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The impact of green spaces, urban settings, seasonal changes, and pollutants on dissemination of antimicrobial genes in air

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The dissemination of antimicrobial resistance genes (ARGs) in the air poses a significant public health challenge. Little is known about the role of vegetation in reducing the dissemination of aerosolised ARGs. DNA extracts obtained directly from the air sample were used to detect nine antimicrobial-resistant genes using qPCR and 16S rRNA sequencing. The average relative abundance of nine aerosolised ARGs (*blaTEM*, *mecA*, *sul3*, *ermB*, *ermC*, *aac(6')-Ib*, *tetM*, *tetW*, and *sul1*) detected in urban spaces with high vegetation coverage (0.0083 (ARGs/16S rRNA), was slightly lower than in those with low or no vegetation (0.0135 (ARGs/16S rRNA)) ($P > 0.05$). The relative abundance of aerosolised ARGs around urban heat islands (UHI) significantly decreased ($t = 2.06$, $P = 0.04$) after central heating was switched off. To our knowledge, this is the first study to report the relative abundance of the *mecA* gene in urban air. UHIs may be a reservoir of ARGs in urban air, hence, planting non-allergenic trees and bushes or hedgerows could be an effective way to decrease the dissemination of aerosolised ARGs. We also detected ARG reduction in high-vegetation-covered areas. Further study is required to explore the vegetation-mediated reduction of ARGs in the air.

Keywords Antimicrobial resistance genes, Aerosolised, Distribution, Dissemination, Influencing factors, Air

The rise of antimicrobial resistance (AMR) is a global challenge that threatens the lifesaving role of antibiotics, upon which modern medicine is highly dependent. The World Health Organization (WHO) considers antimicrobial resistance to be one of the most significant public health challenges[1]. AMR infections are increasing worldwide, and in many cases can only be treated by antimicrobials of last resort[2]. A recent paper published in *The Lancet* reported the most comprehensive analysis to date, based on data from 204 countries, and highlighted that there were 1.27 million deaths in 2019 due solely to AMR (> HIV/AIDS or malaria) and that AMR infections played a decisive role in the deaths of 4.95 million individuals worldwide[3].

Numerous drivers, such as the unregulated sale of antibiotics through pharmacies; poor hygiene practices in hospitals, food production and community settings; overuse of antibiotics in agriculture and livestock; and use of harsh cleaning products in public settings, have been identified as contributing to AMR selection and transmission[4–10]. Qiao et al. (2018) reviewed the current literature and showed that not only pathogens but also antimicrobial resistance genes (ARGs) are present in hospitals, solid waste landfills, sewage plants and rivers[11]. The selection and transmission of AMR is further contributed to by various vectors, including water sources, surfaces (fomite transmission) and a newly described transmission vector: the air[12,13]. The respiratory tract is the primary route through which ARGs in bioaerosols enter the human body; these bioaerosols carry both antimicrobial resistant bacteria (ARBs) and ARGs[14]. This dissemination of ARGs aggravates their transmission in the environment[15].

Antimicrobial resistance genes have been found to be widely distributed in water, soil, livestock and compost of farming and urban communities[11]but until recently their presence in and dissemination through the air has not received as much attention, although aerosolised ARGs are known to be disseminated in the air of farms,

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hospitals and wastewater treatment plants[16]. The concentration of ARGs in outdoor aerosols changes depending on the level of $PM_{2.5}$(particulate matter below 2.5 micrometres in diameter)[17], and seasonal climate[18]. However, studies investigating the impact of temperature and air quality on the dissemination of aerosolised ARGs in urban settings usually consider one of these factors in isolation, missing opportunities to understand how they interact with one another to exacerbate AMR risk[19]. Moreover, interventions suggested by such studies to decrease the dissemination of ARGs in the air, such as planting more diverse urban forests with trees selected specifically for their ability to filter ARGs[19], have not been implemented. It is widely acknowledged that planting trees is an effective intervention to improve air quality[20], however, whether such an intervention has positive effects in decreasing the dissemination of ARGs in the air in urban settings remains to be explored. Such an understanding is crucial to developing a better understanding of the phenomena, as this can help to inform urban planning policy in the crucial period to 2050, when the current rapid increases in urban growth are expected to plateau: the next two decades are a crucial period in which good urban design, which leaves space for ecosystem services, can be locked in[21,22].

In this study, a real-time sampling protocol was used to investigate the effect of vegetation coverage (by comparing high vegetation coverage sites (HV), such as gardens and parks, with low- or no vegetation sites (LNV), such as playgrounds and sites that are known to emit high levels of $PM_{2.5}$, and from urban heat islands (UHI) – urban areas where temperatures can be higher than surrounding areas due to high energy use, heat absorbent surfaces or lack of shade). We consider the effects of temperature, humidity, wind speed, bacterial community composition, and atmosphere pollutant levels on the dissemination of aerosolised ARGs. Our study expands the understanding of factors that may aid in decreasing the dissemination of ARGs through air.

Methods

Sample collection

Air samples were collected from high vegetation coverage sites (HV) (North latitude N39°10'74.56"; East longitude E117°18'43.78"), low or no-vegetation sites (LNV) (North latitude N39°10'87.18"; East longitude E117°18'42.18"), and from the outdoor environment of an urban heat island (UHI) known for its of high levels of $PM_{2.5}$[23] (North latitude N39°10'75.45"; East longitude E117°18'33.63") of Tianjin Medical University. The metrics of vegetation coverage are included in the supplementary material (Table S1). Air samples were collected from Oct 2021 to June 2022, including during autumn (Oct 2021, over 3 days), winter (from Nov to Dec 2021, over 7 days), spring (from March to May 2022, over 17 days), and in summer (Jun 2022, over 6 days).

A volume of 600 L air was collected using a Coriolis µ air sampler (Bertin Technologies) at a flow rate of 200 L/min for 3 min at 1.5 m vertical distance from the ground[24,25]. All samples were collected into 2 ml solution A, part of the genomic DNA extraction kit (Solarbio Technology Ltd, China).

Air quality

Meteorological data and air pollution levels were obtained from the air quality institutes of China (https://www.aqistudy.cn/) (Table S2). Dali Road was the nearest monitoring site (distance:1.8 km) to Tianjin Medical University. Meteorological and air pollution levels were obtained from the monitoring site located on Dali Road at the time the aerosolised samples were collected.

DNA extraction

DNA of aerosolised samples was extracted using a genomic DNA extraction kit according to the manufacturer's guidelines (Solarbio Technology Ltd, China). An ultrafine spectrophotometer (Nanodrop2000, Thermo, USA) and ultraviolet spectrophotometry method were used to measure the concentration of DNA in each sample. Genomic DNA was subsequently diluted in double distilled water to a final concentration of 200 ng/ml.

Quantification PCR

All samples were tested for nine ARGs that have previously been reported to have clinical importance[26]. These included *aac(6')-Ib* (quinolone), *ermB* (macrolide), *ermC* (macrolide), *tetM*(tetracycline), *tetW* (tetracycline), *sul1* (sulfonamides), *sul3* (sulfonamides) *blaTEM* (β-lactams) and *mecA* (β-lactams). 16S rRNA gene was used as a reference for results obtained by the quantification PCR (qPCR). All reactions were performed in triplicate. Antimicrobial resistance genes were amplified by Roche LightCycler480II. SYBRTM Green PCR Master Mix (Novo Start® SYBR qPCR Super Mix Plus, China) was used in the qPCR reaction system of a total 10 µL, including 5 µL of 2×Novo Start® SYBR qPCR Super Mix Plus, 3.5 µL of ddH_2O, 0.5 µL of each primer and 0.5 µL of templates. Melting curve analyses were automatically generated by the Light Cycler R 480 Software 1.2.1.62. $2^{-\triangle CT}$method was used for the ARGs relative abundance assessment[27] (Table S3 and S4). $\Delta C_T = C_{T(ARG)} - C_{T(16\,S\,rRNA)}$, where C_T(ARG) and C_T(16s rRNA) were the amplification threshold cycles, respectively. 16S rRNA was used for reference purposes. The relative abundance of ARGs was determined as the number of copies of genes normalised to the copy number of 16 S rRNA genes.

Identification of bacterial communities

Three replicates of aerosol DNA samples were extracted and mixed, and the V3-V4 hypervariable region of the bacteria 16 S rRNA gene was sequenced by the Illumina Miseq™/Hiseq™ platform, and the image data file was converted into the sequences through base calling (Sangon Biotech, Shanghai, China). Reads were assembled by PEAR (version 0.9.8)[28], and the sequencing quality control was assessed using PRINSEQ (version 0.20.4)[29]. Chimeric sequences were determined and filtered by UPARSE-OTU algorithm command to achieve 97% Operational Taxonomic Units (OTU) clustering (cluster_aggd command (drive5.com)). Taxonomic classification was identified by RDP 16 S database. OTU were clustered at the 97% similarity level by Usearch, and OUT was annotated by the Basic Local Alignment Search Tool (BLAST®).

Statistical analysis

The relative abundance of ARGs conformed to a normal distribution by logarithmic transformation. One-way analysis of variance (ANOVA) tests and independent t-test was conducted by R 4.1.0. software, and $P<0.05$ suggested that the difference has statistical significance. The "ggcor" package was used to analyse the correlation between the relative abundance of aerosolised ARGs and the various influencing factors. Average relative abundance was used to represent the average amount of ARGs determined in different locations, seasons and haze levels.

Results

Aerosolised samples

A total of 65 aerosolised samples were collected between Oct 2021 and Jun 2022. This included 31 samples collected from the LNV sites, 16 samples collected from the outdoor environment of the UHI site and 18 samples from the HV sites. The DNA concentrations that were extracted from aerosolised samples ranged from 0.2 to 35.4 ng/μL (Table S5).

The range of relative amount of aerosolised ARGs

Relative abundance ranges of each aerosolised ARGs subtypes were as follows: *blaTEM* (0.0007–28.5408 (ARGs/16S rRNA)), *mecA* (0.0039–4.4449 (ARGs/16S rRNA)), *sul3* (0.0002–3.9770 (ARGs/16S rRNA)), *ermB* (0.0022–0.0498 (ARGs/16S rRNA)), *ermC* (0.0004–0.1228 (ARGs/16S rRNA)), *aac(6')-Ib* (8.0476×10^{-5}–0.0491 (ARGs/16S rRNA)), *tetM* (0.0006–0.0232 (ARGs/16S rRNA)), *tetW* (9.9893×10^{-5}–0.1205 (ARGs/16S rRNA)), *sul1* (0.0007–0.0046 (ARGs/16S rRNA)) Moreover, the relative abundance of *blaTEM* and *mecA* genes were significantly higher than that of other ARGs ($F=6.126$, $P<0.001$) (Fig. 1, Table S5).

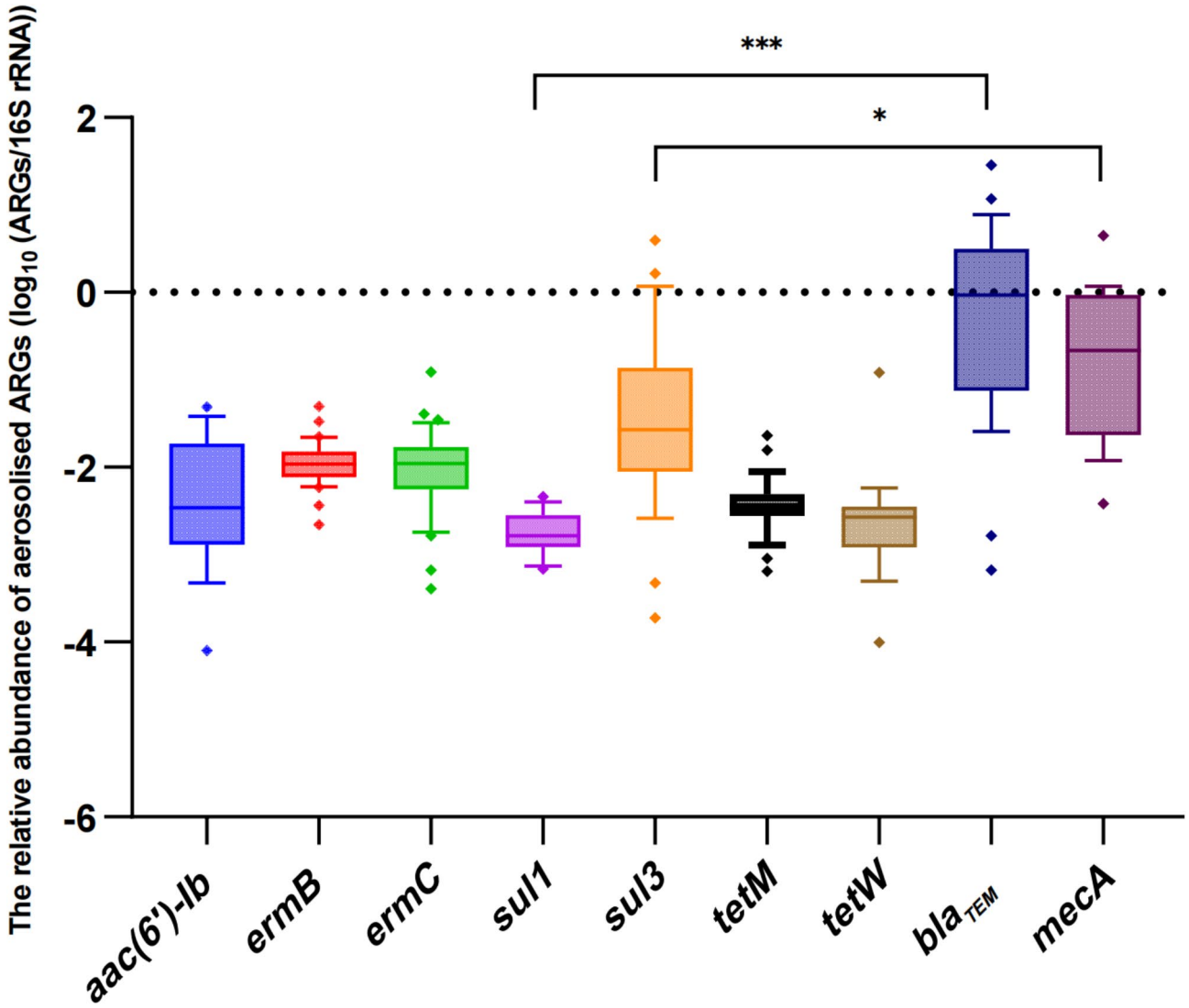

Fig. 1. Statistical analysis of the relative abundance of each ARG subtypes.

Aerosolised ARGs in different locations

We further measured the average relative abundance for each of the aerosolised ARGs in seasons requiring heating (Nov 2021-Mar 2022) and those that do not (Apr-Oct 2022) (Table 1). In the season that required heating, the average relative abundance of nine aerosolised ARGs was as follows: UHI site (0.0280 (ARGs/16S rRNA)), LNV site (0.0213 (ARGs/16S rRNA)) and HV site (0.0140 (ARGs/16S rRNA)). After central heating was switched off, the average relative abundance of 9 ARGs was as follows: LNV (0.0135 (ARGs/16S rRNA)), UHI (0.0083 (ARGs/16S rRNA)) and the HV (0.0083 (ARGs/16S rRNA)). The relative abundance of ARGs in the HV areas was slightly lower than around the UHI and LNV playground sites. However, no statistical significance was observed ($F = 1.27$, $P = 0.29$). Moreover, the relative abundance of nine aerosolised ARGs outside the UHI site was slightly higher than in the LNV and the HV sites in the heating season, but not of statistical significance ($F = 0.77$, $P = 0.46$). (Table 2; Fig. 2).

Aerosolised ARGs in different seasons

The average relative abundance of eight aerosolised ARGs in autumn, winter, spring and summer were 0.0028 (ARGs/16S rRNA), 0.0098 (ARGs/16S rRNA), 0.0161 (ARGs/16S rRNA) and 0.0097 (ARGs/16S rRNA) respectively. The relative abundance of aerosolised ARGs was found to be different during all four seasons ($F = 7.3$, $P = 0.001$). The relative abundance of aerosolised ARGs in spring exhibited significantly higher relative abundance than in autumn ($t = 6.79$, $P < 0.001$) and winter ($t = 3.73$, $P = 0.003$). Moreover, the relative abundance of aerosolised ARGs around the UHI site significantly decreased compared with the heating season (which usually runs from November to March; the non-heating season usually runs between April to October) ($t = 2.06$, $P = 0.04$). In addition, no significant reduction of ARGs was observed in the aerosolised samples collected from the LNV ($t = 0.02$, $P = 0.99$) and HV sites ($t = 1.07$, $P = 0.29$) between the heating season and the non-heating season (Table 2).

Meteorological factors and atmosphere pollution	Season, location and haze level	Average relative abundance, average (min- max) (ARGs/16s rRNA)								
		aac(6')-Ib	*ermB*	*ermC*	*sul1*	*sul3*	*tetM*	*tetW*	bla_{TEM}	*mecA*
Season	Autumn	0.0012 (0.0010–0.0015)	0.0093 (0.0037–0.0174)	0.0089 (0.0057–0.0195)	0.0018 (0.0016–0.0023)	0.0054 (0.0005–0.205)	0.0033 (0.0006–0.0232)	0.001 (0.0005–0.0025)	0.001 (0.0007–0.0017)	-
	Winter	0.0056 (0.0021–0.0347)	0.0159 (0.0077–0.0498)	0.0143 (0.0066–0.041)	0.0015 (0.0007–0.0032)	0.0082 (0.0002–1.0254)	0.0057 (0.0035–0.0157)	0.0027 (0.0012–0.0042)	0.4679 (0.1103–1.991)	-
	Spring	0.0097 (0.0033–0.0279)	0.0096 (0.0059–0.0212)	0.0101 (0.0007–0.035)	0.0046 (0.0046–0.0046)	0.0355 (0.0026–0.1425)	0.0031 (0.0022–0.0037)	0.005 (0.003–0.0184)	1.872 (0.0259–28.5408)	-
	Summer	0.0033 (0.0080–0.0491)	0.0111 (0.0022–0.0335)	0.0043 (0.0004–0.0066)	0.0017 (0.0008–0.0037)	0.3177 (0.1254–4.085)	0.0029 (0.0009–0.0057)	0.0024 (1×10^{-4}–0.0058)	0.1427 (0.03299–20.82)	-
Heating season	LNV	0.0137 (0.0033–0.0279)	0.009 (0.0059–0.0146)	0.0145 (0.00747–0.035)	0.0046 (0.0046–0.0046)	0.019 (0.0026–0.0685)	0.003 (0.002–0.004)	0.0058 (0.003–0.0184)	2.5733 (0.8281–9.5916)	0.1334 (0.0039–4.4449)
	UHI	0.0297 (0.0278–0.0318)	0.0099 (0.0074–0.0157)	0.015 (0.0086–0.0482)	0.1023 (0.0046–0.4838)	0.0348 (0.0179–0.0663)	0.003 (0.002–0.004)	0.0021 (0.0019–0.0024)	1.6376 (0.6644–5.1)	0.0667 (0.0178–1.0387)
	HV	0.0039 (0.0039–0.0039)	0.0088 (0.0062–0.0104)	0.016 (0.0073–0.0986)	0.002 (0.002–0.002)	0.0171 (0.0036–0.0442)	0.0034 (0.0023–0.0047)	0.0018 (0.0018–0.0018)	2.3394 (1.5468–3.4389)	0.0773 (0.0135–2.041)
Non-heating season	LNV	0.0033 (8.048×10^{-5}–0.0491)	0.0111 (0.0022–0.0179)	0.0043 (0.0004–0.1228)	0.0017 (0.0008–0.0037)	0.207 (0.003–4.094)	0.003 (0.0009–0.0057)	0.0025 (1×10^{-4}–0.1205)	0.2339 (0.0259–28.5408)	0.1064 (0.01351–0.9358)
	UHI	0.0027 (0.0002–0.0503)	0.0063 (0.0017–0.0122)	0.0035 (0.0007–0.0225)	0.0008 (0.0002–0.0023)	0.0844 (0.0022–0.9358)	0.0035 (0.0008–0.234)	0.0017 (0.0004–0.0045)	0.08277 (0.01439–9.1237)	0.0907 (0.0041–7.2521)
	HV	0.004 (0.0004–0.0594)	0.005 (0.0004–0.0127)	0.0039 (0.0007–0.069)	0.0014 (0.0008–0.0045)	0.0703 (0.001–0.234)	0.003 (0.001–0.006)	0.0017 (0.0002–0.0132)	0.1363 (0.0202–8.1879)	0.0335 (0.0016–0.7018)
Haze level	Non-haze day†	0.004 (8.048×10^{-5}–0.0346)	0.0107 (0.0022–0.0498)	0.0082 (0.0004–0.1228)	0.0018 (0.0007–0.0046)	0.0464 (0.0005–4.094)	0.004 (0.001–0.0232)	0.0026 (1×10^{-4}–0.1205)	0.4679 (0.0007–28.5408)	0.1351 (0.005–1.1472)
	Haze day†	0.0036 (0.0015–0.0089)	0.0123 (0.0069–0.0174)	0.0144 (0.0057–0.041)	0.0015 (0.0014–0.0016)	0.0072 (0.0002–0.0685)	0.0026 (0.0006–0.0069)	0.0022 (0.0008–0.0034)	0.2339 (0.0017–3.8834)	0.0585 (0.0039–4.3513)

Table 1. Average relative abundance of each aerosolised ARGs in different seasons, locations and haze levels. †Non-haze day: < 75 μg/m^3; Haze day: $PM_{2.5}$ ≥ 75 μg/m^3. ARGs: antimicrobial-resistant genes. -: not detected, UHI: urban heat island, HV: high vegetation coverage (garden), LNV: Low or no vegetation (playground).

Variation	ARGs, season, location and haze level	Average relative abundance of 9 ARGs (ARGs/16s rRNA)	Statistical value	*P*
Season	Autumn [a]	0.0028	*F* = 7.30	0.001
	Winter [a]	0.0098		
	Spring [a]	0.0161		
	Summer [a]	0.0098		
Heating season	LNV	0.0213	*F* = 0.77	0.46
	UHI	0.028		
	HV	0.014		
Non-heating season	LNV	0.0135	*F* = 1.27	0.29
	UHI	0.0083		
	HV	0.0083		
Heating vs. non-heating season	UHI	0.0213	*t* = 2.06	0.04
		0.0135		
	LNV	0.028	*t* = 0.02	0.99
		0.0083		
	HV	0.014	*t* = 1.07	0.29
		0.0083		
Haze level	Non-haze day† [b]	0.0142	*t* = 0.80	0.42
	Haze day† [b]	0.0097		

Table 2. Statistical analysis of the average relative abundance of nine ARGs in different locations, seasons and haze levels. ARGs: antimicrobial-resistance genes; a: the average relative abundance of eight ARGs detected in the playground; b: the average relative abundance of nine ARGs detected in the playground. †Non-haze day: < 75 μg/m^3; Haze day: $PM_{2.5}$ ≥ 75 μg/m^3, bold: the difference has statistical significance. UHI: urban heat island, HV: high vegetation coverage (garden), LNV: Low or no vegetation (playground).

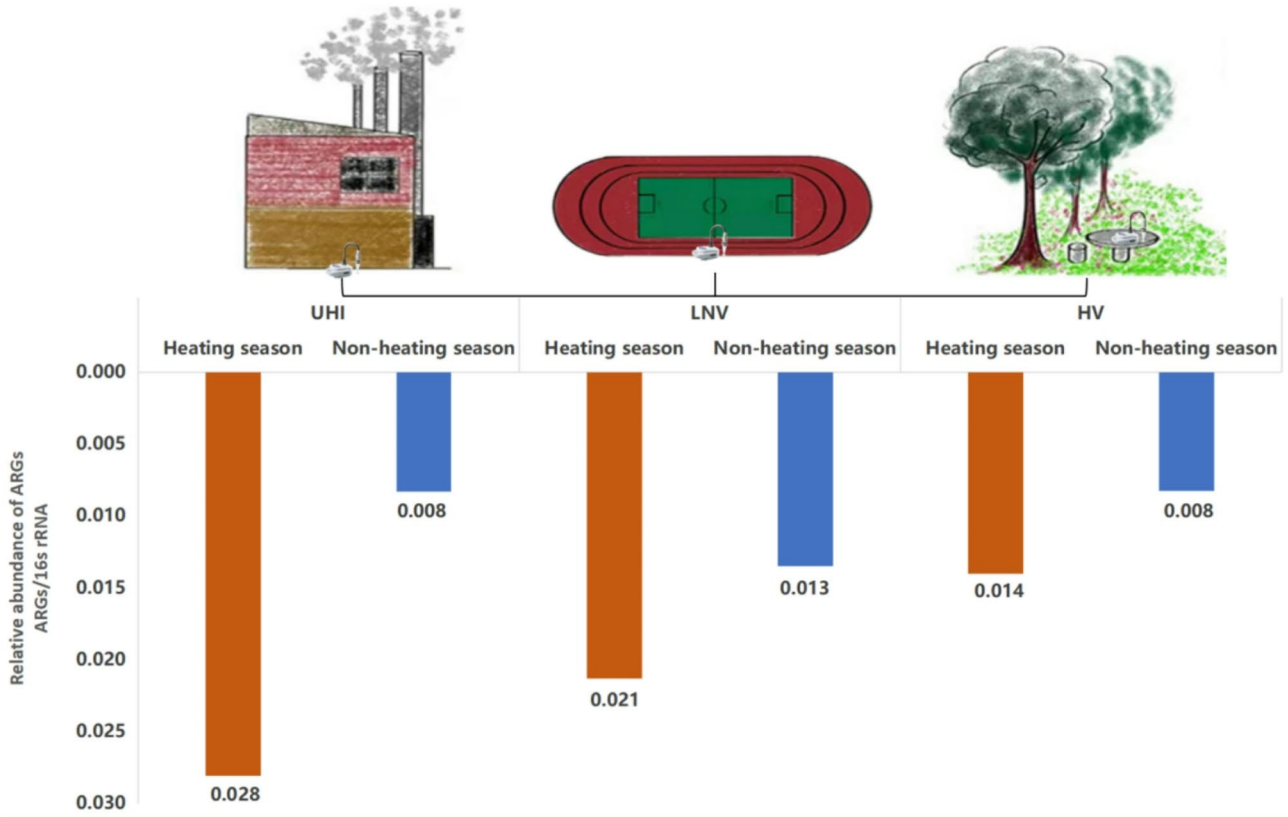

Fig. 2. The relative abundance of ARGs in different sites during the heating and non-heating season. UHI: urban heat island, HV: high vegetation coverage (garden), LNV: Low or no vegetation (playground).

Aerosolised ARGs in different temperatures and humidity

We found that the relative amount of *blaTEM* (r=−0.35, P<0.05) and *ermC* (r=−0.49, P<0.01) significantly decreased when the temperature increased (Table 3). Moreover, the relative abundance of *aac(6')-Ib*(r=−0.13, P>0.05), *ermB*(r=−0.03, P>0.05), *sul1*(r=−0.09, P>0.05), *tetM*(r=−0.26, P>0.05), *tetW*(r=−0.14, P>0.05) and *mecA*(r=−0.01, P>0.05) were negatively correlated with temperature. In contrast, *sul3*(r=0.38, P<0.05) was positively correlated with temperature.

We found that the relative amount of *blaTEM* and humidity showed a significantly positive correlation (r=0.4, P<0.05) (Table 3, Figure S1). In contrast, *ermB* (r=−0.11, P>0.05), *tetM*(r=−0.31, P>0.05), *tetW*(r=−0.02, P>0.05) and *mecA*(r=−0.04, P>0.05) decreased with increased humidity. Moreover, *aac(6')-Ib*(r=0.03, P>0.05), *ermC*(r=0.23, P>0.05), *sul1*(r=0.29, P>0.05), and *sul3*(r=0.18, P>0.05) increased with increasing humidity.

Aerosolised ARGs on haze and non-haze days

According to a previous report[13], haze days are defined as $PM_{2.5} \geq 75$ μg/m^3, while non-haze days are defined as $PM_{2.5} < 75$ μg/m^3. The average relative abundance of nine aerosolised ARGs on haze days (0.0097 (ARGs/16S rRNA)) was lower than that on non-haze days (0.0142 (ARGs/16S rRNA)). We found that *ermB*(r=0.13), *ermC*(r=0.11) and *sul1*(r=0.05) were positively correlated with the concentration of $PM_{2.5}$, but no statistical significance was observed (P>0.05). In contrast, we observed that *aac(6')-Ib*(r=−0.05), *sul3*(r=−0.20), *tetM*(r=−0.30), *tetW*(r=−0.11), *blaTEM*(r=−0.08) and *mecA*(r=−0.02) were negatively correlated with $PM_{2.5}$ concentration, but no statistical significance was identified (t=0.80, P=0.42) (Table 2 and S6, Figure S2).

Aerosolised ARGs in different wind speed

We found that the relative amount of *aac(6')-Ib*(r=−0.19, P>0.05), *ermB*(r=−0.02, P>0.05), *ermC*(r=−0.27, P>0.05), *sul1*(r=−0.32, P>0.05), *sul3*(r=−0.10, P>0.05), *tetM*(r=−0.10, P>0.05), *tetW*(r=−0.15, P>0.05) and *blaTEM*(r=−0.16, P>0.05) slightly decreased with increased wind speed (Table 3). Moreover, the relative abundance of the *mecA* (r=0.18, P>0.05) gene was increased with elevated wind speed.

Aerosolised ARGs in different concentrations of ozone, NO_2 and SO_2

aac(6')-Ib(r=−0.03, P>0.05), *sul3*(r=−0.40, P<0.05), *tetM*(r=−0.21, P>0.05), *tetW*(r=−0.17, P>0.05), *blaTEM*(r=−0.24, P>0.05) and *mecA*(r=−0.16, P>0.05) were negatively correlated with NO_2. In addition, *sul1*(r=−0.12), *sul3*(r=−0.22), *tetM*(r=−0.18), *blaTEM*(r=−0.05) and *mecA*(r=−0.19) were negatively correlated with SO_2, However, the *ermB*(r=0.20, P>0.05) and *ermC*(r=0.22, P>0.05) were positively correlated with NO_2, and *aac(6')-Ib*(r=0.18, P>0.05), *ermB*(r=0.01, P>0.05), *ermC*(r=0.06, P>0.05) and *tetW*(r=0.10, P>0.05) were positively correlated with SO_2. In addition, the relative abundance of *ermB*(r=−0.13, P>0.05), *ermC*(r=−0.46, P<0.01), *tetM*(r=−0.18, P>0.05) and *blaTEM*(r=−0.07, P>0.05) were negatively correlated with ozone concentration (data obtained from Deli Road). In contrast, the relative abundance of *aac(6')-Ib*(r=0.14, P>0.05), *sul1*(r=0.01 P>0.05), *sul3*(r=0.42, P<0.05), *tetW*(r=0.31 P>0.05) and *mecA*(r=0.14, P>0.05) were all positively correlated with ozone concentration.

Microbial taxa

The quantification of bacterial communities for different locations and seasons resulted in identification of 52 bacterial genera (Table S7). The dominant genera identified in the HV sites were *Lactobacillus* and *Lactococcus*. The main genera identified in spring and summer were *Candidatus* and *Vibrio*, whereas *Lactobacillus* and *Lactococcus* were the main species detected in autumn. Moreover, *Lactobacillus*, *Staphylococcus* and *Lactococcus* showed the highest relative abundance in winter (Fig. 3, Table S7).

Discussion

In this study, the varied relative abundance of aerosolised antimicrobial resistance genes was determined in different types of location (LNV, HV and UHI), seasons, temperature, humidity, haze levels, wind speeds, and concentrations of $PM_{2.5}$, ozone, NO_2 and SO_2 etc. We found that the relative abundance of aerosolised ARGs was different at different vegetation coverage sites. Seasonal variation of relative abundance of ARGs in air was determined and, we discussed the impact of various factors on the relative abundance of ARGs in air, showing that the relative abundance is multifactorial.

Variables	*aac(6')-Ib*	*ermB*	*ermC*	*sul1*	*sul3*	*tetM*	*tetW*	bla_{TEM}	*mecA*
T	−0.13	−0.03	**−0.49****	−0.09	**0.38***	−0.26	−0.14	**−0.35***	−0.01
RH	0.03	−0.11	0.23	0.29	0.18	−0.31	−0.02	**0.40***	−0.04
WS	−0.19	−0.02	−0.27	−0.32	−0.10	−0.10	−0.15	−0.16	0.18
$PM_{2.5}$	−0.05	0.13	0.11	0.05	−0.20	−0.30	−0.11	−0.08	−0.02
NO_2	−0.03	0.20	0.22	0.00	**−0.40***	−0.21	−0.17	−0.24	−0.16
SO_2	0.18	0.01	0.06	−0.12	−0.22	−0.18	0.10	−0.05	−0.19
O_3	0.14	−0.13	**−0.46****	0.01	**0.42***	−0.18	0.31	−0.07	0.14

Table 3. Correlation matrix of the analysed parameters. - Negative correlated; *Correlation significant at the 0.05 level; **Correlation significant at the 0.01 level; T: temperature; RH: relative humidity; WS: wind speed; ARGs: antimicrobial-resistance genes.

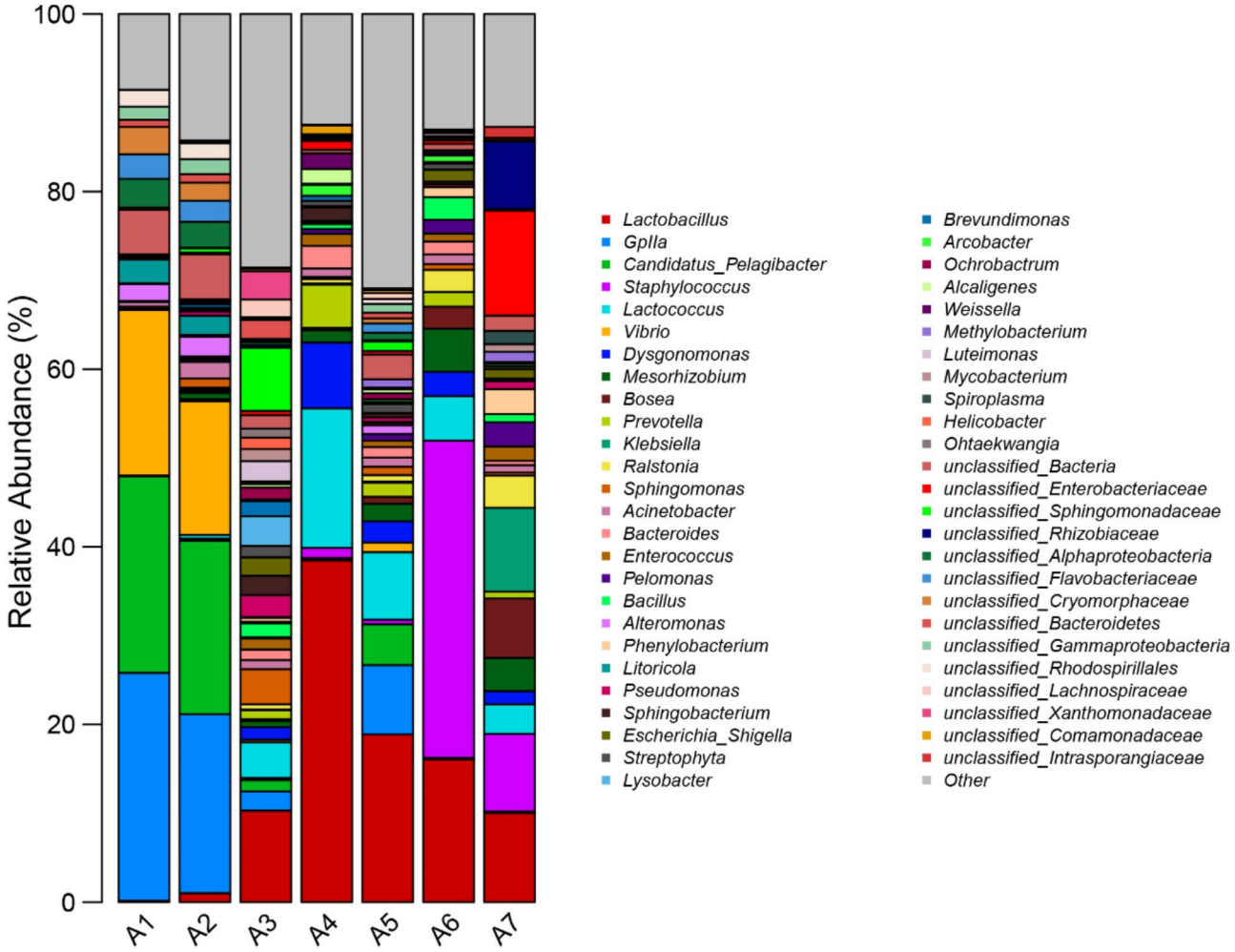

Fig. 3. Diversity and Relative abundance of bacteria in each sample. Note: A1 playground: non-green space (playground) LNV (Spring); A2 playground (summer); A3 urban heating island: UHI (summer); A4 high vegetation coverage (garden): HV (summer); A5 playground: (Autumn); A6 playground (Winter); A7 Intra Hospital.

The relative abundance of the aerosolised ARGs during the study period ranged from 1.3413×10^{-4}–47.57 (ARGs/16S rRNA)/m^3. The lowest amount of ARGs was lower than that previously reported by Zhang et al. (2023) (3.85×10^{-3} (ARGs/16S rRNA)/m^3)[17] but in contrast, the highest amount of ARGs was higher than in Zhang's report (0.014315 (ARGs/16S rRNA)/m^3)[17]. We therefore hypothesise that Coriolis air sampler, used in our study but not in Zhang's, is more sensitive for the detection of ARGs in air samples. This may well be due to the fact that it uses a cyclonic approach to collect and concentrate biological particles into a liquid. We showed that the relative abundance of *blaTEM* and *mecA* genes was significantly higher than that of other ARGs. Our findings were consistent with previous studies that reported the *blaTEM*gene to be the dominant ARG in the urban air of Tianjin and Beijing[30,31]. Moreover, *blaTEM* has also determined to be the most dominant ARG identified in the air of the inner hospital environment[32]. Although Adams et al. (2020) reported that the *mecA*gene could spread via air in the hospital environment[33], no relative abundance of the *mecA* gene in aerosol in the urban air has been reported previously. To our knowledge, this is the first study to report the relative abundance of the *mecA* gene in urban air. The potential risk of dissemination of ARGs from hospitals to the surrounding areas by air is apparent due to *blaTEM* and *mecA*genes being identified both in hospital and urban areas[32,33]. In this study, the average relative abundance of the eight ARGs detected in the playground was significantly elevated in the spring, surpassing levels observed in both autumn and winter. In addition, the average relative abundance of nine ARGs in the vicinity of the UHI site exhibited a significant decline during the non-heating season compared to the heating season. The relative abundance of *blaTEM* was significantly reduced with rising temperatures and increased humidity.

Our results showed that after the central heating was switched off, the relative abundance of aerosolised ARGs around the UHI site significantly decreased. In contrast, no significant reduction of the ARGs was observed in the aerosolised samples collected from the LNV and HV sites between the heating season (Nov to March) and non-heating season (April to Oct). Therefore, UHI sites may be a potential reservoir of ARGs in urban air during the heating season. Previous studies have shown that methicillin-resistant *Staphylococcus aureus*(MRSA)

outbreaks in hospitals in the UK often occurred in October, when the central heating was switched on and hot air currents disseminated bacteria living in heating vents/behind radiators[34]; the findings of this study suggest this effect may also be happening at a citywide level. In addition during this time, released smoke dust may well act as carriers for ARGs[14], and thus contribute to the increased amount of ARGs.

It has been reported that trees can reduce human exposure to particulate matter (PM) from traffic[35,36]. Although the relative abundance of ARGs in the HV was slightly lower than the UHI and LNV, the difference was not statistically significant; however, the reduction of ARGs in HVs may reduce human exposure to ARGs. Thus, planting trees, bushes and hedgerows may be an effective way to reduce the dissemination of aerosolised ARGs, though non-allergenic vegetation must be chosen, as the impact of allergens including pollen on human health need to be considered. We hypothesize that such phenomenon may be due to the ability of plants reduce the particle pollution in the air[37], consequently reducing ARGs that were carried by dust in that air. We found that the Operational Taxonomic Unit (OTU) abundance of *Lactobacillus* and *Lactococcus* accounted for more than 50% of the total bacteria in the HV site, which was higher than that identified in the UHI site and LNV site (Table S7). *Lactobacillus*is plant-associated lactic acid bacteria and is essential for the tolerance of plants against biotic and abiotic stress[38]. Moreover, it has previously been reported that *Lactobacillus* and *Lactococcus spp.*showed antibacterial properties against antimicrobial-resistant bacteria (ARB)[39,40]. Therefore, the dominance of *Lactobacillus* and *Lactococcus* in the air may inhibit the survival and propagation of ARB and thus contribute to the lowest relative abundance of ARGs in the HV areas.

The dominant ARGs subtype varied in different seasons. Quinolone (*aac(6')-Ib*), macrolide (*ermB*, *ermC*), tetracycline (*tetM*, *tetW*), sulfonamides (*sul1*, *sul3*), and β-lactams (*blaTEM*, *mecA*) are antimicrobial drugs that are commonly used in the clinic and thus the presence of these free antimicrobial resistance genes aggravated the horizontal antibiotic-resistant gene transfer in the environment[15]. The *ermB* gene was the dominant ARGs in autumn, *blaTEM* was the most dominant gene in winter and spring, and *sul3*was the main ARGs that was detected in summer. This is consistent with previous studies, in which seasonal variations of aerosolised ARGs have also been observed[18,41]. *Cyanobacteria*, *Candidatus* and *Vibiro* accounted for 66% and 55% of total bacteria found in aerosolised samples of spring and summer respectively, however, the relative amount of *Cyanobacteria*, *Candidatus* and *Vibiro* reduced dramatically in autumn and winter samples. *Candidatus* and *Vibiro*were known to be carriers of multiple ARGs[42,43]. In contrast, *Lactobacillus* and *Lactococcus* were barely found in aerosolised samples of spring and summer, and the relative abundance of *Lactobacillus* and *Lactococcus* increased up to 26.5% and 21% respectively of the total bacteria detected in the aerosol samples of autumn and winter (Table S7). Increased *Lactobacillus* and *Lactococcus*in autumn and winter might be due to the changes of plant fermentation processes in autumn and winter[44]. The relative abundance of dominant bacterial species varied between seasons, consistent with previously published studies[18] but the bacterial species found to be dominant during each season were different from those reported previously; this may be associated with the sampling procedure, i.e. the height and methods used.

In this study, *blaTEM* and *ermC*were negatively correlated to temperature. The findings were consistent with previously published works which have also reported a negative correlation between ARGs and temperature[45]. Li et al. also demonstrated that temperature rise could affect the growth of bacteria, and thus result in a decrease in ARGs[46]. In this study, *sul3* was significantly positively correlated with temperature, and *blaTEM* and humidity showed a significantly positive correlation. In general, when the temperature was high and humidity was low, the relative abundance of aerosolised ARGs was low but when the temperature and humidity were low, the relative abundance of ARGs gradually increased. When the humidity was high and the temperature was low, the relative abundance of ARGs increased dramatically. When the temperature was high and humidity was low, the relative abundance of the total ARGs gradually decreased. Therefore, attention should be paid to the monitoring of aerosolised ARGs in low temperature and high-humidity environments. The relationship of these climate conditions to the levels of ARGs present in the air should also be considered alongside emerging novel understandings of disease risk under different climate conditions, for example, high temperature and humidity are correlated with increased risk of septicaemia, anthrax, and black quarter outbreaks in livestock[12]. The levels of ARGs in the air may be influencing the levels of disease risk observed.

In contrast with a previously published study in which the authors demonstrated that wind speed reduced the relative abundance of ARGs[45], no significant decrease of *aac(6')-Ib*, *ermB*, *ermC*, *sul1*, *sul3*, *tetM*, *tetW* and *blaTEM*was observed with increased wind speed. Bai et al. reported that wind promotes the transmission of aerosolised ARGs from hotspots to the surrounding environment[47]. Therefore, the increased relative abundance of the *mecA* gene in this study might be due to the wind-associated transmission from the hotspot. An air filter system for hot areas might be an effective way to eliminate the emission of ARGs from the hotspot into the surrounding environment.

A number of previously published studies reported that atmospheric particulate matter (PM) can be a carrier of aerosolised ARGs, and thus promote ARGs dissemination[14,30,48]. Moreover, it has been reported that $PM_{2.5}$ can impact the dissemination of ARGs due to low levels of integron on hazy days[49], which may explain the negative correlation between ARGs and $PM_{2.5}$. However, no statistically significant association between $PM_{2.5}$ and ARGs was observed.

It has been reported that ARGs may be destroyed by NO^{3-}, and SO_4^{2-}[49], while other studies report that antimicrobial-resistant bacteria might acquire their nutrients from SO_2 and NO_2 [30]. In this study, we found that *sul3* was significantly decreased with increased concentration of NO_2. However, no statistically significant association between NO_2 and other ARGs was observed. The oxidation effect of O_3 might reduce ARGs in the environment[50]. In contrast with a previous study[50], we found that *sul3* significantly decreased with a reduced concentration of ozone. However, no statistically significant association between ozone and other ARGs was observed. Therefore, the influence of air pollutants on the dissemination of aerosolised ARGs was complex due to multiple factors that affected the relative abundance of aerosolised ARGs in combination as well as

individually. Further studies are warranted to fully investigate the impact of these factors and their intersection on the dissemination of aerosolised ARGs (Table 3).

Although we observed that HV could reduce the amount of ARGs, further study is necessary to confirm this result due to the small sample size of this study. Moreover, the collected air samples in this study were approximately equal to the amount of air inhaled by an individual in a single breath[51]. In addition, we found an even higher relative abundance of ARGs/m^3 compared with the traditional methods (i.e. PM sampler), which suggested that the Coriolis air sampler may well be a more effective method for studying ARGs in the air.

Conclusion

In conclusion, compared with autumn and winter, the average relative abundance of the ARGs detected in the playground was significantly elevated in spring. Moreover, we observed a reduction of ARGs in high vegetation coverage sites. Therefore, planting non-allergenic trees, bushes, and hedgerows may be an alternative way to reduce the dissemination of ARGs through the air. We also identified that the relative abundance of aerosolised ARGs around the UHI site was significantly reduced when central heating was switched off.The relative abundance of bla_{TEM} demonstrated a significant reduction with rising temperatures, yet experienced an increase with the elevation of humidity. The consideration of the impact of different environmental and climate conditions, and the use of climate change risk mapping tools to predict increased risk during specific weather conditions, could help public health offices to plan communication campaigns and predict when increased cases of resistant infections are likely to occur.

Data availability

Sequence data that support the findings of this study have been deposited in the Genome Sequence Archive with the primary accession code CRA019942, which is publicly accessible at https://bigd.big.ac.cn/gsa/browse/CRA019942.To request the data from this study, Zhen Xu (xuzhen@tmu.edu.cn) could be contacted.

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Competing interests

The authors declare no competing interests.

Ethical approval

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Consent to participate

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Consent to publish

The authors confirm that it is not under consideration for publication elsewhere.

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