Science of the Total Environment 913 (2024) 169625

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Alternations of antibiotic resistance genes and microbial community dynamics on shared bicycles before and after pandemic lockdown

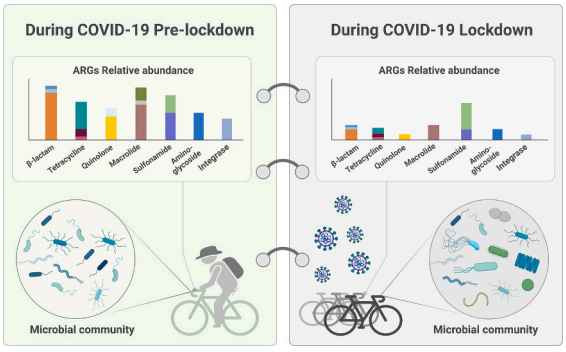
Leshan Xiu [a,b,c,*,1], Haodong Liu [a,b,1], Yi Xie [a,b,1], Qinqin Hu [a,b], Huimin Li [a,b], Fumin Chen [a,b], Chenxi Wang [a,b], Yuqian Zhang [d,e], Liyuan Hou [f,g,**], Kun Yin [a,b,***]

[a] School of Global Health, Chinese Center for Tropical Diseases Research, Shanghai Jiao Tong University School of Medicine, Shanghai, China
[b] One Health Center, Shanghai Jiao Tong University-The University of Edinburgh, Shanghai, China
[c] State Environmental Protection Key Laboratory of Environmental Health Impact Assessment of Emerging Contaminants, Shanghai Academy of Environment Sciences, Shanghai, China
[d] Department of Surgery, Division of Surgery Research, Mayo Clinic, Rochester, MN 55905, USA
[e] Microbiome Program, Center for Individualized Medicine, Mayo Clinic, Rochester, MN 55905, USA
[f] Department of Civil and Environmental Engineering, Utah State University, UT 84322, USA
[g] Utah Water Research Laboratory, 1600 Canyon Road, Logan, UT 84321, USA

HIGHLIGHTS

- Shared bicycles act as potential reservoirs for diverse ARGs and potential pathogens.
- COVID-19 lockdown impacts microbial diversity and ARGs abundance on shared bicycles.
- Persistent ARGs and pathogens found despite reduced bicycle usage during the lockdown.
- Highlights the need for better hygiene to mitigate the risks in shared transportation

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Ewa Korzeniewska

Keywords:
COVID-19 lockdown
Shared bicycles
Antibiotic resistance genes

ABSTRACT

The prevalence of shared bicycles has raised concerns over their potential to transmit pathogens and microbes harboring antibiotic resistance genes (ARGs), which pose significant human health risks. This study investigated the impact of anthropogenic activities on the composition of ARGs and microbial communities on shared bicycles during the COVID-19 pandemic and subsequent lockdown when shared bicycle usage was altered. A total of 600 swab samples from shared bicycle surfaces were collected in Shanghai before and during COVID-19 lockdown

* Correspondence to: L. Xiu, School of Global Health, Chinese Center for Tropical Diseases Research, Shanghai Jiao Tong University School of Medicine, Shanghai, China.
** Correspondence to: L. Hou, Department of Civil and Environmental Engineering, Utah State University, UT 84322, USA.
*** Correspondence to: K. Yin, School of Global Health, Chinese Center for Tropical Diseases Research, Shanghai Jiao Tong University School of Medicine, Shanghai, China.
E-mail addresses: xiuls001@hotmail.com (L. Xiu), liyuan.hou@usu.edu (L. Hou), kunyin@sjtu.edu.cn (K. Yin).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.scitotenv.2023.169625
Received 17 October 2023; Received in revised form 20 December 2023; Accepted 21 December 2023
Available online 27 December 2023

Microbial community
Surveillance

periods. Even during lockdown, 12 out of 14 initially detected ARG subtypes persisted, indicating their tenacity in the face of reduced anthropogenic activities. These ARGs displayed significantly higher absolute and relative abundance levels before the lockdown. In addition, the percentage of potential pathogens in the total microbial abundance remained at 0.029 % during the lockdown, which was lower than the pre-lockdown percentage of 0.035 % and suggested that these risks persist within shared bicycle systems. Interestingly, although microbial abundance decreased without the consecutive use of shared bicycles during lockdown, the microbial diversity increased under the impact of restricted anthropogenic activities ($p < 0.001$). This emphasizes the need for continuous monitoring and research to comprehend microbial community behaviors in various environments. This study uncovered the underlying impacts of the COVID-19 lockdown on the microbial and ARG communities of shared bicycles, providing comprehensive insights into the health management of shared transportation. Although lockdown can decrease the abundance of ARGs and potential pathogens, additional interventions are needed to prevent their continued spread.

1. Introduction

Shared transportation is an innovative and environmentally-friendly model promoting the transformation of low-carbon development as part of global responses to address climate change (Kim, 2023; Zhu and Lu, 2023). In recent years, shared bicycles have become a popular option for personal mobility in 1590 cities across 92 countries worldwide, with China, Europe, and North America accounting for over 99 % of the total bicycle sharing service (https://bikesharingworldmap.com). Shared bicycles are highly sought-after due to their flexibility, low cost, low carbon footprint, and sustainability as an efficient public transportation mode (Guo et al., 2022), particularly in metropolitan areas. For local residents, shared bicycles also effectively bridge the first- and last-mile traffic gap between home and work locations or metro stations (Fu and Guo, 2018). In China, the number of shared bicycles has exceeded 23 million, with an average daily order of over 45.7 million in 2020 (Guo et al., 2022; Hayes et al., 2022).

Shared bicycles, generally considered as a safe mode of public transportation, are raising concerns about their potential to disseminate pathogens and antibiotic-resistant bacteria (ARB) (Gu et al., 2020; Sun et al., 2020; Xu et al., 2019; Zou et al., 2019). This is due to their open placement and repeated use by multiple riders. The handles and brakes, in particular, serve as contact points for ARB, turning shared bicycles into potential vectors for transmitting antibiotic resistance genes (ARGs) (Gu et al., 2020; Sun et al., 2020; Zou et al., 2019). The prevalence of ARB and ARGs on shared bicycles is attributed to some riders carrying them (Gu et al., 2020; Xu et al., 2019; Zou et al., 2019), and their prevalence and concentration can escalate with the rapid expansion of shared bicycles across a city. Previous studies have identified antibiotic-resistant *Staphylococci*, *Enterococci*, and *Enterobacteriaceae* on shared bicycles from Tianjin (Xu et al., 2019), Beijing (Zou et al., 2019) and Chengdu (Gu et al., 2020), China, using culture-based approaches. Additionally, a study using 16S rRNA sequencing techniques has shown that the richness and diversity of microbial species and ARGs carried by shared bicycles are significantly higher than in the surrounding environment (Sun et al., 2020). Therefore, shared bicycles pose an underestimated risk of inter-individual transmission of pathogens and ARB, and this risk persists over an extended period without proper disinfection.

Anthropogenic activities have contributed to the distribution of ARGs (Czatzkowska et al., 2022) from animal and human sources to natural environments. ARGs have been frequently detected in a wide range of environments, such as soils (Delgado-Baquerizo et al., 2022), ambient air (Ginn et al., 2021), and wastewater (Hayes et al., 2022), posing a significant public health threat. Horizontal gene transfer (HGT) is the most effective pathway for the spread of ARGs among microorganisms in the environment, with the central role of mobile genetic elements (MGEs) in transferring ARGs throughout microbes (Zhao et al., 2021). There is accumulating evidence that anthropogenic activities such as environmental sanitation strongly impact microbial abundance or diversity in the surrounding environment (Jiang et al., 2022; Zhao et al., 2022). For instance, intense anthropogenic disturbance has significantly changed the composition of the roadside soil microbial community (De Silva et al., 2021). Previous research has demonstrated that anthropogenic activities directly or indirectly caused changes in microbial community compositions in sediments along the Yangtze Estuary and its coastal area (Guo et al., 2019). Furthermore, another study observed a significant decrease in microbial populations and river heavy metal levels when anthropogenic activities are restricted during the COVID-19 lockdown (Karunanidhi et al., 2021).

Given the influence of anthropogenic activities on shared bicycle usage (Gu et al., 2020; Sun et al., 2020; Xu et al., 2019; Zou et al., 2019), it is reasonable to hypothesize that a substantial reduction in human activity could significantly impact the dynamics of ARGs and microbial communities on shared bicycles. However, the diversity and abundance of ARGs and pathogens in shared bicycles affected by anthropogenic activities have not been studied, and their potential correlation with the carried microbial community is not well understood (Sun et al., 2020). In March 2022, an outbreak of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) Omicron BA.2 variant rapidly spread throughout Shanghai, China (Chen et al., 2022), resulting in a series of containment strategies and restrictions, including quarantine, social distancing, and widespread lockdown (Zhang et al., 2023). The population of Shanghai was under lockdown from March 28 to June 1, 2022, restricting travel and transportation for several months (Chen et al., 2022; Zhang et al., 2023). This lockdown provides a unique opportunity to observe the dynamics of ARGs and microbial communities on shared bicycles with less influence of anthropogenic activities. Importantly, recent studies have shown that even in the absence of significant anthropogenic activities, as is the case in environments such as spacecraft and the International Space Station (Be et al., 2017; Lu et al., 2023), ARGs can be transported through airborne particulate matter and dust (Feng et al., 2022; Zhou et al., 2021). These ARGs can also continue to migrate among microbial communities through MGEs (Feng et al., 2022). Thus, the extent to which lockdown measures and altered human behavior effectively reduce the presence of ARGs and pathogens in an environment remains a question of considerable scientific and public health importance.

As the world continues to navigate the challenges presented by infectious diseases and antibiotic resistance, understanding the factors that influence the dynamics of ARGs and microbial communities is significant to ensuring the safety and sustainability of shared transportation systems (Bai et al., 2023). Furthermore, a critical gap exists in our understanding regarding the impact of anthropogenic activities on the diversity and abundance of ARGs and pathogens within shared bicycles. To address these vital questions, we employed quantitative PCR techniques to investigate the diversity, abundance, and co-occurrence of ARGs and MGEs. In addition, we applied 2bRAD-M, a novel, cutting-edge species-resolved sequencing approach, to provide a comprehensive metagenomic characterization of the microbiomes on shared bicycles. 2bRAD-M, based on type IIB restriction endonuclease cleavage, produces uniformly short DNA fragments, enabling precise screening and species abundance estimation through unique tag distribution (Sun et al., 2022). In particular, the aim of this study was to determine how

anthropogenic activities impact the composition of microbial communities and ARGs on shared bicycles during the COVID-19 pandemic and subsequent lockdown when shared bicycle usage was altered. The results from this study will provide an insight into the importance of following management strategies that not only effectively reduce the transmission of pathogens and ARGs to riders but also contribute to the overall improvement of public health associated with shared transportation.

2. Materials and methods

2.1. The study area and sampling sites

We selected five representative locations (i.e., a school, a community, a metro station, a shopping mall, and a hospital), marked as S, C, MS, SM, and H, respectively, in the Jing'an district in Shanghai, Eastern China (Fig. 1). The sampling period was from February 12 to May 31, 2022, with the COVID-19 pre-lockdown period running from February 12 to March 27 and the COVID-19 lockdown period from March 28 to May 31. Before sample collection, researchers were instructed to wear disposable nitrile gloves and dip the swab in the sterilized PBS solution with a pH of 7.0. A standard operating procedure was developed for the sample collection. Briefly, researchers thoroughly rubbed the swab across the surface of handles and brakes, covering an approximately 6 × 6 cm area, using both sides and various angles to ensure the maximum yield of genomic DNA. Each shared bicycle was sampled with a single swab. Ten shared bicycles were randomly selected and tested at each of the selected locations on the same day (Fig. 1).

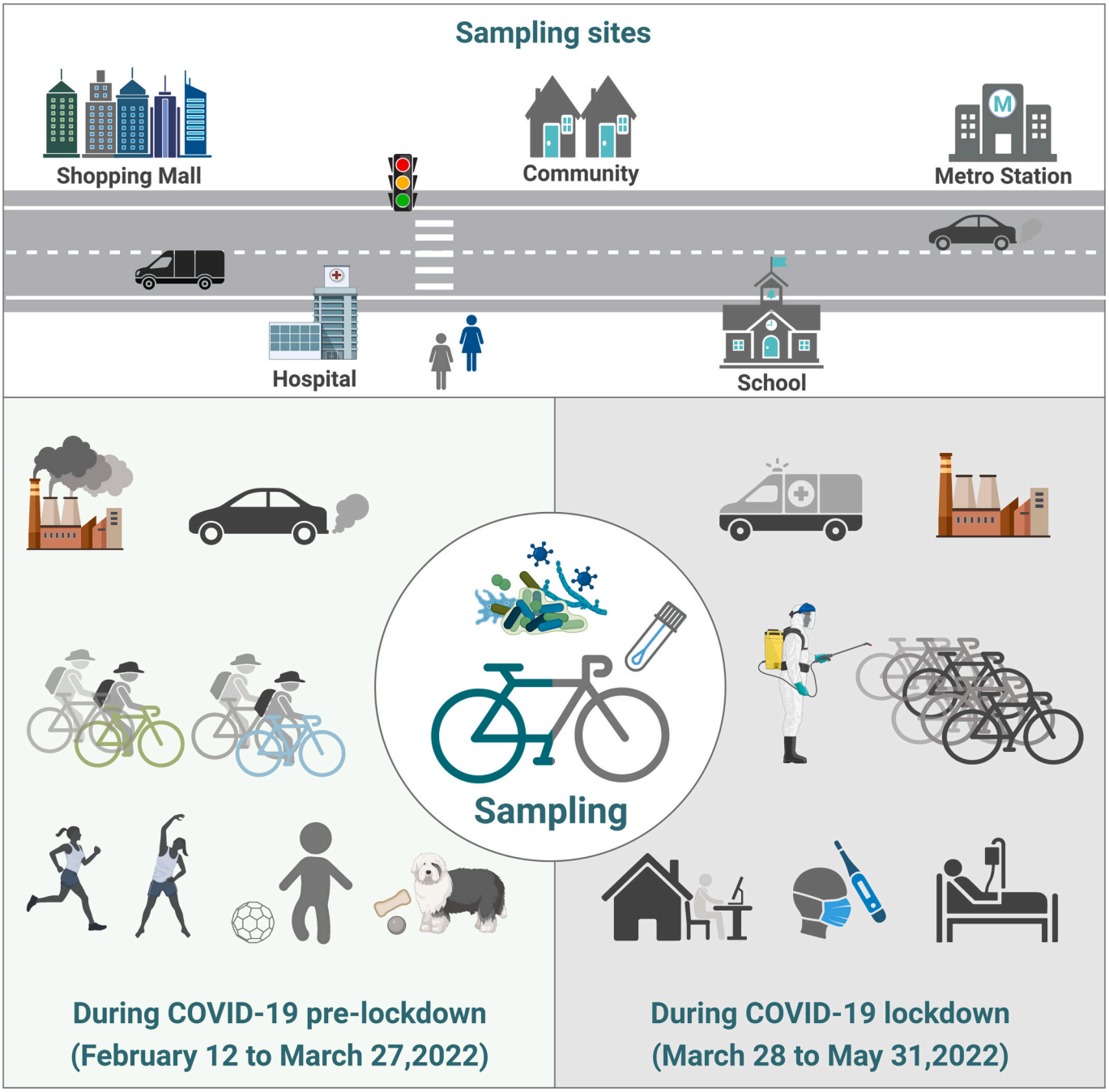


Fig. 1. Study periods and sampling sites of shared bicycles in this study.

Thus, a total of 600 swabs were collected from pre-lockdown (n = 300) and COVID-19 lockdown (n = 300) periods. All samples were collected in sterilized bottles, immediately packed with ice to minimize biological activity during collection, and transported to the laboratory for further analysis. A comprehensive set of environmental factors (Table 1), including temperature, relative humidity, UV Index, wind speed, visibility, pressure, and dew point, were also recorded according to the Dark Sky (https://darksky.net).

2.2. Sample processing and DNA extraction

To overcome the challenge of low genomic DNA yield from individual swabs, we adopted a strategy where ten swabs collected from shared bicycles at the same site during each sampling day were pooled into composite tubes containing 10 mL of 1 × PBS. Subsequently, these composite tubes underwent vigorous mixing on a vortex mixer, ensuring comprehensive homogenization of the samples. This process resulted in the creation of 60 pools derived from the 600 individual swabs, with each set of 30 pools representing shared bicycles sampled during the pre-lockdown and lockdown periods. To capture and concentrate the bacteria from the swabs, filtration was performed using a 47 mm diameter PES membrane with a 0.22 μm pore size. The membranes were then stored at −20 °C before DNA extraction. Genomic DNA extractions from the membranes were performed using the DNeasy PowerSoil Kit (Qiagen, Hilden, Germany) following the manufacturer's protocols. Quantity and quality of DNA were measured using the Qubit-4 analyzer and NanoDrop-100 Spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA), respectively.

2.3. Real-time qPCR quantification of ARGs

The quantification of ARGs was determined by real-time qPCR assays. A total of 19 most detected ARGs associated with six antibiotic classes were tested in this study, including *aacC2*, bla_{OXA-1}, bla_{TEM-1}, bla_{SHV-1}, bla_{CTX-M}, *ermB*, *ermF*, *mefA*, *qepA*, *qnrA*, *qnrS*, *sul1*, *sul2*, *sul3*, *tetA*, *tetC*, *tetM*, *tetQ*, and *tetW*. Furthermore, *intI1* (class I integrase) and the 16S rRNA gene were included as indicators for HGT and total microbial abundance, respectively. The qPCR assays were performed to calculate the copy numbers of ARGs using a Roche Light-Cycler 480 (Roche Applied Science, Germany) based on the standard curves. Each 20 μL qPCR consisted of 10 μL of 2 × LightCycle 480 SYBR Green I Master (Roche Applied Sciences, USA), 1 μL each of forward and reverse primers, 2 μL of template DNA, and 6 μL of nuclease-free water (Thermo Fisher Scientific). The thermal cycling conditions consisted of an initial denaturation at 95 °C for 2 min, followed by 40 cycles at 95 °C for 10 s, annealing temperatures of each gene for 30 s, 72 °C for 30 s, and a final step for the automatically generated a melting curve. Standard curves were generated for each run, using a 10-fold serial of target ARG fragments with known copy numbers (ranging from 10^6 to 1 gene copies/μL). Absolute gene abundance is the concentration of specific ARGs per unit surface area. It is determined by converting the copy number of target ARGs in the samples via qPCR, taking into account the dilution factor. The unit is copies/cm^3. The relative abundance (copies/16S rRNA copies) was calculated as the absolute abundance of ARG divided by the absolute abundance of 16S rRNA. Each sample and standard were tested in triplicate, including the positive and negative controls. Targets with false melting temperatures or multi-peaks were regarded as negative. To ensure accuracy, only data with a square of the related coefficient (R^2) higher than 0.99 for the standard curve was used for each target ARG.

2.4. Library construction and 2bRAD-M sequencing

The 2bRAD-M libraries were constructed by adapting the protocol (https://github.com/z0on/2bRAD_denovo, accessed 18 August 2022) proposed by Wang et al. (Wang et al., 2012). In brief, high-quality genomic DNA (approximately 200 ng) from each swab pool was digested with the type IIB restriction enzyme *BcgI* (New England BioLabs, UK). Subsequently, the generated IIB-REase-specific and uniform 36 base pair fragments with random overhangs were multiplexed using custom barcoded ligation adapters. After purifying PCR products, 2bRAD-M sequencing was performed on the Illumina Nova PE150 platform (Illumina, San Diego, USA) according to the standard protocols from Qingdao OE Biotech Co., Ltd. (Qingdao, China). Raw sequence data from all tested samples were deposited at the NCBI BioProject archive under the accession number PRJNA954366. Detailed information regarding the specific sample sequencing data and their accession numbers can be found in Supplementary Table S1.

2.5. Bioinformatics and statistical analysis

Raw sequencing reads were processed using the customized Perl scripts from the 2bRAD pipeline v2.0 (https://github.com/z0on/2bRAD_denovo, accessed 18 August 2022) to trim adaptors, remove duplicated and filter out reads with ≥90 % of bases with a minimum quality score of 20 (input quality ASCII offset of 33). A genomic reference dataset was developed using 173,165 microbial genomes (including bacteria, fungi, and archaea) from the NCBI RefSeq database. Using a built-in Perl script (Xue et al., 2021), quality-filtered reads representing microbial communities were mapped against the genomic reference dataset, which contains all 2bRAD tags theoretically unique to each of 26,163 microbial species. Reads coverage for each identified genome was determined, and then the relative abundance of each microbial species was estimated using a previously described formula (Lam et al., 2022; Xue et al., 2021). This generated a taxonomic abundance profile. According to the pathogen list released by the National Health

Table 1
Sampling information and meteorological conditions in the pre-lockdown and lockdown period.

	Sampling date	Sampling site					Meteorological conditions					
		School	Community	Metro station	Shopping mall	Hospital	T (°C)	RH (%)	UVI	W (m/s)	P (hPa)	DPt (°C)
During the pre-lockdown	2022-Feb-12	S1	C1	MS1	SM1	H1	9	75	4	4	1024	5
	2022-Feb-26	S2	C2	MS2	SM2	H2	15	43	7	4	1023	3
	2022-Mar-5	S3	C3	MS3	SM3	H3	15	30	7	6	1021	−2
	2022-Mar-13	S4	C4	MS4	SM4	H4	24	63	4	3	1010	16
	2022-Mar-19	S5	C5	MS5	SM5	H5	12	73	4	6	1016	7
	2022-Mar-26	S6	C6	MS6	SM6	H6	16	68	5	6	1016	10
During the lockdown	2022-Apr-13	L_S1	L_C1	L_MS1	L_SM1	L_H1	16	86	4	6	1011	13
	2022-Apr-22	L_S2	L_C2	L_MS2	L_SM2	L_H2	29	41	5	3	1012	14
	2022-Apr-27	L_S3	L_C3	L_MS3	L_SM3	L_H3	20	53	5	6	1018	10
	2022-May-7	L_S4	L_C4	L_MS4	L_SM4	L_H4	27	57	5	5	1017	17
	2022-May-19	L_S5	L_C5	L_MS5	L_SM5	L_H5	23	60	4	5	1017	15
	2022-May-31	L_S6	L_C6	L_MS6	L_SM6	L_H6	28	18	6	5	1010	2

T: average temperature; RH: relative humidity; UVI: UV Index; W: wind speed; V: visibility; P, pressure; DPt, Dew Pt.

Commission of the People's Republic of China, a search was conducted within the GTDB database (v20220826), which currently includes 894 bacterial and 36 fungal species. The absolute abundances of potential pathogens (found in the list) were divided by the absolute abundances of the total community to get their relative abundances. The relative abundance of identified pathogenic bacterial species in the samples was cumulatively summed to create a Pathogenicity Index. This index served as an assessment of the pathogen abundance within the microbial community (Lam et al., 2022). Here, the higher the index score, the higher the abundance of pathogens. The correlation between microbial community and meteorological conditions was analyzed using redundancy analysis (RDA) and network analysis (Barberan et al., 2012; Liu et al., 2024). Descriptive statistics were generated using GraphPad Prism software or Microsoft Excel 2010 (Microsoft Corp., USA). Significance was determined using analysis of variance (ANOVA), with a p value $<$ 0.05 considered significant and a p value $<$ 0.001 considered highly significant.

3. Results

3.1. Profiling of microbial community composition

After assembling and quality filtering, a total of 319,423,331 and 235,433,013 high-quality sequences were obtained from shared bicycles in the pre-lockdown and lockdown periods, respectively (Figs. S1 and S2). Proteobacteria, Actinobacteriota, Ascomycota, Firmicutes, and Bacteroidota were the five dominant phyla, accounting for over 95.00 % and 93.00 % of the total bacteria in both periods (Fig. 2). Actinobacteriota and Proteobacteria were the most prevalent phyla, comprising around 42.72 % and 47.05 % of the total microbial community in pre-lockdown and lockdown periods, respectively. The heatmap at the phylum level revealed two main clusters, grouping pre-lockdown samples and lockdown samples, respectively (Fig. S2). The relative abundance of the microbial community at the genus level was different between the two periods, although they shared the most abundant genus (Fig. 2B). For example, *Cutibacterium* and *Staphylococcus* were significantly more abundant across all samples collected from pre-lockdown (22.40 % and 4.05 %, respectively) than during the COVID-19 lockdown (3.29 and 1.07 %, respectively). The composition and structure of the microbial community on the shared bicycles were also analyzed at the species level. A total of 19,352 microbial species were identified in all 60 samples, including 18,854 bacteria, 433 fungi, and 65 archaea. A marked dissimilarity in microbial community composition was observed between samples collected in pre-lockdown and lockdown (Fig. 2C). Among the top 15 ranking microbial species (Fig. 2C), *Cutibacterium acnes* and *Methylobacterium brachiatum* were the most abundant species detected on the surface of the shared bicycles from pre-lockdown. In contrast, the dominant species during the COVID-19 lockdown were *Aureobasidium melanogenum* and *Ralstonia sp001078575*.

3.2. Comparison of microbial diversity between pre-lockdown and COVID-19 lockdown periods

The microbial community structure on shared bicycles was significantly impacted by the COVID-19 lockdown, as indicated by the alpha diversity analysis. During the COVID-19 pre-lockdown, consecutive use of shared bicycles dramatically enriched the microbial populations (17,777 species) compared to the COVID-19 lockdown (15,779 species). There were no significant differences in Chao1 diversity analyses between pre-lockdown and lockdown (Fig. 3A; $p = 0.044$). However, the Shannon index, which estimates richness and evenness, was statistically ($p < 0.001$) higher in samples from COVID-19 lockdown (5.41) than pre-lockdown (4.46) (Fig. 3B). This indicates that the increased microbial diversity during the COVID-19 lockdown is possibly attributed to the reduced anthropogenic influence and an influx of microbes from other sources, such as soil and air. Despite the increase in microbial diversity during the lockdown period, the beta diversity analysis showed distinctive clustering, indicating the variation in the structure and composition of microbial community across the studied samples (Fig. 3). The differences in microbial community composition between pre-lockdown and lockdown periods were highly significant ($p < 0.001$), as confirmed by NMDS ordination (Fig. 3C). Principal coordinate analysis (PCoA) of microbial communities revealed unique clustering by the first and second components, explaining 29.60 % and 12.26 % of the variation, respectively (Fig. S3A). The Unweighted pair-group method with arithmetic mean (UPGMA) clustering tree also revealed differences in microbial communities between the pre-lockdown and lockdown periods (Fig. S3B). Although each sample exhibits different diversity and abundance relative to each other, the differences between microbial communities between pre-lockdown and lockdown periods were noticeable.

3.3. Dynamics of microbial community under the stress of the lockdown

Based on our results, the COVID-19 lockdown and anthropogenic activities significantly affected microbial biomass dynamics on the surface of handles and brakes of shared bicycles. We employed the linear discriminant analysis (LDA) effect size (LEfSe) method to determine the statistical significance of differentially abundant taxa and the biological relevance of species during both pre-lockdown and lockdown periods. LEfSe analysis indicated that the 201 microbial taxa exhibited significant differences between the two periods (LDA score > 3): 129 bacteria taxa were found during the lockdown period, and 72 microbial taxa were found during the pre-lockdown period (Fig. 4). The LDA scores also indicated that opportunistic pathogenic microorganisms, such as *Mycobacteriaceae*, *Staphylococcaceae*, and *Moraxellaceae*, were more enriched during pre-lockdown than in lockdown (Fig. 4A). Our results showed that during the lockdown period, most of the studied microbial fractions were significantly decreased ($p < 0.05$). For instance, the relative abundance of *Staphylococcus* and *Corynebacterium* genus on the shared bicycles decreased by 3.77- and 2.74-fold during the lockdown period, respectively (Fig. 4C). In contrast, the phyla of *Proteobacteria* (1.42-fold increase in the relative abundance), *Cyanobacteria* (3.95-fold), and *Bacteroidota* (1.38-fold) significantly increased on the surface of shared bicycles during the lockdown period compared to the pre-lockdown period (Fig. 4B).

3.4. Changes in ARGs and pathogens properties of shared bicycles

To explore the diversity and abundance of ARGs, 19 typical ARG subtypes conferring resistance to six antibiotics, along with MGE and 16S rRNA genes, were measured. Out of the 19 ARG subtypes, 14 were detected on the surface of handles and brakes in shared bicycles. 12 of these ARGs were still detectable during the lockdown period, highlighting their persistence. *Sul1*, *sul2*, and *tetW* were the most dominant subtypes, while other ARGs were found in <50 % of cases. Samples collected during the pre-lockdown period showed a higher number of ARGs (Fig. 5). ARG subtypes conferring resistance to macrolides (*ermF* and *mefA*) and quinolone (*qnrA*) were only present during the pre-lockdown period. No ARG subtypes exclusive to the lockdown period were observed. The restrictions on human activities during the COVID-19 lockdown had a significant impact on the relative abundance of ARGs. For instance, the relative abundance of most detected ARG subtypes during the pre-lockdown period was significantly higher than during the lockdown period. A similar observation was also found for class 1 integron. In addition, the percentage of tested samples containing ARGs decreased during the lockdown period, accounting for only 6.67 % to 40 % of the total bicycles collected during the lockdown period.

In addition to characterizing ARGs, the abundance and prevalence of pathogenic microorganisms that could cause allergies, respiratory diseases, and infections in humans were investigated. The percentage of potential pathogens comprised around 0.035 % and 0.029 % of the total

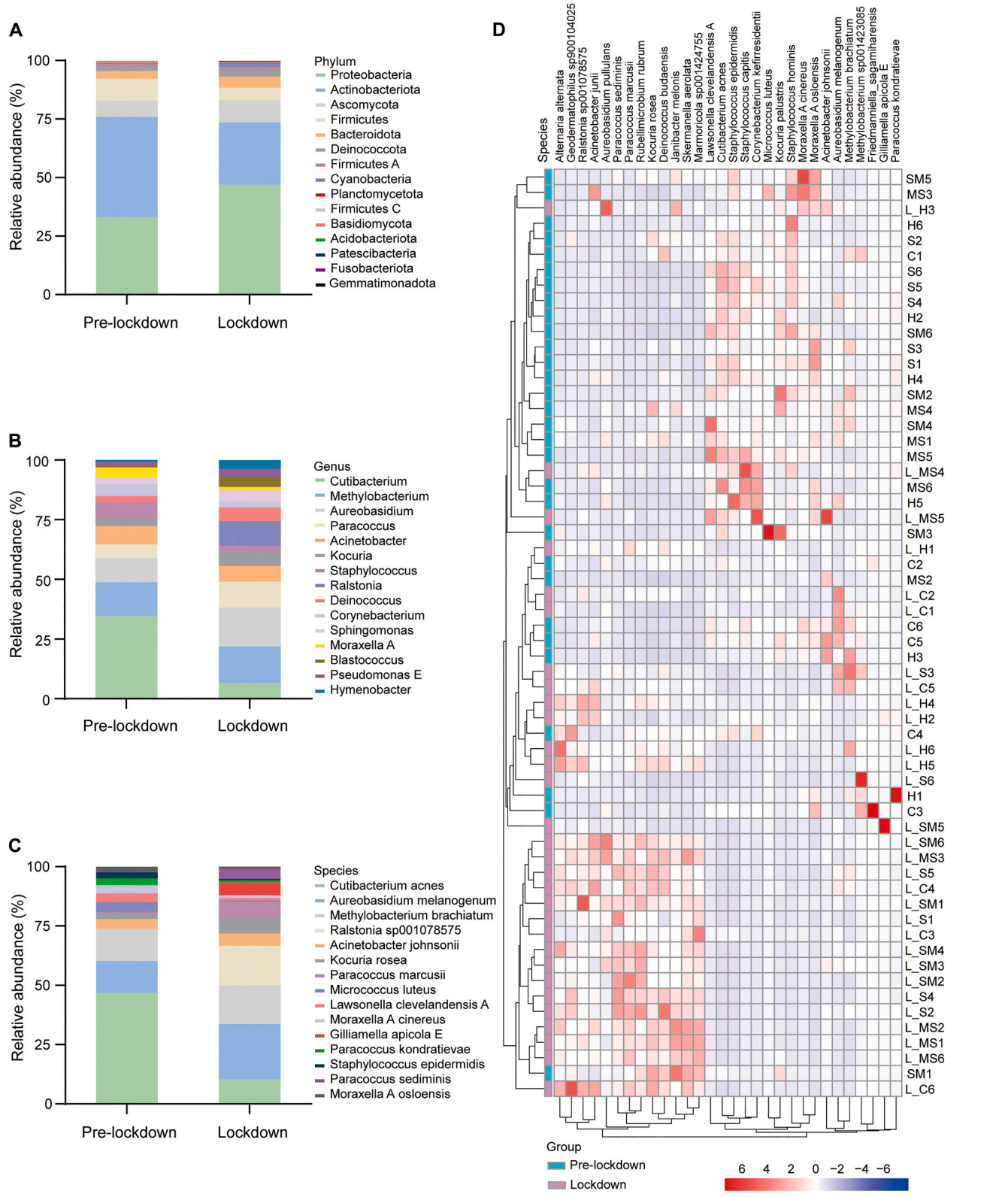


Fig. 2. Characteristics of microbial community composition in shared bicycles. (A) Variations in microbial community composition of samples at the phylum level (the top 15 phylum are presented). (B) Variations in microbial community composition of samples at the genus level (the top 15 genus are presented). (C) Variations in microbial community composition of samples at the species level (the top 15 species were presented). (D) Heatmap and hierarchical clustering of microbial community composition of all samples at the species level (only the top 30 species are selected for visualization). The letters S, C, MS, SM, and H represent the school, community, metro station, shopping mall, and hospital. The numbers indicate the specific sample numbers collected from each location. The letter L is used to denote the sampling period during the COVID-19 lockdown. Therefore, sample names on the x-axis can be understood as follows: L_S1, L_S2, L_S3, etc. represent samples collected from the school during the COVID-19 lockdown period, while S1, S2, S3, etc. represent samples collected from the school during the COVID-19 pre-lockdown period.

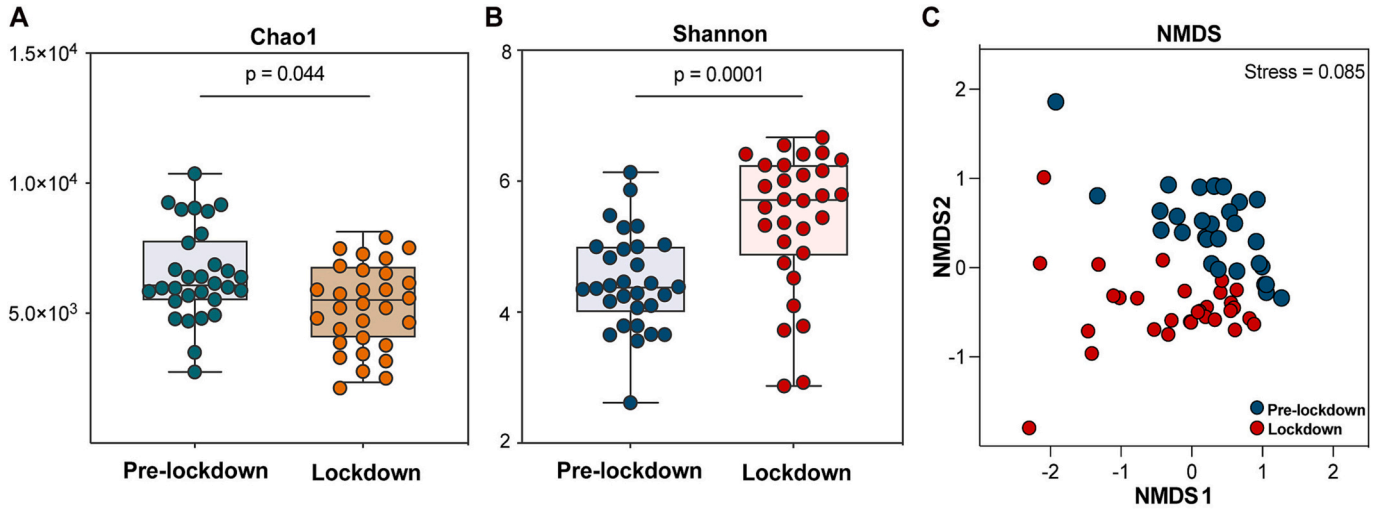


Fig. 3. Comparison of alpha and beta diversity of microbial communities between pre-lockdown and COVID-19 lockdown periods. The Chao1 (A) and Shannon (B) diversity index on shared bicycles. (C) The nonmetric multidimensional scaling (NMDS) ordination in all shared bicycles.

microbial communities in pre-lockdown and lockdown periods, respectively. The pathogenicity index of 0.035 and 0.029 for shared bicycles collected during the pre-lockdown and lockdown periods, respectively (Table S2). It was found that the lockdown due to the spread of COVID-19 reduced the pathogenicity index by 17.14 % compared to that during the pre-lockdown period. However, it remains to be seen whether these pathogens can cause infections after human exposure to shared bicycles.

3.5. Changes in microbial community composition and ARGs among sampling sites

The shared bicycles from all six sampling sites exhibited higher microbial abundance before the lockdown compared to the lockdown period, whereas microbial diversity showed the opposite trend (Fig. S4). Moreover, the Chao1 and Shannon indexes indicated no significant differences in microbial abundance and diversity at different sampling sites during the pre-lockdown and lockdown periods (Fig. S4). However, minor differences in microbial abundance were observed during the lockdown period, particularly at the school sampling site compared to the other four sites. At the species level, there were distinct differences in the proportions of the top 15 microbial species at each sampling site, highlighting the microbial communities' variations among different sampling locations (Fig. 6A).

The absolute abundances of ARGs exhibited a notable decrease, ranging from 370.52 to 429.46 copies/cm^2 in the pre-lockdown period, in contrast to a lower range of 37.12 to 175.25 copies/g during the lockdown period, respectively. In general, the abundance of predominantly detected ARGs in five sampling sites during the pre-lockdown period followed this order: community (429.46 copies/cm^2) > hospital (428.86 copies/cm^2) > school (428.82 copies/cm^2) > shopping mall (425.26 copies/cm^2) > metro station (370.52 copies/cm^2). Nevertheless, no significant differences in the absolute abundance of ARGs were observed among the various sampling sites during the pre-lockdown period. In contrast, the prevalent ARGs exhibited significant differences among various sampling sites during the lockdown period, following this trend: metro station (175.25 copies/cm^2) > community (152.09 copies/cm^2) > hospital (87.53 copies/cm^2) > shopping mall (68.59 copies/cm^2) > school (37.12 copies/cm^2). Before the lockdown, target ARGs were present across all sampling sites, with a predominant concentration in shared bicycles parked at metro stations, shopping malls, and hospitals. The absolute abundance of β-lactam, tetracycline, and macrolide resistance genes was consistently high at all five sampling sites. In metro stations, shopping malls, and hospitals, the dominant classes of ARGs were quinolone and aminoglycoside resistance genes (Fig. 6B). Contrastingly, at the community and school sampling sites, shared bicycles contributed higher absolute levels of sulfonamide resistance genes (Fig. 6B). During the lockdown period, there was a sharp decline in the absolute abundance of all six classes of ARGs at all six sampling sites. And relatively high concentrations of ARGs persisted at metro stations and hospitals (Fig. 6B).

3.6. Correlation between microbial community and environmental factors on shared bicycles

We further investigated the correlation between environmental factors and the microbial community in shared bicycles. Our results revealed a weak degree of correlation between meteorological conditions and microbial community composition. Specifically, redundancy analysis (RDA) identified the two components that explained 17.85 % and 2.23 % of the variation in environmental factors affecting microbial community composition at the species level and 15.93 % and 1.70 % at the genus level (Fig. 7A and Fig. S5A). RDA also revealed no influence of microbial community with varying environmental factors such as relative humidity, UV Index, wind speed, visibility, and dew point. However, the temperature had a significant effect on the microbial community. Specifically, among these microbes, *Ralstonia* and *Blastococcus*, *Paracoccus kondratievae*, *Ralstonia sp001078575*, *Paracoccus marcusii*, and *Kocuria rosea* were the most affected by temperature (Figs. 7A). Additionally, pressure had a particular impact on several species, including *Cutibacterium acnes*, *Micrococcus luteus*, *Moraxella A cinereus*, and *Moraxella A osloensis*.

To gain further insights into the influence of environmental factors on microbial community composition at the species level, we employed network analysis. Among the top 200 bacterial species, the temperature was found to have a positive impact on 42 species and a negative impact on 28 species (Fig. 7 and Table S3). Interestingly, *Cutibacterium acnes*, the most abundant species on shared bicycles, had a negative correlation with temperature, whereas *Ralstonia sp001078575*, the third most abundant species, had a positive correlation with temperature (Fig. 7B, Fig. S5B, and Table S3). Our results suggest that temperature is a crucial environmental factor that shapes the microbial community composition on shared bicycles. The temperature explained <20 % of the overall variation. Microbial community variations before and during lockdown periods are not solely determined by temperature; anthropogenic activities may significantly influence differentiating the microbial

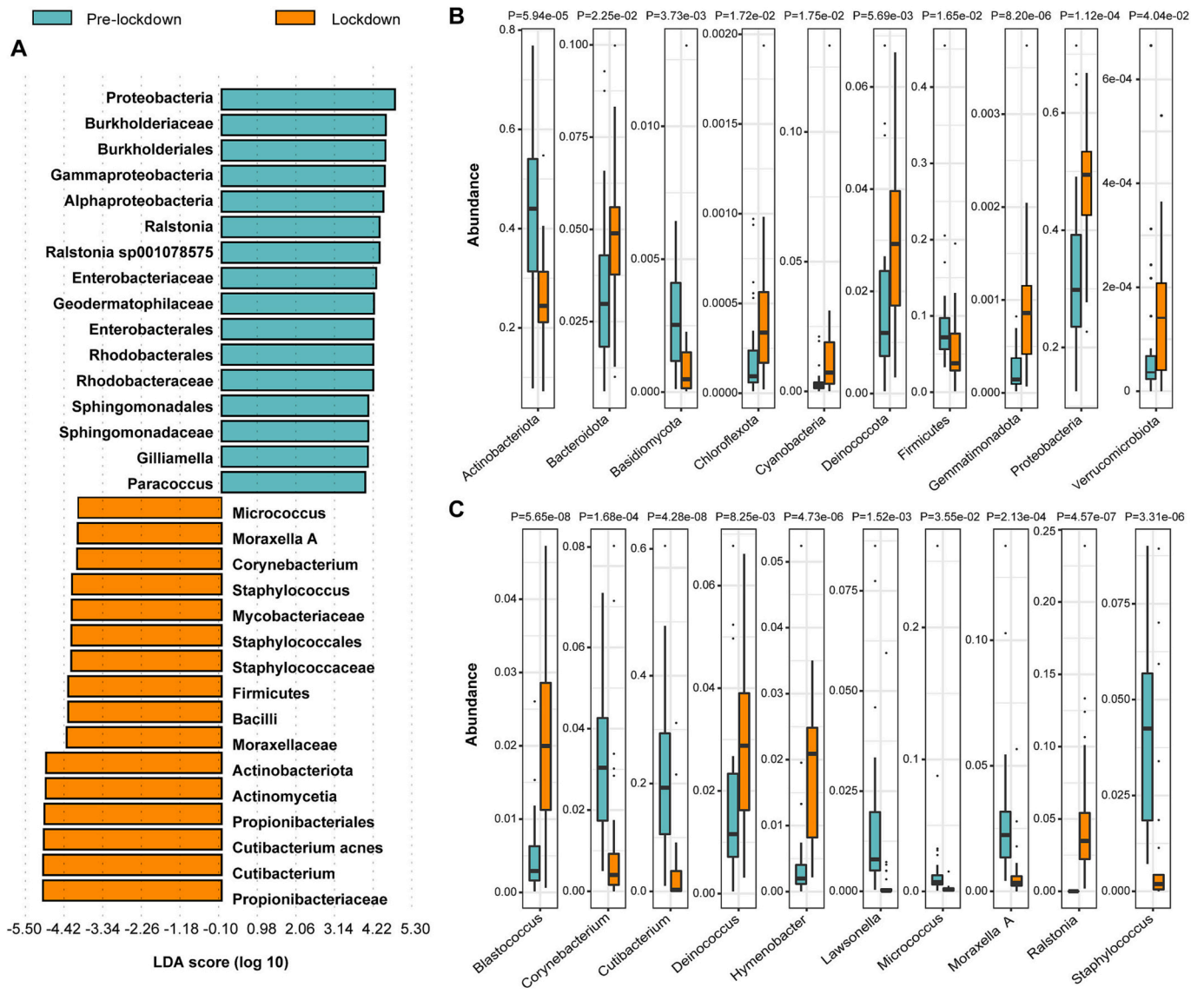


Fig. 4. Dynamics of microbial community between pre-lockdown and lockdown periods. (A) Liner discriminant analysis between pre-lockdown and lockdown periods. The abundance of dominant bacteria that exhibit significant differences at the phylum (B) and genus (C) level between the two periods.

community during these periods.

4. Discussion

To date, few studies have examined the impact of the pandemic on the abundance of potential pathogens and ARGs on shared bicycles. The extent to which lockdown measures and human activities can contribute to eliminating ARGs and pathogens remains uncertain. To address this knowledge gap, we investigated the effects of the lockdown on the composition of microbial communities and ARGs and the potential differences resulting from anthropogenic activities. Our findings provide valuable insights into the indirect impacts of lockdown on the environment and offer a comprehensive understanding of managing shared bicycles during the pandemic.

Routine monitoring and quantification of microbial communities in public transportation systems with urban environments could help to identify and mitigate potential disease risks (Qin et al., 2020). Although 16S rRNA gene amplicon sequencing has traditionally been used to estimate the composition of bacterial and archaeal communities (Johnson et al., 2019), this method typically only provides genus- or family-level sensitivity for metagenomic studies of microbial populations (Earl et al., 2018; Johnson et al., 2019). In contrast, 2bRAD-M sequencing can provide accurate taxonomic profiles at the species level (Sun et al., 2022), even for challenging samples with low microbial biomass. In this study, we investigated the microbial diversity on shared bicycles affected by the COVID-19 lockdown using 2bRAD-M sequencing, which provides sufficient taxonomic resolution and accuracy to perform at the species level. Our results showed a significant difference in bacterial community structure on shared bicycle surfaces between the pre-lockdown and lockdown periods. The dominant bacterial phyla were *Proteobacteria*, *Actinobacteriota*, *Ascomycota*, *Firmicutes*, and *Bacteroidota*, which accounted for over 95 % and 93.0 % of the total bacteria in both periods. These findings are consistent with those reported in a recent study using 16S rRNA gene sequencing (Sun et al., 2020). Notably, the human skin bacterium *Cutibacterium acnes* was present in significant amounts in all samples collected during the pre-lockdown period (21.5 %), similar to the detection rate of 19 % in the mass transit system (Guevarra et al., 2022). It is important to note that bacterial genera with high antibiotic resistance rates (Fessler and Schwarz, 2017; Lutz et al., 2014; Van Looveren et al., 2004), such as *Acinetobacter*, *Corynebacterium*, and *Staphylococcus*, maintained a higher prevalence both pre-lockdown and lockdown period. These genera have been

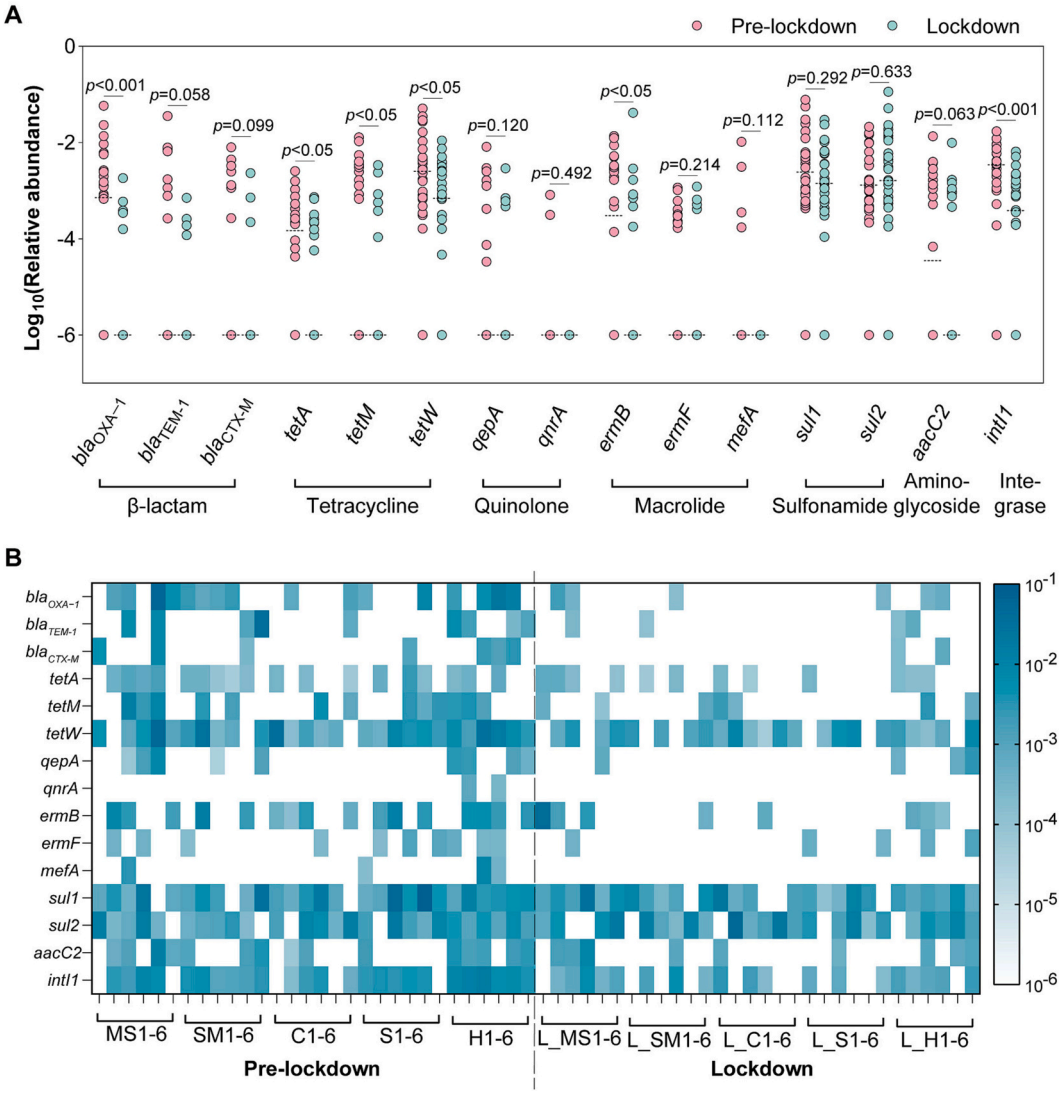


Fig. 5. ARG subtypes and *intI1* profiles on shared bicycles. (A) Relative abundances of detected ARGs and *intI1* were normalized to the 16S rRNA gene on shared bicycles. (B) Heatmap of the relative abundances of ARGs and *intI1* on shared bicycles. The color in the heatmap ranges from blackish green to white, representing the transition from high to low relative abundance.

demonstrated to spread through the environment (Huijbers et al., 2015), posing a risk of disease transmission to humans. However, the unexpectedly high abundance of bacterial species observed during the lockdown period was associated with widespread environmental bacteria typically found in plants and soil. The shifts in bacterial community composition may be due to long-term outdoor exposure and inhibition of anthropogenic activities. We acknowledge that 2bRAD-M sequencing only targets and reads the tags of enzyme-digested DNA fragments found in bacteria, archaea, and fungi (Sun et al., 2022), indicating that this method cannot capture microorganisms like the virus. Thus, investigating the microbial community as a potential reservoir of pathogens is essential. Developing a standardized and comprehensive taxonomic approach for metagenomic analysis would be beneficial in assessing the public health risks associated with shared bicycles.

The spread of antimicrobial-resistant microbes in public transportation has emerged as a major public health concern (Guevarra et al., 2022; Yao et al., 2022). Recent studies (Gu et al., 2020; Zou et al., 2019) have assessed the contamination of antimicrobial-resistant microbes on shared bicycles through culture-dependent phenotypic testing. Unfortunately, these techniques have low throughput and long turn-around time and preclude rapid screening of a large number of ARG profiles (Xiu et al., 2020). Thus, we utilized more efficient real-time qPCR assays to perform ARG profiling of shared bicycles and detect shifts in abundance and types caused by anthropogenic activities. During the lockdown, shared bicycle services were either suspended or entirely halted. Initially, there was a hypothesis that this operational adjustment could lead to a reduction in ARGs due to decreased usage and the implementation of surface disinfection practices. Our study validated this hypothesis, demonstrating a decrease in the abundance of ARGs during the lockdown period in comparison to the pre-lockdown period. The relative abundance of most detected ARG subtypes was significantly lower during the lockdown period. One possible explanation for ARGs' lower richness and diversity during the lockdown is the inhibition of anthropogenic activities that increase the types and abundances of microorganisms (Zhang et al., 2018). Additionally, the reduction in ARGs may have contributed to the increase in dust concentrations on shared bicycles during the lockdown, which generates unfavored conditions for ARB to grow. Our study also revealed a positive correlation between the diversity and abundance of ARGs and the levels of the MGE gene (i.e., the class I integron), which is consistent with previous research (Di Cesare et al., 2016). The decreased abundance of MGE also suggests that there was likely less HGT occurring among species during the lockdown period. It is crucial to acknowledge one of the limitations of our study, which focused solely on 19 ARGs associated with six antibiotic classes.

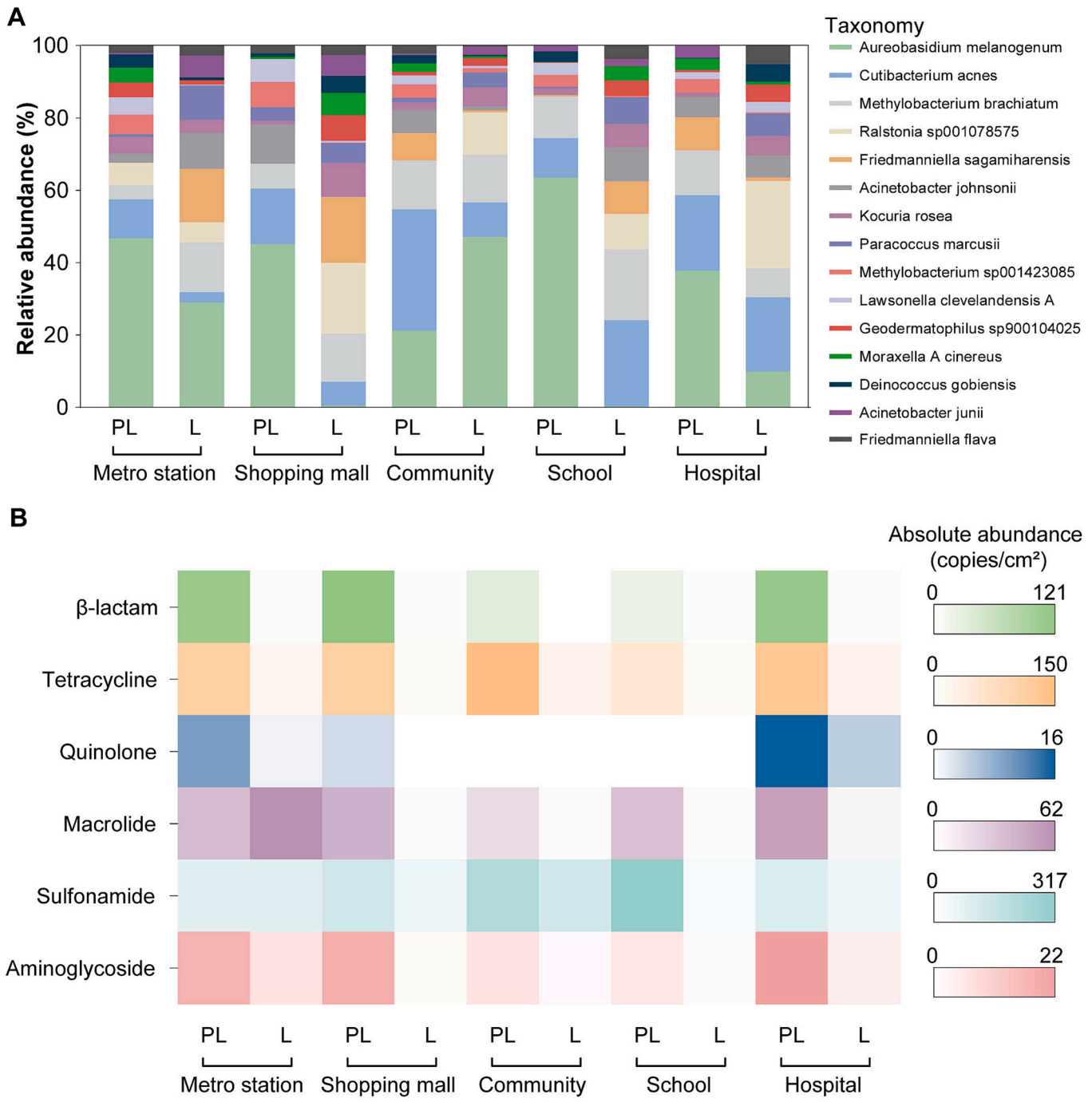


Fig. 6. Profiles of microbial communities and ARGs between sampling sites. (A) Relative abundances of detected ARGs and *intI1* were normalized to the 16S rRNA gene on shared bicycles. (B) Heatmap of the absolute abundances of ARGs and *intI1* on shared bikes. The letters PL and L represent the period of pre-lockdown and lockdown, respectively.

Consequently, further surveillance studies employing high-throughput qPCR to target a broader spectrum of ARGs are warranted. This approach would contribute to a more comprehensive understanding of the distribution patterns of ARG profiles and shed light on the influence of anthropogenic activities and intervention strategies in the context of shared bicycles.

Furthermore, our findings suggested that even with reduced human activities during the lockdown, shared bicycles continued to harbor a notable proportion of opportunistic pathogenic microorganisms. Specifically, we observed a higher prevalence of potential pathogens during the pre-lockdown period (0.035 %) compared to the lockdown period (0.029 %). Humans and the environment are major sources of these pathogenic populations on the surface of shared bicycles. Given that human activities dramatically enriched the microbial species in the pre-lockdown period, the decreased prevalence of potential pathogens during the lockdown period is not surprising. The opportunistic pathogenic microorganisms harboring ARGs, such as *Mycobacteriaceae*, *Staphylococcaceae*, and *Moraxellaceae*, which were detected in our study, have also been found to be enriched in other public transportation (Grydaki et al., 2021; Siriarchawatana et al., 2023; Zamudio et al., 2015). Our results suggest that the potentially causative agents of infectious diseases on shared bicycles can change and spread further with consecutive use. Nevertheless, conducting a pathogen surveillance study on a more extensive sampling scale would be necessary to assess the

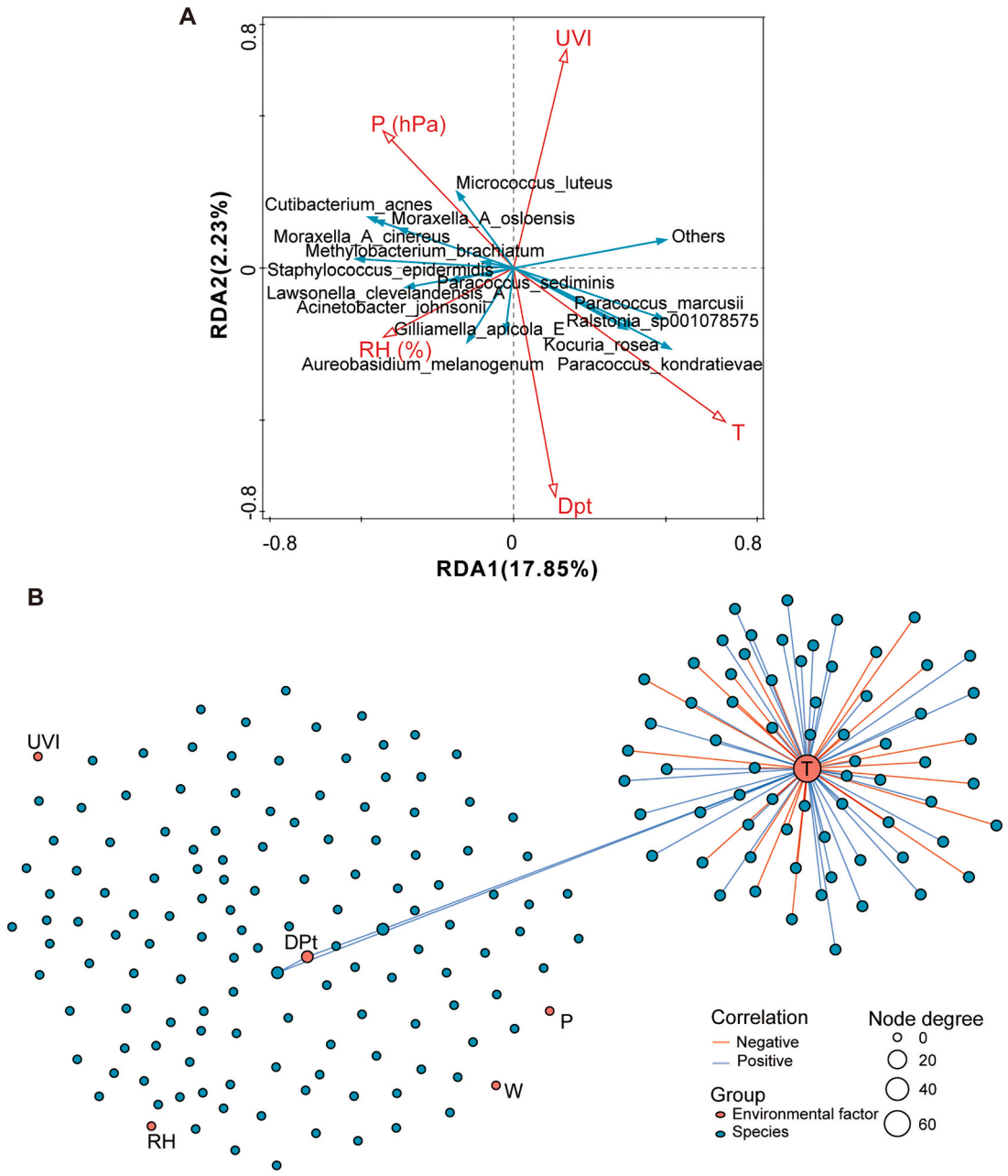


Fig. 7. Correlations between microbial community and environmental factors on shared bicycles. (A) Redundancy analysis (RDA) of microbial community at the species level and environmental factors. (B) Network analysis of the microbial community at the species and environmental factor. T: average temperature; RH: relative humidity; UVI: UV Index; W: wind speed; V: visibility; P, pressure; DPt, Dew Pt.

risks fully (Liu et al., 2020). These results hold significant implications for understanding the ecological dynamics of microbial communities and designing effective strategies to control pathogenic presence in shared bicycle systems, especially concerning the potential role in the dissemination of ARGs.

Although some recent studies (Gu et al., 2020; Sun et al., 2020; Zou et al., 2019) have investigated the concentrations of antimicrobial-resistant and pathogenic bacteria in shared bicycles, little is known about the impact of anthropogenic activities on ARGs and microbial communities on shared bicycles. Our study fills this gap by evaluating the impact of anthropogenic activities on the dynamic of ARGs and microbial communities in shared bicycles during the COVID-19 lockdown in Shanghai. Our investigation reveals significant differences in the microbial communities and ARG profiles of shared bicycles at various sampling sites. Before the lockdown, ARGs were detected across all sampling sites, indicating a widespread presence. The higher prevalence of ARGs at the metro station, shopping mall, and hospital may be linked to increased human traffic, diverse microbial sources, or unique microbial reservoirs associated with these specific environments. The prevalence of antibiotic-resistant microbes in hospital environments (Azuma et al., 2024), for instance, is well-documented, and the presence of shared bicycles in these areas may serve as a reservoir for ARGs. Interestingly, during the lockdown period, a sharp decline in the absolute abundance of all six classes of ARGs was observed at all sampling sites. This overall reduction suggests that the altered human activities during the lockdown, such as reduced usage and potential changes in cleaning practices, had a general suppressive effect on the abundance of ARGs. Despite this overall decline, relatively higher concentrations of ARGs were still noted at the metro station and hospital during the lockdown. The persistence of higher ARG levels at these locations could

be attributed to various factors, including the persistence of resistant microbial strains, local antibiotic usage patterns, or unique environmental conditions that favor the survival and dissemination of antibiotic resistance determinants. Future studies could focus more profoundly on the specific contributors to these variations, shedding light on the dynamics of antibiotic resistance in urban shared transportation systems.

Considering that the pandemic is now over, our study offers valuable insights that can guide our daily lives. As shared transportation systems resume regular use, implementing routine monitoring programs for microbial composition and ARG presence can serve as an early warning system. This allows for timely interventions in case of emerging threats, contributing to the overall safety and sustainability of shared bicycles. Public awareness campaigns on the importance of personal hygiene, especially in shared spaces, can complement these measures. Encouraging users to practice good hygiene, such as handwashing and using personal protective equipment, can further mitigate the risk of disease spread.

5. Conclusions

In this study, we provide novel evidence and quantification of the impact of lockdown practices on the dynamics of ARGs and microbial communities on shared bicycles. Although microbial abundance decreased without the consecutive use of shared bicycles during lockdown, the microbial diversity increased under the impact of restricted anthropogenic activities ($p < 0.001$). Our findings reveal that, even during lockdown, 12 out of 14 initially detected ARG subtypes persisted, indicating their tenacity in the face of reduced anthropogenic activities. Additionally, the relative abundance of potential pathogens did not decrease much (0.029 %) during the lockdown compared to the pre-lockdown (0.035 %), suggesting that these risks persist within shared bicycle systems. Notable variations in microbial communities and ARG profiles among the sampling sites have been observed, indicating that local environmental factors, human activities, and other variables contribute to the observed differences. The presence of ARGs, coupled with the ongoing prevalence of potential pathogens, requires urgent attention toward enhanced disinfection protocols and hygiene measures for shared transportation systems to mitigate the risk of disease spread, especially during periods of increased usage. Furthermore, establish routine monitoring programs to assess the microbial composition and ARGs on shared transportation, enabling early detection of emerging threats and helping implement timely interventions. Additional interventions aimed at mitigating microbial risks are imperative to ensure the safety and sustainability of shared bicycles.

Funding statement

This study was supported by grants from the China Medical Board (23-526), the School of Global Health Technological Innovation Projects (SGHKJCX2022-03), State Environmental Protection Key Laboratory of Environmental Health Impact Assessment of Emerging Contaminants (SEPKL-EHIAEC-202206) and the Natural Science Foundation of Shanghai (22ZR1436200).

CRediT authorship contribution statement

Leshan Xiu: Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Haodong Liu:** Formal analysis, Data curation. **Yi Xie:** Methodology, Formal analysis. **Qinqin Hu:** Data curation. **Huimin Li:** Software. **Fumin Chen:** Data curation. **Chenxi Wang:** Methodology. **Yuqian Zhang:** Methodology. **Liyuan Hou:** Writing – review & editing, Data curation, Conceptualization. **Kun Yin:** Writing – review & editing, Supervision, Project administration, Investigation, Conceptualization.

Declaration of competing interest

The authors have declared that no competing financial interests exist.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.169625.

References

Azuma, T., Usui, M., Hayashi, T., 2024. Inactivation of antibiotic-resistant bacteria in hospital wastewater by ozone-based advanced water treatment processes. Sci. Total Environ. 906, 167432.
Bai, H., He, L.Y., Gao, F.Z., Wu, D.L., Yao, K.S., Zhang, M., et al., 2023. Airborne antibiotic resistome and human health risk in railway stations during COVID-19 pandemic. Environ. Int. 172, 107784.
Barberan, A., Bates, S.T., Casamayor, E.O., Fierer, N., 2012. Using network analysis to explore co-occurrence patterns in soil microbial communities. ISME J. 6, 343–351.
Be, N.A., Avila-Herrera, A., Allen, J.E., Singh, N., Checinska Sielaff, A., Jaing, C., et al., 2017. Whole metagenome profiles of particulates collected from the International Space Station. Microbiome 5, 81.
Chen, Z., Deng, X., Fang, L., Sun, K., Wu, Y., Che, T., et al., 2022. Epidemiological characteristics and transmission dynamics of the outbreak caused by the SARS-CoV-2 omicron variant in Shanghai, China: a descriptive study. Lancet Reg. Health West Pac. 29, 100592.
Czatzkowska, M., Wolak, I., Harnisz, M., Korzeniewska, E., 2022. Impact of anthropogenic activities on the dissemination of ARGs in the environment—a review. Int. J. Environ. Res. Public Health 19.
De Silva, S., Ball, A.S., Shahsavari, E., Indrapala, D.V., Reichman, S.M., 2021. The effects of vehicular emissions on the activity and diversity of the roadside soil microbial community. Environ. Pollut. 277, 116744.
Delgado-Baquerizo, M., Hu, H.W., Maestre, F.T., Guerra, C.A., Eisenhauer, N., Eldridge, D.J., et al., 2022. The global distribution and environmental drivers of the soil antibiotic resistome. Microbiome 10, 219.
Di Cesare, A., Eckert, E.M., D'Urso, S., Bertoni, R., Gillan, D.C., Wattiez, R., et al., 2016. Co-occurrence of integrase 1, antibiotic and heavy metal resistance genes in municipal wastewater treatment plants. Water Res. 94, 208–214.
Earl, J.P., Adappa, N.D., Krol, J., Bhat, A.S., Balashov, S., Ehrlich, R.L., et al., 2018. Species-level bacterial community profiling of the healthy sinonasal microbiome using Pacific biosciences sequencing of full-length 16S rRNA genes. Microbiome 6, 190.
Feng, T., Han, Q., Su, W., Yu, Q., Yang, J., Li, H., 2022. Microbiota and mobile genetic elements influence antibiotic resistance genes in dust from dense urban public places. Environ. Pollut. 311, 119991.
Fessler, A.T., Schwarz, S., 2017. Antimicrobial resistance in *Corynebacterium* spp., *Arcanobacterium* spp., and *Trueperella* pyogenes. Microbiol. Spectr. 5.
Fu, C., Guo, Q., 2018. Road traffic injuries in shared bicycle riders in China. Lancet Public Health 3, e111.
Ginn, O., Nichols, D., Rocha-Melogno, L., Bivins, A., Berendes, D., Soria, F., et al., 2021. Antimicrobial resistance genes are enriched in aerosols near impacted urban surface waters in La Paz, Bolivia. Environ. Res. 194, 110730.
Grydaki, N., Colbeck, I., Mendes, L., Eleftheriadis, K., Whitby, C., 2021. Bioaerosols in the Athens Metro: metagenetic insights into the PM_{10} microbiome in a naturally ventilated subway station. Environ. Int. 146, 106186.
Gu, J., Xie, X.J., Liu, J.X., Shui, J.R., Zhang, H.Y., Feng, G.Y., et al., 2020. Prevalence and transmission of antimicrobial-resistant Staphylococci and Enterococci from shared bicycles in Chengdu, China. Sci. Total Environ. 738, 139735.
Guevarra, R.B., Hwang, J., Lee, H., Kim, H.J., Lee, Y., Danko, D., et al., 2022. Metagenomic characterization of bacterial community and antibiotic resistance genes found in the mass transit system in Seoul, South Korea. Ecotoxicol. Environ. Saf. 246, 114176.
Guo, X.P., Yang, Y., Niu, Z.S., Lu, D.P., Zhu, C.H., Feng, J.N., et al., 2019. Characteristics of microbial community indicate anthropogenic impact on the sediments along the Yangtze Estuary and its coastal area, China. Sci. Total Environ. 648, 306–314.
Guo, Y., Yang, L., Chen, Y., 2022. Bike share usage and the built environment: a review. Front. Public Health 10, 848169.
Hayes, A., May Murray, L., Catherine Stanton, I., Zhang, L., Snape, J., Hugo Gaze, W., et al., 2022. Predicting selection for antimicrobial resistance in UK wastewater and aquatic environments: ciprofloxacin poses a significant risk. Environ. Int. 169, 107488.
Huijbers, P.M., Blaak, H., de Jong, M.C., Graat, E.A., Vandenbroucke-Grauls, C.M., de Roda Husman, A.M., 2015. Role of the environment in the transmission of antimicrobial resistance to humans: a review. Environ. Sci. Technol. 49, 11993–12004.

Jiang, X., Wang, C., Guo, J., Hou, J., Guo, X., Zhang, H., et al., 2022. Global meta-analysis of airborne bacterial communities and associations with anthropogenic activities. Environ. Sci. Technol. 56, 9891–9902.
Johnson, J.S., Spakowicz, D.J., Hong, B.Y., Petersen, L.M., Demkowicz, P., Chen, L., et al., 2019. Evaluation of 16S rRNA gene sequencing for species and strain-level microbiome analysis. Nat. Commun. 10, 5029.
Karunanidhi, D., Aravinthasamy, P., Subramani, T., Setia, R., 2021. Effects of COVID-19 pandemic lockdown on microbial and metals contaminations in a part of Thirumanimuthar River, South India: a comparative health hazard perspective. J. Hazard. Mater. 416, 125909.
Kim, K., 2023. Discovering spatiotemporal usage patterns of a bike-sharing system by type of pass: a case study from Seoul. Transportation (Amst.) 1–35.
Lam, T., Chew, D., Zhao, H., Zhu, P., Zhang, L., Dai, Y., et al., 2022. Species-resolved metagenomics of kindergarten microbiomes reveal microbial admixture within sites and potential microbial hazards. Front. Microbiol. 13, 871017.
Liu, J., Zhu, Y., Jay-Russell, M., Lemay, D.G., Mills, D.A., 2020. Reservoirs of antimicrobial resistance genes in retail raw milk. Microbiome 8, 99.
Liu, Y., Wang, F., Wang, Z., Xiang, L., Fu, Y., Zhao, Z., et al., 2024. Soil properties and organochlorine compounds co-shape the microbial community structure: a case study of an obsolete site. Environ. Res. 240, 117589.
Lu, Y., Yang, J., Zhang, L., Chen, F., Han, P., Fu, Y., 2023. Characteristics of bacterial community and ARG profiles in the surface and air environments in a spacecraft assembly cleanroom. Environ. Pollut. 329, 121613.
Lutz, J.K., van Balen, J., Crawford, J.M., Wilkins 3rd, J.R., Lee, J., Nava-Hoet, R.C., et al., 2014. Methicillin-resistant *Staphylococcus aureus* in public transportation vehicles (buses): another piece to the epidemiologic puzzle. Am. J. Infect. Control 42, 1285–1290.
Qin, N., Liang, P., Wu, C., Wang, G., Xu, Q., Xiong, X., et al., 2020. Longitudinal survey of microbiome associated with particulate matter in a megacity. Genome Biol. 21, 55.
Siriarchawatana, P., Pumkaeo, P., Harnpicharnchai, P., Likhitrattanapisal, S., Mayteeworakoon, S., Boonsin, W., et al., 2023. Temporal, compositional, and functional differences in the microbiome of Bangkok subway air environment. Environ. Res. 219, 115065.
Sun, C., Yuan, T., Chen, L., Xie, Z., Shen, Z., 2020. Occurrence of potentially pathogenic bacteria on shared bicycles. Int. J. Hyg. Environ. Health 224, 113442.
Sun, Z., Huang, S., Zhu, P., Tzehau, L., Zhao, H., Lv, J., et al., 2022. Species-resolved sequencing of low-biomass or degraded microbiomes using 2bRAD-M. Genome Biol. 23, 36.
Van Looveren, M., Goossens, H., ARPAC Steering Group, 2004. Antimicrobial resistance of *Acinetobacter* spp. in Europe. Clin. Microbiol. Infect. 10, 684–704.
Wang, S., Meyer, E., McKay, J.K., Matz, M.V., 2012. 2b-RAD: a simple and flexible method for genome-wide genotyping. Nat. Methods 9, 808–810.
Xiu, L., Li, Y., Wang, F., Zhang, C., Li, Y., Zeng, Y., et al., 2020. Multiplex high-resolution melting assay for simultaneous identification of molecular markers associated with extended-spectrum cephalosporins and azithromycin resistance in *Neisseria gonorrhoeae*. J. Mol. Diagn. 22, 1344–1355.
Xu, Z., Liu, S., Chen, L., Liu, Y., Tan, L., Shen, J., et al., 2019. Antimicrobial resistance and molecular characterization of methicillin-resistant coagulase-negative staphylococci from public shared bicycles in Tianjin, China. J. Glob. Antimicrob. Resist. 19, 231–235.
Xue, Z., Han, Y., Liu, B., Gu, Y., Tian, W., Whiting-Wagner, N., et al., 2021. Bacterial diversity in ballast water and sediments revealed by 2b-RAD sequencing. Mar. Pollut. Bull. 169, 112523.
Yao, H., Jiang, J., Wang, H., Wei, T., Sangeetha, T., Sun, P., et al., 2022. An emerging unrated mobile reservoir for antibiotic resistant genes: does transportation matter to the spread. Environ. Res. 213, 113634.
Zamudio, C., Krapp, F., Choi, H.W., Shah, L., Ciampi, A., Gotuzzo, E., et al., 2015. Public transportation and tuberculosis transmission in a high incidence setting. PloS One 10, e0115230.
Zhang, K., Chen, R., Cai, Z., Hou, L., Li, X., Xu, X., et al., 2023. The effect of ozone short-term exposure on flow-mediated dilation: using data before and after COVID-19 lockdown in Shanghai. Sci. Total Environ. 881, 163485.
Zhang, S., Yang, G., Hou, S., Zhang, T., Li, Z., Liang, F., 2018. Distribution of ARGs and MGEs among glacial soil, permafrost, and sediment using metagenomic analysis. Environ. Pollut. 234, 339–346.
Zhao, J., Jin, L., Wu, D., Xie, J.W., Li, J., Fu, X.W., et al., 2022. Global airborne bacterial community-interactions with Earth's microbiomes and anthropogenic activities. Proc. Natl. Acad. Sci. U. S. A. 119, e2204465119.
Zhao, Q., Guo, W., Luo, H., Xing, C., Wang, H., Liu, B., et al., 2021. Deciphering the transfers of antibiotic resistance genes under antibiotic exposure conditions: driven by functional modules and bacterial community. Water Res. 205, 117672.
Zhou, Z.C., Liu, Y., Lin, Z.J., Shuai, X.Y., Zhu, L., Xu, L., et al., 2021. Spread of antibiotic resistance genes and microbiota in airborne particulate matter, dust, and human airways in the urban hospital. Environ. Int. 153, 106501.
Zhu, Z., Lu, C., 2023. Life cycle assessment of shared electric bicycle on greenhouse gas emissions in China. Sci. Total Environ. 860, 160546.
Zou, Z.Y., Lei, L., Chen, Q.Y., Wang, Y.Q., Cai, C., Li, W.Q., et al., 2019. Prevalence and dissemination risk of antimicrobial-resistant Enterobacteriaceae from shared bikes in Beijing, China. Environ. Int. 132, 105119.