds and interact with other passengers. Microbial exchange is quite fast; the hand microbiome changes within minutes, and after cleaning the handrails, the bacteria are re-established within minutes. To investigate new taxa and metabolic pathways of subway microbial communities, several high-quality metagenomic-assembled genomes (MAG) have been described. Subways are harsh environments unfavorable for microorganism growth. However, recent studies have observed a wide diversity of viable and metabolically active bacteria. Understanding which bacteria are living, dormant, or dead allows us to propose realistic ecological interactions. Questions regarding the relationship between humans and the subway microbiome, particularly the microbiome effects on personal and public health, remain unanswered. This review summarizes our knowledge of subway microbiomes and their relationship with passenger microbiomes.

KEYWORDS
built environment, metaSUB, metro, microbiome, subway, urban

1 | INTRODUCTION

Subways are among the most crowded built environments. Millions of commuters and billions of their associated microorganisms ride subways daily, sharing human-associated airborne bacteria, namely, microbial clouds (Meadow et al., 2015). Commuting promotes the formation of continuous interaction networks. Although interactions are brief and intermittent, the networks involve millions of people and their microbiomes. The subway microbiota reflects the microbiome of the urban population and its environment. Interestingly, public health policies (antibiotic selection) and their responses are measurable in subway microbiome profiles (Casimiro-Soriguer et al., 2019; Danko et al., 2021; Kang et al., 2018; Zhelyazkova et al., 2021). In the future, it might be possible to use subway microbiome profiles to track and test hypotheses regarding changes in sociocultural customs or urban growth. Understanding subway microbiomes and their structure, interactions, and changes over time yields relevant ecological questions. Additionally, understanding human-microbial interactions is beneficial for public health practice. This review summarizes our knowledge of subway microbiomes and their relationship with passenger microbiomes. Additionally, we highlighted several associated challenges and forthcoming opportunities.

An increasing number of people live in cities and require transportation systems. In 2018, the United Nations reported that 55% of the world's population lives in cities, which is expected to increase to 68% by 2050 (United Nations, U, 2018). Owing to the growth of cities, it is extremely important to understand the composition and dynamics of their microbiomes and the manner in which the microbiomes affect people. In 2018, the Union Internationale des Transports Publics (UITP) reported 182 subway systems worldwide that transported on an average 168 million passengers per day. In particular, Asia has the most comprehensive and widely used subway systems globally; this region has also shown the highest recent growth in km of tracks. In 2018, the longest subway was the Shanghai subway, followed by the Beijing subway (639 and 590 km, respectively). Moreover, the busiest subway was the Tokyo subway, followed by the Moscow subway (9.5 and 6.5 million passengers per day, respectively; UITP, 2018; Table 1). In all regions worldwide, the most frequent stations are underground stations (67%), followed by elevated stations (19%), and at-grade or trench stations (UITP, 2018).

Studying the subway microbiome consists of several methodological and conceptual stages. In particular, studies of cultivable organisms sought to isolate bacteria from polluted air (Szám et al., 1979). The first culture-free study was performed using 16S rRNA gene amplicons sequenced using Sanger sequencing and 454 pyrosequencing, and it described bacterial diversity within each community (i.e., alpha diversity) (Robertson et al., 2013). Further, various studies on 16S amplicons sequenced using Illumina focused on the comparison of different microbial communities. Based on analysis of similarity and dissimilarity of communities (i.e., beta diversity), the relationships among microbial communities and environmental factors, as well as microorganism sources were investigated (Gohli et al., 2019; Leung et al., 2014; Triadó-Margarit et al., 2017). During this stage, the MetaSUB consortium was formed; its goal is to study subways worldwide; therefore, standardized handling of the samples was essential to obtain comparable data (The MetaSUB International Consortium, 2016). Sampling methods and sample handling can lead to the identification of different taxa; in particular, this can affect reproducibility for air samples, and no standardization is currently available (Bøifot, Gohli, Moen, et al., 2020; Bøifot, Gohli, Skogan, et al., 2020). Recently, metagenomes from numerous cities have been analysed. These studies sought a more in-depth understanding of beta diversity and its possible predictions, the alpha diversity of different strains, metabolic and cellular functions, and metagenome assembly (Danko et al., 2021; Leung et al., 2021; Wu et al., 2021).

As a first approximation, the subway microbiome is similar to that of any urban indoor environment. Much of our understanding of other buildings, such as houses, classrooms, hospitals, and gyms, applies to subways. The main difference between subway systems and other buildings is their high population density. Subways are urban networks; passengers from different locations constantly enter and exit subways, spending relatively little time in them (a few minutes or hours per day). Additionally, the use of space and objects is planned and evident in subways. Stairs, corridors, tunnels, and platforms are intended to guide the flow of people who touch handrails, ticket selling machines, and kiosks. Ventilation systems are carefully designed, particularly in cities with cold winters or underground lines. Finishing materials are resistant and easy to clean. In addition, rush hour can lead to overcrowding. Tunnels and recesses allow urban animals, such as rats, cockroaches, or pigeons, to live in subways. Subways are much more than a transport system; they are public spaces that represent an element of the city's identity. People appropriate them as cultural spaces, and they also serve as homeless or bomb shelters in some cities.

Highlights

- There is a global subway microbiome.
- Each city has its microbiome.
- The subways are a reservoir for antibiotic resistance.
- The subway is a microbiome exchange hub.

1.1 | The built environment microbiome

The built environment microbiomes are determined by their occupants and their relationship with the outdoors (Leung et al., 2016; Meadow et al., 2014). Other factors, such as building design and intended use, are also involved (Flores et al., 2011; Kembel et al., 2014; Wood et al., 2015). Built environments consist of rooms and objects with different microbial communities. For example, different bathroom surfaces microbiomes have different microbial sources; the floor resembles the soil, faucets resemble the skin, and the toilet contains microbial signals from the human gut microbiome (Flores et al., 2011). In contrast, rough and porous materials promote the accumulation of dust and moisture, allowing more organisms to grow (Lax et al., 2019). However, smooth materials and paint coatings only allow the viability of some organisms (Hu et al., 2019).

Moreover, the indoor microbiome depends on architectural design, people flow, airflow dynamics, and whether the ventilation is natural or mechanical. When buildings are well-ventilated, the air microbiome resembles that outside. The outdoor microbiomes depend on the land use in the immediate vicinity, including microbial signals from the soil, plants, and human skin. Furthermore, indoor CO_2 concentration is a good indicator of adequate ventilation and the contribution of outdoor microbiomes to those inside (Zhou et al., 2020). In contrast, in buildings that are not well-ventilated, microbial signals from humans, animals, plants, and possibly molds are clearer. Interestingly, indoor bacterial communities depend more on occupants, whereas indoor fungal communities resemble those outdoors (Lee et al., 2021).

Interactions between people and the environment can be direct upon physical contact with a surface, or indirect, such as bioaerosol exhalation or skin flaking (Meadow et al., 2014). Dispersion and filtering, the two types of interactions between people and spaces and/or objects, cause similarities between microbiomes on some

TABLE 1 Top 10 busiest subway systems

	Subway system	Daily ridership (millions)†	System length (km)†
1	Tokyo	9.5	382
2	Moscow	6.5	348
3	Shanghai	5.6	639
4	Beijing	5.4	590
5	Seoul	5.2	466
6	New York City	4.9	401
7	New Delhi	4.9	348
8	Guangzhou	4.7	371
9	Mexico City	4.6	201
10	Hong Kong	4.4	204

†UITP (2018).

surfaces. For example, the bench microbiomes in two different gyms are more similar than the bench and free weight microbiomes in the same gym (Wood et al., 2015).

Each person has a microbial cloud (Meadow et al., 2015), as shown in Figure 1. The microbial cloud consists of microorganisms expelled by breathing, coughing, and talking, and those in the skin and hair scales shed by desquamation (Noble et al., 1976; Xie et al., 2009). Studies conducted in clean chambers have indicated that humans emit approximately 1–10 million particles per hour, without speaking and during low physical activity (Bhangar et al., 2016; You et al., 2013). Likewise, the number of particles emitted depends on human age, relative humidity, and clothing type (Johnson & Morawska, 2009; Licina & Nazaroff, 2018; Zhou et al., 2017). Large particles fall onto the floor within minutes, whereas bioaerosols (particles of <5 μm) remain in the air for hours, drifting with air currents. In some circumstances, the droplets (>5 μm) dry quickly, giving rise to "droplet nuclei," which keep microorganisms in the air (Fernstrom & Goldblatt, 2013; Liu et al., 2017; Wells & Stone, 1934; Xie et al., 2007). Moreover, large particles are incorporated into floor dust, a reservoir of human-associated bacteria that can be easily resuspended by people passing or air currents (Hospodsky et al., 2012). Thus, bacteria can travel via skin scales, as clusters in droplet nuclei, or individually (Tham & Zuraimi, 2005). Numerous studies have modeled the movement of bioaerosols and their potential impact on severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) transmission (Coyle et al., 2021; Fierce et al., 2021; Nazaroff, 2022; Rencken et al., 2021). In urban outdoor studies, the count of suspended particles (particulate matter >2.5 μm) is used as a measure of industrial pollution; increasing attention is being paid to the impact of bioaerosols on human health (Xie et al., 2021; Yue et al., 2020). Exciting studies on the manner in which microbiomes interact between people and the spaces in which they coexist have been published. The microbiomes of people living together match each other and thier homes (Flores et al., 2013; Lax et al., 2014; Wilkins et al., 2017). A microbial cloud surrounds an individual and leaves a trail of particles detected in the air and on surfaces (Meadow et al., 2015).

People living in Western urban settings have a less diverse microbiome than those living in rural settings (Clemente et al., 2015; Hanski et al., 2012). These differences in microbial diversity have been related to hygiene habits and reduced exposure to diverse microbial environments, such as soil, plants, and animals (McCall et al., 2020). A decrease in skin microbial diversity has been associated with diseases such as atopic dermatitis, acne, and psoriasis (Kong et al., 2012). Exposure to highly diverse environmental microbiomes at an early age is essential for proper immune system development, which may prevent some inflammation-associated noncommunicable diseases (Kirjavainen et al., 2019; Lehtimäki et al., 2021; Nurminen et al., 2021; Rook et al., 2014; Roslund et al., 2020). The biodiversity hypothesis proposes that contact with the soil and green spaces must be maintained because the human microbiome depends on the environmental microbiome (Von Hertzen et al., 2011). Therefore, since 2008, "the Finnish Allergy Programme," a public health programme in Finland, has been implemented to develop immune tolerance through increased human exposure to nature (Haahtela, 2019; Haahtela et al., 2012).

In addition to human microbiomes, the microbiomes of urban houses are less diverse than those of rural houses (Parajuli et al., 2018). The indoor dust correlates with the diversity of surrounding plants and percentage of vegetation area (Ding et al., 2020; Hui et al., 2019). Furthermore, the diversity of indoor microbiomes negatively correlates with the expression of interleukin 10, a protein associated with the propensity for allergies, in adolescents (Hanski et al., 2012). In particular, the "Microbiome Rewilding Hypothesis" proposes that increasing green areas in cities could help restore the biodiversity of environmental microbiome and thereby improve human health and microbiome (Mills et al., 2017). Many observations have suggested an association between rural and soil exposures and increased microbiome biodiversity, and good health (Mills et al., 2019). However, much remains to be understood regarding the causality of these situations (Rothman & Greenland, 2005). For instance, in a study of elderly people residing in rural houses, microbial diversity was lower than that of the urban control group (Saarenpää et al., 2021).

Numerous indoor environments are desert-like because they have very low humidity, which impairs microbial growth (Chase et al., 2016; Lax et al., 2019). A propidium monoazide study has confirmed this arrested bacterial development, showing that less than 10% of organisms detected in indoor environments are viable (Vaishampayan et al., 2013). Studies comparing rRNA and rDNA have shown low proportions of the most metabolically active microorganisms and certain inactive dominant organisms (Gomez-Silvan et al., 2018; Zhou et al., 2021).

1.2 | The skin microbiome

To comprehend the subway microbiome, we need to understand one of its primary sources, the skin microbiome. The skin is the largest human organ, hosting multiple microenvironments. Similar to the gut microbiome, the skin microbiome varies in different sites (Byrd

FIGURE 1 Microbial clouds. People and animals emit particle cloud, leaving their microbiome trail

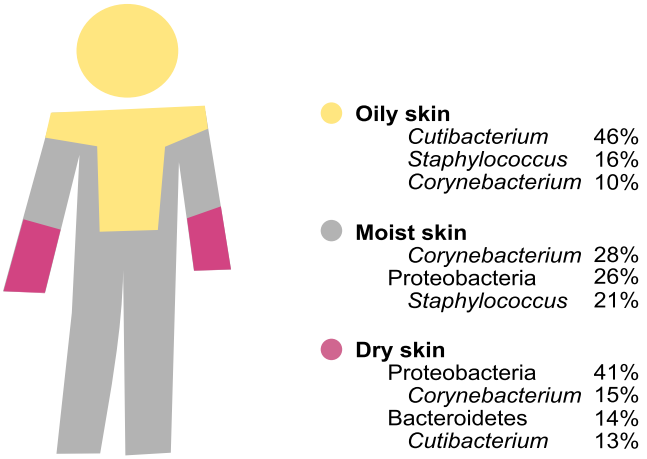

FIGURE 2 Skin microbiota composition by areas. The most abundant taxa are shown (adapted from Chen & Tsao, 2013)

et al., 2018; Grice & Segre, 2011). The sebaceous microbiome is dominated by the genus *Cutibacterium* (formerly *Propionibacterium*). *Cutibacterium*, *Staphylococcus*, and *Corynebacterium* dominate moist areas. In addition, dry area (palm and forearm) microbiomes are the most diverse, with a greater abundance of Proteobacteria (Grice et al., 2009). The most abundant fungus on the skin is *Malassezia*. In general, fungal diversity is very low, except on the feet (soles, nails, and toes; Findley et al., 2013; Figure 2).

The skin microbiome begins to form at birth and matures with months and years. Its diversity increases and becomes area-specific; however, site specificity is lost in old age (Capone et al., 2011; Dominguez-Bello et al., 2010; Luna, 2020; Zhai et al., 2018). Intriguingly, the skin microbiome is stable for months or even years in healthy adults (Hillebrand et al., 2021). However, routine cleaning and cosmetic products can modify bacterial diversity, which can be re-established by adopting the previous routine, even after the use of antimicrobials (Bouslimani et al., 2019; Callewaert et al., 2014). Moreover, the least diverse areas are the most stable ones (Oh et al., 2016). Previous studies have proposed that the deeper skin layers and hair follicles function as reservoirs as the microbial community re-establishes itself (Nakatsuji et al., 2013; Zeeuwen et al., 2012). The skin microbiome changes with direct human contact and indirect contact via surfaces (Neckovic et al., 2020). Additionally, its diversity increases upon contact with animals (Wetzels et al., 2021). The microbiome is also affected by various environmental factors; people cohabitating in a house normalize their microbiomes, and microbial signals from the land around the houses are evident in children (Lehtimäki et al., 2017; Leung et al., 2018). Whether these changes are permanent and whether microbial communities are re-established upon eliminating external sources remains unclear.

Furthermore, the skin microbiome is mainly composed of commensal and symbiotic microorganisms (Grice & Segre, 2011). *Cutibacterium acnes* is facultatively anaerobic and the most abundant bacterium in oily skin areas (Grice et al., 2009). Most strains of *C. acnes* are commensal and produce propionic acid, which inhibits the growth of other bacteria (Dreno et al., 2018). Acne-associated strains can produce proinflammatory cytokines and stimulate sebum production (Beylot et al., 2014; Jugeau et al., 2005). Furthermore, *Staphylococcus epidermidis* dominates the moist areas of healthy individuals (Grice et al., 2009). *S. epidermidis* can inhibit skin inflammation, and the growth of *S. aureus*, promote wound healing, and produce antimicrobial peptides (Leonel et al., 2019; Li et al., 2019). Although *S. aureus* has been associated with the exacerbation of atopic dermatitis, it is expected to be present in the skin of healthy individuals (Ramsey et al., 2016). The microbiome also depends on the lifestyle of each culture; for example, in China, the genus *Enhydrobacter* is more abundant (Leung et al., 2015). Other studies also noted ethnicity as a variable associated with the skin microbiome and classified the variables as lifestyle (e.g., cleansing products and smoking), physiological response to porphyrin (proinflammatory metabolite) production, and aging (Dimitriu et al., 2019).

Currently, experiments involving gut microbiome treatments are underway. Probiotics, prebiotics, and transplantation and autotransplantation techniques are being developed to promote a microbiome that improves skin health and, incidentally, certain cosmetic issues (Boxberger et al., 2021; Callewaert et al., 2021; Pistone et al., 2021).

Analysis of the skin microbiome of selected mammals (N = 38 species) revealed that the human skin microbiome was significantly less diverse and distinctive for each taxa (Ross et al., 2018). Moreover, the same study revealed evidence for phylosymbiosis in mammals, which is the congruence between host phylogeny and microbial community relatedness, suggesting coevolutionary patterns. Other factors influencing the human and vertebrate skin microbiome include human-pet interaction and cohabitation, maternal transfer, captivity status, diet, geographic location, biological sex, and environment (Ross et al., 2019).

The appeal of skin microbiome specificity has led to the proposal to use the skin microbiome in forensic techniques. Methods

such as hidSkinPlex are used to identify individuals using 286 phage and bacterial markers (Schmedes et al., 2018). Although microbial communities are stable in healthy adults, forensic analysis of the skin microbiome has limitations and must be performed accordingly; unlike fingerprints, the skin microbiome is not static (Wilkins et al., 2017).

2 | SUBWAY CITIZEN

2.1 | Who is out there?

Globally, there are fewer than 20 studies on subway microbiomes performed using high-throughput sequencing (HTS; Table 1). Two types of samples were analysed: surface and air samples. Surface samples were swabbed from various objects in the stations and railcars. Furthermore, air samples were collected from platforms and railcars using filter-based or continuous vacuum instruments. In these studies, most of the bacteria belonged to the phyla Actinobacteria, Firmicutes, and Proteobacteria (Table 2). The quantity and diversity of microorganisms are mainly dependent on anthropogenic activities and environmental context.

In particular, eight studies were conducted on surfaces. In the subways of Boston, Hong Kong, Oslo, and Mexico City, the dominant genera are related to those prevalent on the skin (e.g., *Cutibacterium*, *Staphylococcus*, *Streptococcus*, and *Corynebacterium*) (Gohli et al., 2019; Hernández et al., 2020; Hsu et al., 2016; Kang et al., 2018). In the New York and Moscow subways, the dominant bacteria are related to those often found in the soil (e.g., *Stenotrophomonas* and *Pseudomonas*) (Afshinnekoo et al., 2015; Klimenko et al., 2020). Moreover, two studies included samples from 60 different cities (Danko et al., 2021; Wu et al., 2021). Various surfaces, such as floors, seats, handrails, walls, kiosks, and benches, were sampled; the surface type is the most significant determinant of community beta diversity (Hsu et al., 2016; Klimenko et al., 2020; Vargas-Robles et al., 2020). This diversity pattern is explained by two characteristics: object use and material composition. Interestingly, seats made from different materials harbour different microbial communities. However, seatbacks and seats made of the same material also harbour different microbial communities (Hsu et al., 2016). Thus, surface functionality determines which organisms arrive (dispersion), and materials provide different adherence and growth environments for bacteria (filtering). The floor is the most diverse surface, with more unique taxa and the most significant environmental signals (Klimenko et al., 2020; Vargas-Robles et al., 2020). In Boston, the diversity (Shannon index) of the surfaces correlates with the relative humidity (56%–85%) (Hsu et al., 2016). In Hong Kong, morning microbial communities are more diverse, and afternoon microbial communities are more similar to each other and the hand microbiome (Kang et al., 2018; Leung et al., 2014; Figure 3).

Furthermore, desiccation prevents bacterial growth indoors, and deposited bacteria can be observed (Chase et al., 2016; Lax et al., 2019; Wood et al., 2015). This could explain why the dominant bacteria in most subways correspond to those on the skin. However, subways have numerous microenvironments, and some have sufficient moisture to grow environmental bacteria. Microenvironments could explain why the New York City and Moscow subways are dominated by soil microbiome rather than skin microbiome.

Six studies on airborne bacteria in New York, Hong Kong, Barcelona, Oslo, and Athens, and a multicity study (New York, Hong Kong, Oslo, Denver, London, and Stockholm) have been reported. In New York, Hong Kong, Oslo, and multicity studies, the dominant genera corresponded to abundant and prevalent bacteria on human skin (i.e., skin-associated) (*Staphylococcus*, *Micrococcus*, *Kocuria*, *Cutibacterium*, and *Enhydrobacter*) (Afshinnekoo et al., 2015; Gohli et al., 2019; Leung et al., 2014, 2021); whereas in Barcelona and Athens studies, the dominant genera were bacteria often found in the soil (e.g., *Methylobacterium*, *Bradyrhozobium*, *Paracoccus*, *Arthrobacter, and Rubellimicrobium*) (Grydaki et al., 2021; Triadó-Margarit et al., 2017). The estimation of bacteria in the air using quantitative polymerase chain reaction (qPCR; 2.2–4.5 $\times$ 10^4 cells/m^3) is similar to that in different systems (Grydaki et al., 2021; Robertson et al., 2013; Triadó-Margarit et al., 2017). Air bacterial communities of each city do not significantly differ between themselves or from those outside the subways (Leung et al., 2014; Robertson et al., 2013; Triadó-Margarit et al., 2017). Although indoor and outdoor air microbiomes are not homogeneous, their resemblance suggests good ventilation in all systems. The microbiomes of the closest stations were more similar than those of the distant stations (Leung et al., 2014). Furthermore, in Hong Kong, an increase in diversity was observed at lower temperature (24°–30°) or higher relative humidity (50%–90%; Leung et al., 2014). In Oslo, the opposite was observed; however, at different temperature (−5°–25°) and relative humidity (30%–76%) (Gohli et al., 2019). The abundance of *Enhydrobacter* in Hong Kong is higher than that in other systems (3.1% vs. <0.5%) (Leung et al., 2014, 2021) which corresponds with the microbiome of the Chinese skin characterized by the abundance of this bacterium (Leung et al., 2015).

A strong correlation between microbial communities detected on surfaces and those in air has been observed (Gohli et al., 2019; Leung et al., 2021; Pochtovyi et al., 2022). However, air and surface bacteria have different taxa abundances. Random forest analysis indicated that *Ralstonia* and *Streptomyces* are more abundant in the air (Gohli et al., 2019). The interaction between these two environments is hypothesized to be bidirectional. Aerosols settle on surfaces, and surface microbiomes are resuspended in air. However, the genera *Rubrobacter*, *Pseudonocardia*, and *Nesterenkonia* are highly abundant in the air and do not appear to be deposited on surfaces (Gohli et al., 2019).

2.2 | Subways and their cities

Multicity studies have proposed a pan and core microbiome of the subway system. A study of 4,728 surface samples from 60 cities worldwide recognized 4,424 taxa as the urban pan-microbiome.

TABLE 2 HTS Microbiome studies in different subway systems

City/year	Sample	Approach	Findings	Reference
New York, 2013	Air	16S and 18S rRNA amplicons	The bacterial composition is similar throughout the system and between the indoor and outdoor air Dominated by human associated bacteria like *Staphylococcus* strains	Robertson et al. (2013)
Hong Kong, 2014	Air	16S rRNA amplicon	Diversity is influenced by temperature and relative humidity conditions Human skin associated bacteria: *Micrococcus*, *Enhydrobacter*, *Cutibacterium*, *Staphylococcus* and *Corynebacterium*	Leung et al. (2014)
New York, 2015	Surfaces	Shotgun metagenomics	Dominant bacteria are associated with skin, airways, urogenital and gastrointestinal tract Most abundant bacteria are *Pseudomonas stutzeri*, *Stenotrophomonas maltophilia*, *Enterobacter* and *Acinetobacter* strains	Afshinnekoo et al. (2015)
Boston, 2016	Surfaces	16S rRNA amplicon Shotgun metagenomics	Main differences in microbial composition are due to surface type. Low presence of antibiotic resistance and virulence factors genes All surfaces are dominated by human skin and oral bacteria such as *Cutibacterium*, *Corynebacterium*, *Staphylococcus* and *Streptococcus*	Hsu et al. (2016)
Barcelona, 2016	Air	16S rRNA amplicon	Airborne bacteria are dominated by *Methylobacterium*, *Chitinophagaceae* and *Bradyrhizobium* Low presence of potentially pathogenic bacteria	Triadó-Margarit et al. (2017)
Hong Kong, 2018	Passengers' hands	Shotgun metagenomics	Human skin bacteria are present after a trip, such as *Cutibacterium*, *Micrococcus*, *Acinetobacter* and *Staphylococcus* It suggests that clinically important ARG families increase from AM to PM	Kang et al. (2018)
Oslo, 2019	Air/surface	16S rRNA amplicon	The main phyla are the same in bioaerosols and surfaces, Actinobacteria and Proteobacteria Main airborne bacteria: *Micrococcus*, *Staphylococcus* and *Rubrobacter* Main surfaces bacteria: *Staphylococcus*, *Sphingomonas* and *Streptococcus* Seasonal variation with high diversity in spring and summer	Gohli et al. (2019)
Mexico City, 2020	Surface	16S rRNA amplicon	420 bacterial genera were universal to all the system. Dominated by human skin bacteria: *Cutibacterium*, *Corynebacterium*, *Streptococcus* and *Staphylococcus*	Hernández et al. (2020)
Moscow, 2020	Surface	16S rRNA amplicon	The main bacteria sources are skin and soil. Most abundant bacteria: Stenotrophomonas, *Pseudomonas*, *Dietzia* and *Brevundimonas*.	Klimenko et al., 2020.
Mexico City, 2020	Surface passengers' hands	16S rRNA amplicon	After a subway trip, passengers' hands are more similar to the bacterial composition of the subway surfaces	Vargas-Robles et al. (2020)

TABLE 2 (Continued)

City/year	Sample	Approach	Findings	Reference
Athens, 2021	Air	16S rRNA amplicon	The main sources are environmental; the outdoor air affects the composition inside the subway. Most airborne abundant bacteria: *Paracoccus*, *Sphigomonas*, *Kocuria*, *Acinetobacter* and *Staphylococcus*	Grydaki et al. (2021)
Multicity (6 cities), 2021	Air	Shotgun metagenomics	There are 17 core taxa in the air Xenobiotic metabolism is City specific. *C.acnes* and *M. luteus* strains are City specific. Skin, soli and wastewater are the source of subway resistome	Leung et al. (2021)
Multicity (60 cities), 2021	Surfaces	Shotgun metagenomics	There are 31 core taxa at surfaces. They detected 11,614 DNA Virus. Some abundant bacteria are not prevalent	Danko et al. (2021)
Multicity (60 cities), 2021	Surfaces	Shotgun metagenomics	1,448 high-quality MAG were assembled. 732 new species were found	Wu et al. (2021)
Moscow, 2022	Air/surfaces	16S rRNA amplicon	There are 15 core genera in the air and surfaces of stations	Pochtovyi et al. (2022)

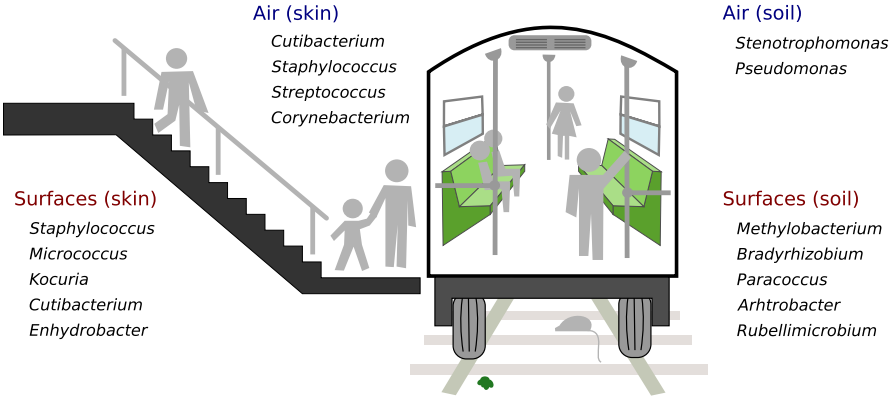

FIGURE 3 Dominant bacteria in the world's subways microbiomes. Most of the bacteria detected in subways have skin or soil origins

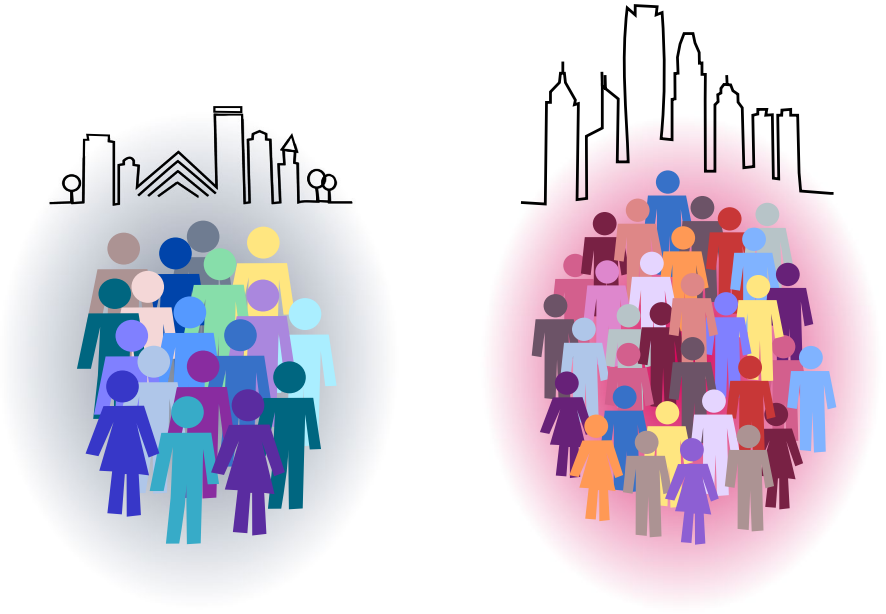

FIGURE 4 Each city subway is distinguishable by a particular microbiome signature. The subway microbiome may be a proxy of the City microbiome itself

However, rarefaction analysis suggested that more taxa are present. The core microbiome comprises 31 taxa with a prevalence greater than 97% and a subcore microbiome of 1,145 taxa with a prevalence between 70% and 97%. The core microbiome consists exclusively of bacteria, and the subcore microbiome consists of all bacteria, and the yeast *Saccharomyces cerevisiae*. *Cutibacterium acnes* (skin commensal) is the most abundant bacterium, followed by *Bradyrhizobium* sp. (nitrogen fixer in soil and plants). However, numerous sequences could not be assigned to any species; 34.6% of the reads did not have similarities in the National Centre for Biotechnology Information (NCBI) RefSeq database, and some of these sequences only had similarities with those of the environmental microbiome of the subway system (Danko et al., 2021).

Subway microbial communities share numerous characteristics; however, the abundance of microorganisms and presence of rare organisms in air and surface samples differ by city. These differences could be ascribed to peculiarities in skin microbiomes associated with distinct local lifestyles (Leung et al., 2015) and geographic characteristics, such as of coastal or tropical cities (Zhang et al., 2021).

The subway-related identity of a city has been profiled in different ways by analysing 16S rRNA amplicons and metagenomes (Walker et al., 2018; Zhu et al., 2019). The differences in alpha diversity allow us to predict climatic characteristics of the samples, such as coastal proximity, temperature, and humidity (Zhang et al., 2021). A decrease in diversity has also been observed in cities farther from the equator (Danko et al., 2021). According to some authors, species with very different abundances define the microbial pattern of each city (Walker et al., 2018). According to other authors, low-abundance taxa define the microbial profile of each city (Chen & Tyler, 2020). Various methodologies are used to identify subway microbiome signals in different cities; therefore, each city has a distinct bacterial signature (Figure 4).

2.3 | Technical details and the development of bioinformatic methodologies

The initial step towards studying subway microbiomes is to obtain metagenomic DNA from environmental samples (1–500 ng). DNA can then be used to build shotgun libraries to perform proper metagenomics or as a template for PCR to amplify targeted sequences such as 16S and 18S rRNA and the internal transcribed spacer (ITS). Briefly, 16S and 18S rRNA and ITS are used to analyse the meta-taxonomy of prokaryotes (16S) and eukaryotes (18S and ITS). Shotgun sequencing of metagenomic DNA allows full microbial community profiling (viruses, prokaryotes, and eukaryotes) and is used to determine microbial coding sequences, and thus microbial metabolic functions. Multiple studies have described metagenomic concepts, strengths, weaknesses, pitfalls, workflows, and pipelines (Breitwieser et al., 2018; New & Brito, 2020; Quince et al., 2017; Wooley et al., 2010). Multiple strategies have been used for subway microbiome analysis (Table 2). Although major trends (taxa and coding genes) in different studies could be compared, every experimental and analytical step could bias the results (e.g., DNA extraction, sequencing technology, coverage).

Studies of subway microbiomes have had a crucial methodological impact on metagenomics. The MetaSUB Consortium has standardized surface sampling and processing methods, allowing the comparison between different microbial systems worldwide (The MetaSUB International Consortium, 2016). In 2017, 2018, 2019, and 2020, the Critical Assessment of Massive Data Analysis (CAMDA) MetaSUB challenges were presented (http://www.camda.info/). In these challenges, some subway metagenome origins were concealed to determine whether the bioinformatic groups could identify them. By releasing shotgun and 16S amplicon sequencing data, MetaSUB has allowed research groups to develop and validate algorithms, optimize pipeline analysis time and results, generate benchmarks, and detect problems and limitations of actual data (Kawulok et al., 2019; Qiao et al., 2018; Zhu et al., 2019). Bioinformatic developers usually work with mock communities (data created in silico from bacterial genome sequences with defined proportions); having access to a large amount of actual data from different parts of the world allows them to test and improve their programs.

In 2017, the CAMDA challenge included data from three cities, and the 2018 and 2019 CAMDA challenges included a mystery sample with unknown location. In 2018, 311 samples from eight known cities and 83 mystery samples from four cities were released. Moreover, in 2019, microbial sequences from 16 known cities and eight mystery cities were made available. Finally, the 2020 CAMDA challenge included 1065 samples from 23 cities and 121 samples from 10 mystery cities, and it encompassed finding patterns with nonbiological data (sample metadata).

Different previously established methods successfully classified (profiled) the samples by city based on bacterial communities (Walker et al., 2018; Zhou et al., 2019). In particular, most pipelines start by comparing the sequences (reads, contigs, or amplicons) with a database (e.g., NCBI-nr) to assign taxonomy (order, family, genus) to each. The tables of taxon abundances are then created, and a machine learning method is used to cluster the samples by city.

Instead of using taxonomic profiles, some laboratories use the abundance of biochemical and cellular functions (e.g., Kyoto Encyclopedia of Genes and Genomes or Comprehensive Antibiotic Resistance Database databases and systems) (Casimiro-Soriguer et al., 2019; Zhu et al., 2019). These functional classifications have demonstrated that samples in different cities can be grouped according to biochemical and cellular characteristics. Notably, this analysis type has yielded exciting results; for example, New York has more photosynthesis-associated genes than do other cities (Zhu et al., 2019). In addition, the metabolites of xenobiotic degradation pathways are detectable in air samples and are a part of a city's signature; for example, nitrotoluene, xylene and caprolactam, and bisphenol degradation in Denver, Hong Kong, and New York, respectively (Leung et al., 2021).

Furthermore, certain variations are intended to reducing the computation time. In particular, instead of using reads directly, researchers used k-mers, constructed by splitting the reads into smaller

sequences such as 24 bp, as classifiers (Anyaso-Samuel et al., 2021; Huang et al., 2020). Some pipelines reduce the data by excluding features from the abundance tables; for example, some genera and features unable to distinguish samples are excluded (Casimiro-Soriguer et al., 2019; Walker et al., 2018), or dimension reduction methods, such as principal component analysis (PCA), are used. Another problem detected upon increasing the number of cities is that several taxa are not represented in each city, and researchers must decide whether to remove them or treat them as zero (Huang et al., 2020).

A limitation of environmental samples, including subway samples, is that numerous sequences are not homologous to those in databases. Such sequences are lost, as they do not resemble those that have been studied in laboratories. For example, in CAMDA 2018 shotgun samples, phyla could only be assigned to between 1% and 80% of the reads of each sample (Ryan, 2019). Therefore, database-free methods were used to avoid this problem; researchers used k-mers directly to construct abundance tables, successfully completing the CAMDA challenge (Kawulok et al., 2019; Qiao et al., 2018).

Some research groups have used the same data to solve other challenges, such as the assembly of low-coverage genomes (Gerner et al., 2018), strain identification using multilocus analysis (Zolfo et al., 2018), design of an antibiotic resistance index (Zhelyazkova et al., 2021), de Bruijn graph sketch (Muggli et al., 2019), and methods to generate in silico "gold standard" microbial communities with the complexity of environmental samples (Gerner et al., 2018).

2.4 | Delicate embroidery, strains, and metagenome-assembled genomes (MAG)

Identifying strains in environmental samples is relevant to understanding the implications of metabolic capacities and identifying possible pathogens. Two classic problems are encountered when searching for strains in metagenomes: shallow coverage owing to unavailable essential sequences, and several mixed strains (Joseph et al., 2016). However, the latter may not be relevant.

The large quantity of metagenomic data has allowed for the identification of strains in subway samples. Several strains of *S. aureus* have been identified in 149 surface samples from New York, and they (CC8 and CC30 strains) correspond to the most common strains of the human microbiome. The methodology consisted of verifying good coverage and analysing single nucleotide polymorphism (SNP) frequencies used to distinguish between the samples (Joseph et al., 2016). Several strains of *C. acnes* and *M. luteus* were detected in air samples. Moreover, a *C. acnes* strain dominated each sample, and the observed strains were similar to those of people without acne. *M. luteus* strains corresponded to those from farms and hospitals. Interestingly, there can be several *M. luteus* strains in a single sample (Leung et al., 2021).

New York surface samples were analysed for the presence of *Bacillus anthracis* based on k-mer profiles. A database was constructed with sequences that distinguished *B. anthracis* from *B. cereus*, and sequences directly involved in the production of the lethal factor were identified. In New York, k-mers that distinguished *B. anthracis* were detected; however, the lethal factor was not detected. These results suggested the presence of new *Bacillus* strains (Petit et al., 2018) and ruled out conclusions on *B. anthracis* reported by Afshinnekoo et al. (2015) and Gonzalez et al. (2016).

MAGs represent another method used to identify strains. Wu et al. (2021) assembled 1,448 high-quality MAGs, of which 732 were new species with new biosynthetic pathways. These new species correspond to *Microbacteriaceae*, *Sphingomonadaceae*, *Xanthomonadaceae*, *Pseudomonadaceae*, and *Burkholderiaceae*. Twenty-six MAGs were assembled from the air samples; some were environmental, whereas others were associated with humans (Leung et al., 2021). Gerner et al. assembled 27 high-quality MAGs using the 2017 CAMDA data. Furthermore, they assembled 14 *C. acnes* strains and 13 MAGs of various genera (*Pseudomonas*, *Stenotrophomonas*, *and Enterobacter*) using the Boston and New York samples, respectively (Gerner et al., 2018). Several MAGs of *C. acnes* have a growth rate index of >1, suggesting that these bacteria are metabolically active on surfaces (Gerner et al., 2018).

2.5 | Other organisms in the microbiome

Only one study on ITS amplicons in the subway mycobiome has been conducted (Grydaki et al., 2021). In the Athens subway, researchers detected 89 fungal genera with the majority belonging to Ascomycota (62.9%) and Basidiomycota (36.4%): the most common genera were *Cladosporium* (23.9%) and *Mycosphaerella* (10.7%), followed by *Antrodia* (7%), *Sistotrema* (2.4%), *Bjerkandera* (2.3%), *Penicillium* (2.3%), *Alternaria* (1.4%), and *Aspergillus* (1.3%), whereas *Malassezia* was present in low abundance (>0.5%). Most fungi in Athens are plant pathogens or saprophytes (Grydaki et al., 2021). *Cladosporium*, *Penicillium*, and *Aspergillus* have been cultivated in the Milan and St. Petersburg subways (Bogomolova & Kirtsideli, 2009; Picco & Rodolfi, 2000). In Boston and Hong Kong, the most abundant fungus is *Malassezia globosa* (Hsu et al., 2016; Kang et al., 2018). Interestingly, in a study of 60 cities, *Saccharomyces cerevisiae* was a part of the subcore microbiome (prevalence 70%–97%) (Danko et al., 2021).

Little is known about the subway virome. Shotgun sequencing studies have shown that 0.03% of viral sequences corresponded to bacteriophages in New York surface samples (Afshinnekoo et al., 2015). Furthermore, In Hong Kong, 3.21% of viral sequences corresponded to DNA viruses (Leung et al., 2021) and most viral sequences in Boston corresponded to *Cutibacterium* bacteriophages (Hsu et al., 2016). Moreover, the abundance of some phages correlated with that of some bacterial strains (Hsu et al., 2016). In the study of 60 cities, 11,614 virus species were assembled, of which 94.1% did not coincide with isolated or environmental viruses. Of the viruses assigned to assembled species, 41% corresponded to bacteriophages of core bacteria, and some have been detected in various cities on different continents (Danko et al., 2021). Numerous health-relevant viruses are RNA viruses; therefore, further studies

on detecting RNA molecules are required. Notably, studies using reverse transcription-qPCR have detected the presence of particular viruses. In the air of Barcelona, influenza was detected in half of the samples during the flu season and its concentration was estimated at 10^2 viruses per m^3 (Triadó-Margarit et al., 2017). In Singapore, adenovirus (10%), syncytial virus (4.5%), and influenza (1%) have been detected (Coleman et al., 2018). In Barcelona in June 2020, SARS-CoV2 was detected in 33% of air samples, with a maximum concentration of 23 viral copies per m^3, implying exposure to 1.5 viral copies per ride per person. Markedly, 40% of the surface samples tested positive for SARS-CoV2 and 20–700 viruses per m^2 were detected (Moreno et al., 2021). Viral studies are challenging because of insufficient knowledge about environmental viruses and the low DNA and RNA concentrations obtained from subway samples.

3 | SUBWAY PASSENGER INTERACTIONS

Subway microbiome analysis demonstrates the interaction between passengers and subways. However, much remains to be done to understand these interactions, their relevance, and the manner in which they occur (Figure 5).

3.1 | What does the subway microbiome contribute to an individual?

In a Hong Kong study, instead of subway surface sampling, passengers' hands were sampled after travelling (Kang et al., 2018). After the participants washed their hands with soap and water, they were in contact with different subway surfaces for 30 min at two different times of the day. This analysis focused on studying antibiotic resistance genes (ARGs) and observed that ARG abundance increased in the afternoon. Noteworthy, the Hong Kong subway is treated with a layer of nanosilver titanium dioxide coating, an antimicrobial paint (Kang et al., 2018).

A study in Mexico City went a step further; the differences between the palms of the hands before and after an 11-station ride (approximately 20 min) were analysed. People modify their microbiomes without losing their microbial signals. Upon exiting a subway, a person's hands microbiome resembles those of other passengers and the subway itself (Vargas-Robles et al., 2020). These results agree with those of Selway et al. (2020) who observed that upon exposure to green spaces for 15 min, people modify their skin microbiome, increasing its diversity and rendering it more similar to that of the soil (Selway et al., 2020). Because human microbial fingerprints are to a certain extent stable, the vast majority of these microbial variations must be transitory.

3.2 | How quickly does the microbiome re-establish itself after cleaning? How often should the handrails be cleaned?

A study of school desk cleaning showed that microorganisms re-established themselves within two to five days. In this study, most microorganisms originated from human skin. In the absence of moisture, the re-establishment process likely occurs owing to bacterial settlement and not the growth of the remaining bacteria (Kwan et al., 2018). A similar analysis of subway handrails showed that bacterial richness and colony forming units were re-established within 5–30 min, which also likely occurs owing to bacterial settlement. Additionally, the passengers likely clean and dirty the handrails at the same time. In Mexico City, handrails are made of stainless steel, and microbial re-establishment on stainless steel could largely differ from that on other materials (Vargas-Robles et al., 2020). No ecological succession was observed in either study.

3.3 | Are subways reservoirs of antibiotic resistance?

Subways represent large reservoirs of antibiotic-resistant bacteria, which has been analysed by cultivating bacteria and detecting ARGs. Subway bacteria have been grown in Petri dishes with numerous antibiotics such as ampicillin, chloramphenicol, ciprofloxacin,

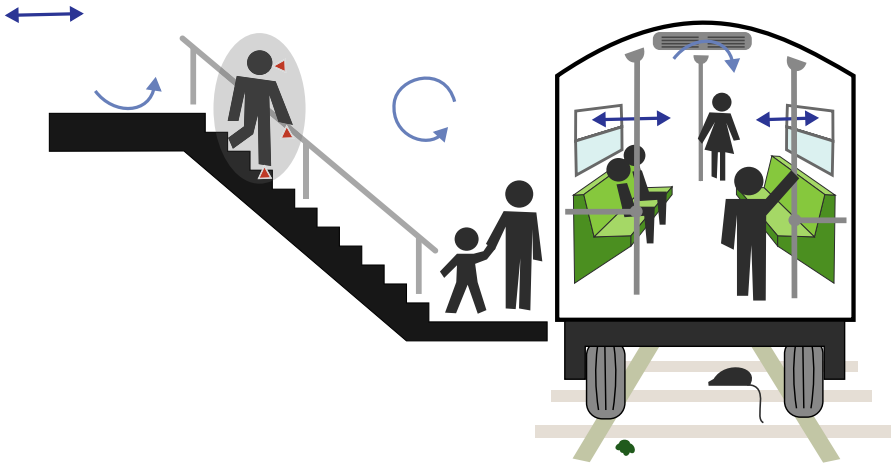

FIGURE 5 Passengers leave their microbiota through their microbial clouds and direct contact by touching surfaces. In the same way, the passengers get the subway microbiome by touching the surfaces and breathing. Air currents (arrows) can allow the exchange of bacteria with the outside environment and the recirculation and resuspension of bacteria. The soil and dust in the shoes also enable exchange with outside metro bacteria

kanamycin, nalidixic acid, nitrofurantoin, penicillin, streptomycin, tetracycline, and trimethoprim (Bactrim) (Afshinnekoo et al., 2015; Gohli et al., 2019; Zhou & Wang, 2013). Gene analysis in a multi-city study demonstrated that the most frequent ARGs were those against macrolides, lincosamides, streptogramins, and beta-lactams. Resistance gene patterns vary in each city in response to local public health strategies (Danko et al., 2021). In Hong Kong, more tetracycline resistance genes were detected in the subway line that connects with mainland China, possibly owing to the use of this antibiotic in pig farms (Kang et al., 2018). Zhelyazkova et al. (2021) propose using the "relative risk" to measure exposure to geolocation-associated ARGs. This index can be employed to evaluate public policies regarding antibiotic use. In Hong Kong, genes coding for efflux pumps and resistance to fluoroquinolone, tetracycline, vancomycin, and erythromycin have been detected, whereas in Boston, genes for efflux pumps and tetracycline and beta-lactam resistance have been detected. The amount of ARGs varied largely between the two studies. On an average, 469 ARGs per million reads were detected in Hong Kong, whereas only 1.17 ARGs per million reads were detected in Boston. Nevertheless, these concentrations are lower than those observed in human gut samples (Hsu et al., 2016; Kang et al., 2018).

4 | IS THE MICROBIOME ALIVE?

Numerous microorganisms do not grow indoors but accumulate passively. The indoor environment is characterized by promoting organism desiccation (Gibbons, 2016). HTS studies do not recognize whether the detected organisms are metabolically active. Therefore, the bacteria detected in these studies may have died or were nonviable. As an indirect measure, the Index of Replication or Growth Rate Index, which is the ratio between the number of genes near the origin of replication and the terminus, can be used (Brown et al., 2016; Emiola & Oh, 2018). In some microbial systems, strains of *C. acnes*, *Paracoccus*, *Deinococcus*, *Kocuria*, *Sphingomonas*, *Staphylococcus*, *and Micrococcus*, etc., are proposed to be active (Gerner et al., 2018; Leung et al., 2021), which requires further validation. An alternative for metabolic validation is transcriptome analysis. In addition, massive cultivation strategies are a reliable way to confirm metabolic viability (Ruiz et al., 2019).

Microbes detected in subways could be classified as living, dormant, or dead cells. Understanding which organisms are metabolically active (i.e., living) raises many other questions. Source tracking, used to search for microbial sources, is a common practice and it assumes that the bacteria are from other sources. We imagined a subway as an environmental microbial sink for several bacteria, a low-quality habitat that does not promote growth. The above is supported by what we know of other indoor spaces; also that many of the dominant bacteria in the subway are abundant and prevalent on human skin; besides the rapid recovery of communities on cleaned surfaces. However, we cannot be certain about the viability or metabolic activity of the subway microbiome. Discriminating living cells from dormant or dead cells is crucial. Additionally, metabolically active bacteria that are thriving in subways might live there permanently. If a bacterium arrives via its host, lands on a surface, and eventually enters dormancy, its source is a holobiont comprising a host and microbes or the soil (Mills et al., 2019). Only living bacteria have ecological interactions, such as cooperation or competence. Co-occurrence networks have been developed in the Moscow Metro to understand the relationships among bacteria (Klimenko et al., 2020). Researchers found small networks of two to four organisms that might not make biological sense. Failure to find clear networks could be attributed to scarce interactions, metabolically inactive bacteria, or dense and heterogeneous interaction networks (Hirano & Takemoto, 2019). Another issue to be solved is conditionally rare taxa (CTR). These taxa are abundant in some samples but not in others (Danko et al., 2021; Leung et al., 2014; Robertson et al., 2013). CTR can be understood as a phenomenon that depends on the outside source if most subway bacteria are dormant or dead. Alternatively, if subway bacteria are viable and active, environmental features that cause these taxa to bloom should be explored.

5 | OPEN CHALLENGES

Subways have been previously proposed as microbial species pools within urban communities (Hernández et al., 2020), representing a playground between the environment, hosts, and microbes (Miller et al., 2018; Zobel et al., 1998). Subway microbiomes are dynamic and can shift within a day (Kang et al., 2018). Furthermore, microbial configuration is multifactorial, ranging from molecules to the entire ecosystem. The remaining challenges regarding the subway microbiome include: determining the bacteria that migrate into subways and that cope with desiccation, the manner in which the bacteria interact inside subway systems, and the bacteria that migrate out and survive on the skin of people.

Two of the most significant differences between subways are human behaviour and cultural differences, which have not been analysed or incorporated into these studies, for example, the difference in commute distances, presence of passengers with service dogs, age and socioeconomic group, whether homeless people are sheltering in the subway tunnels, a variety of culinary and hygienic customs, etc. Integrating these variables is a multidisciplinary challenge for future studies.

Buildings have been labelled "sick" if they promote allergy or asthma diseases, or "healthy" if they promote well-being (Dannemiller, 2019). Historically, and through advertising, cleaning microorganisms has been associated with good health. However, it is not possible to eliminate all microorganisms from indoor environments, as some microorganisms are resistant to cleaning. In addition, we introduce our microbiome upon entering a building. Therefore, the introduction of symbiotic organisms into buildings as complex communities (i.e., dogs or plants) or cultivated probiotics (Horve et al., 2020) has been proposed. We wonder if supplementing the subway interior with probiotic communities such as gardens is suitable.

Two unsolved questions are: (1) Which pathogens are present in subways? (2) Is exposure to these pathogens relevant to the health

of passengers? These questions cannot be answered using the metagenomic analyses mentioned above. Taxonomic analysis of 16S amplicons or reads does not have sufficient resolution to distinguish between pathogenic, symbiotic, or commensal strains. Although analyses of assembled genomes allow us to distinguish between strains and identify virulence factors, they do not indicate whether these strains are viable. Additionally, the presence of viable pathogenic organisms is not sufficient to cause diseases. Host and environmental conditions play an indispensable role in health (Barreto et al., 2006; Engering et al., 2013). Quality of life including access to health facilities, good nutrition, and healthcare systems, completely changes the repercussions of a possible disease (Neiderud, 2015). Moreover, naming a bacterial pathogen is over simplistic because the pathogen must cause measurable damage to its host, and responses in host-microbe interactions are context-dependent (Casadevall & Pirofski, 2014). Metagenomic analysis indicates that the microorganisms that live and travel in subways are either normal skin-associated or soil-associated commensal bacteria. Epidemiological studies are required to understand the health relevance of exposure to the subway microbiome.

Finally, we do not know whether our understanding of the subway microbiome before the coronavirus disease 2019 pandemic remains completely true. The pandemic has changed human relationships with subways; people wear face masks, and wash their hands with alcohol gel. In addition, the way subways are cleaned has changed, and sanitary mats, antibacterial coatings, ultraviolet lamps, and ozone-producing machines have been installed. These differences in behaviour and cleanliness undoubtedly affect the microbiome of these spaces. The impacts of reduced social motility and social distancing on subways and their microbiomes are yet to be elucidated.

These studies suggested that subways are hubs for microbiome exchange. Subway microbiomes reflect user populations and the city. Notably, identifying cities based on human microbiome may soon be possible. We need to fully understand the interactions between the microbiomes of subways and passengers and their relevance to individual and public health, including their role in resilience to and coping with stressful events such as the current pandemic.

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CONFLICT OF INTEREST

The authors have no conflicts of interest.

AUTHOR CONTRIBUTIONS

All authors contributed to the conceptualization, investigation, and writing the manuscript.

DATA AVAILABILITY STATEMENT

No data are associated with the manuscript.

ORCID

Mariana Peimbert https://orcid.org/0000-0002-4881-8587

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