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RESEARCH ARTICLE

Optimized determination of airborne tetracycline resistance genes in laboratory atmosphere

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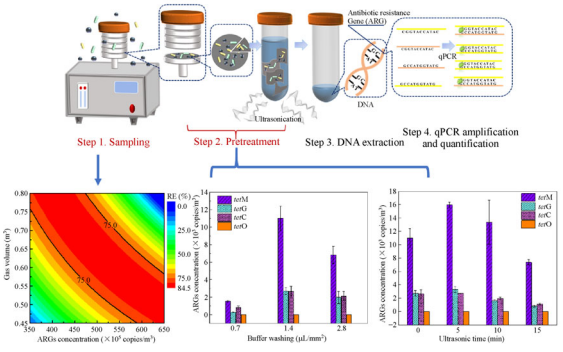
HIGHLIGHTS

- Sampling parameters with high efficiency was determined.
- Operational process to detect airborne ARGs was optimized.
- Providing research basis to control airborne ARGs of a laboratory atmosphere

GRAPHIC ABSTRACT



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ABSTRACT

Antibiotic resistance genes (ARGs) have been detected in various atmospheric environments. Airborne ARGs transmission presents the public health threat. However, it is very difficult to quantify airborne ARGs because of the limited availability of collectable airborne particulate matter and the low biological content of samples. In this study, an optimized protocol for collecting and detecting airborne ARGs was presented. Experimental results showed that recovery efficiency tended to increase initially and then declined over time, and a range of 550–780 copies/mm^2 of capture loading was recommended to ensure that the recovery efficiency is greater than 75%. As the cell walls were mechanically disrupted and nucleic acids were released, the buffer wash protects ARGs dissolution. Three ratios of buffer volume to membrane area in buffer wash were compared. The highest concentrations of airborne ARGs were detected with 1.4 μL/mm^2 buffer wash. Furthermore, the majority of the cells were disrupted by an ultrasonication pretreatment (5 min), allowing the efficiency ARGs detection of airborne samples. While, extending the ultrasonication can disrupt cell structures and gene sequence was broken down into fragments. Therefore, this study could provide a theoretical basis for the efficient filter collection of airborne ARGs in different environments. An optimized sampling method was proposed that the buffer wash was 1.4 μL/mm^2 and the ultrasonication duration was 5 min. The indoor airborne ARGs were examined in accordance with the improved protocol in two laboratories. The result demonstrated that airborne ARGs in an indoor laboratory atmosphere could pose the considerable health risk to inhabitants and we should pay attention to some complicated indoor air environment.

1 Introduction

Antibiotic resistance genes (ARGs), a portion of DNA carrying genetic information encoding resistant to specific substances such as antibiotic, are known as emerging environmental contaminants that pose a threat to public health. Antibiotics are used to treat diseases and promote animal growth. However, misuse and overuse of antibiotics lead to the emergence of great number of resistant bacteria with ARGs. Pathogens in hospital environments are frequently found to be resistant, which could pose a significant health risk to human (Nguyen et al., 2019). In comparison with traditional chemical pollutants, ARGs can be transferred and spread between bacteria of the same

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or different species through mobile genetic elements. These genes are also persistent in the environment and have become a worldwide public health issue (Wang et al., 2019b; Zhang et al., 2019).

Table 1 summarizes the common resistance genes reported in literature. ARGs with resistance to sulfonamides (*sul*) and tetracycline (*tet*) have been widely detected in various environmental media. Moreover, ARGs have been found in a wide range of environmental matrices, including sediments, soils, lakes, rivers, surface water, air, and indoor environments. In studies on ARGs in rivers, 27 types of ARGs, mainly including *sul* and *tet* resistance genes, are detected and have four main sources, namely, WWTP, human waste, aquaculture, and pharmaceutical production wastewater. Sediment samples collected from the Yangtze River Estuary have high concentrations of ARGs, conferring resistant to sulfanilamides and tetracyclines (Lin et al., 2015; Guo et al., 2018). The types of ARGs found in sediments and soil are similar to those in aquatic environments, and they are detected at higher concentrations (Fernando et al., 2016; Cheng and Hong, 2017). However, studies on airborne ARGs are limited.

Airborne ARGs have unique biological activities, persistence, and replicability via conjugation, transduction and transformation. They (ARGs) are more toxic than ordinary particulate matter and have a major effect on human or animal health after they invade the human or animal respiratory tract. For example, ARGs in human pathogens can cause treatment failure, prolong the duration of illnesses and increase mortality rates, exacting high human and economic costs to society (Friedman et al., 2016). Unlike resistance genes in water and soil, airborne ARGs cannot be directly analyzed and detected partly because of the limited availability of collectable airborne particulate matter, the low biological content of samples, and the incompatibility of traditional DNA extraction methods with airborne particulate matter collection methods.

The characteristics of current collection and quantitative methods for ARGs are respectively summarized in Tables S1 and S2. The solid impact sampling method makes samples proliferate, thus, makes it impossible to quantify ARGs concentration. The liquid capture sampling method is only for short-term collection of samples (about 10 min), which makes it impossible to monitor low airborne ARGs concentrations. Filtering sampling resolves this problem in culture method and slightly decreases sample loss caused by impact sampling. In addition, the filtering sampling can achieve the enrichment of low concentration airborne ARGs by extending the sampling time. Quantitative real-time polymerase chain reaction (q-PCR) shows many advantages compared to other quantitative methods of

Table 1 Types and concentrations of ARGs in different environments

Environmental Media	Country	Source type	Target gene	References
River	Spain	WWTP	*sul*1(5.0×10^3 copies/16S rDNA gene copies)	Marti et al. (2013)
River	USA	WWTP	*tet*A(6.3×10^2 copies/mL) *tet*X(1.2×10^3 copies/mL)	Lapara et al. (2011)
Surface water/river	Spain	Human waste	*tet*M *tet*O *tet*Q *tet*W *qnr*D *qnr*S *qep*A *oqx*A *and oqx*B (data not reported)	Rodriguez-Mozaz et al. (2015)
Surface water/river	Spain	WWTP	*bla*TEM *bla*CTX-M *bla*SHV(data not reported)	Sidrach-Cardona et al. (2014)
Surface water/river	Canada	WWTP	sul1 sul2 tetO qnrS(data not reported)	Hayward et al. (2018)
Vidy Bay sediments	Switzerland	WWTP	*sul*1(2.2×10^9 copies/g) *tet*B(1.5×10^6 copies/g)	Czekalski et al. (2014)
Rubbish	China	Urban area	*sul*1($(9.3\pm0.1) \times 10^6$ copies/g) *sul*2($(3.7\pm0.1) \times 10^8$ copies/g) *tet*W($(2.3\pm0.1) \times 10^5$ copies/g)	Li et al. (2015)
Soil	USA	Human waste	*tet*Z(5.9×10^9 copies/ng)	Hong et al. (2013)
Biological organ	Italy	Antibiotic	ystA ystB(data not reported)	Fois et al. (2018)
Biological	USA; Norway	Medicine and farming	strA-strB(data not reported)	Ludvigsen et al. (2018)
Biological	Poland	Fish processing plant	actA fbpA hlyA plcA plcB prfA (data not reported)	Skowron et al. (2018)
Air	USA	Terrestrial agriculture	tetO tetM tetW tetQ(data not reported)	Mceachran et al. (2015)
Air	USA	Indoor environments	*tet*X($1.0 \times 10^2 - 2.0 \times 10^2$ copies/m^3) *tet*W($1.0 \times 10^2 - 4.0 \times 10^2$ copies/m^3)	Ling et al. (2013)
Air	USA	Urban parks	sul1(10^3 copies/m^3)	Echeverria-Palencia et al. (2017)
Air	South Africa	Indoor environments	*sul*1(1.6×10^{-3} copies/m^3)	Pal et al. (2016)
Air	China	Indoor environments	*qep*A (0.3 ± 0.1 copies/16S rRNA gene copies) *bla*TEM (0.2 ± 0.1 copies/16S rRNA gene copies)	Li et al. (2018)

gene concentration, such as high sensitivity, good accuracy, safe and fast process. The protocol in this study is the combination of filtering sampling and qPCR, which can provide insights into environmental resistance genes.

In this study, we developed and applied an optimized protocol to collect and detect airborne ARGs. In this protocol, we integrated several key modifications into the steps of airborne sample collection, sample pretreatment, DNA extraction, and quantification. We showed that such modifications and optimization greatly improved the collection and DNA extraction efficiency of airborne samples. We applied this protocol to study airborne tetracycline resistance genes in an indoor laboratory atmosphere, where we extracted DNA from airborne microbes for ARGs detection. The laboratory environmental quality affects the physical status of researchers because of the experimental work (Kim et al., 2018). We also explored the different characteristics of airborne tetracycline resistance genes in two laboratories.

2 Materials and methods

2.1 Generation of airborne *Escherichia coli*

The schematic of the experimental protocol is presented in Fig. S1 (SI). *Escherichia coli* (CMCC1.3373) was cultured in 100 mL of nutrient broth (Wang et al., 2019a. Table S3) containing 7 μg/L tetracycline at 37°C for 3 days. *E. coli* harbouring tetracycline resistance genes (*tet*R) that grown in a medium supplemented with tetracycline were analyzed through qPCR to detection these genes. *E. coli* containing *tet*R was cultured, enriched, and used to prepare a bacterial suspension. An aerosol generator was utilized to generate airborne *E. coli* containing *tet*R. The airborne *E. coli* were used as standard samples at a concentration of approximately 8.0×10^5 copies/m^3. The inlet of sampler and the outlet of bioaerosol generator was connected with a rubber tube. The low-volume sampler (5 L/min) had glass microfiber filters. All of the samples were treated immediately or stored at −80°C before DNA was extracted (Jiang et al., 2015).

2.2 Operating protocol for airborne ARG sampling and detecting

Figure 1 shows the schematic of the experimental approach. After filter sampling was completed (Step 1), a pretreatment (Step 2) workflow was conducted. In this step, glass microfiber filters were cut into small pieces and ultrasonicated. Then, DNA was extracted (Step 3), and qPCR amplification and quantification were carried out (Step 4). The protocol was further described in detail as follows.

Step 1. Filter sampling

Filter sampling has several advantages, including high capture efficiency, simple operation, and easy promotion. As such, this procedure is extensively used to sample airborne particles or microbes in various settings (hospital, farm, and living environment). In the study, the TH-150F medium-volume samplers (Wuhan Tianhong Instruments Co., Ltd.) with glass microfiber filters was used to collect sample. All of the samples could be treated immediately or stored at 80°C before they were pretreated (Jiang et al., 2015).

Step 2. Pretreatment

(1) Buffer wash

The diameter of the glass microfiber filter (Fuyang bairun laboratory instrument Co. Ltd.) is 90 mm, the thickness is 0.23±0.03 mm, and the pore diameter is 0.22 μm. Before the glass microfiber filter was put into the Lysing Matrix E tube (1.5 ml, MP Biomedicals, USA) with the sodium phosphate buffer (978 μL) and the MT buffer (122 μL), the glass microfiber filter samples were cut into 1/4, 1/8, and 1/16. As the cell walls were mechanically disrupted, nucleic acids were released into the protective buffer. Buffer wash (α) was introduced to evaluate the sample pretreatment efficiency and calculated with Eq. (1):

$$\alpha = \frac{V}{S} = \frac{V}{n \times \pi \times r^2}, \quad (1)$$

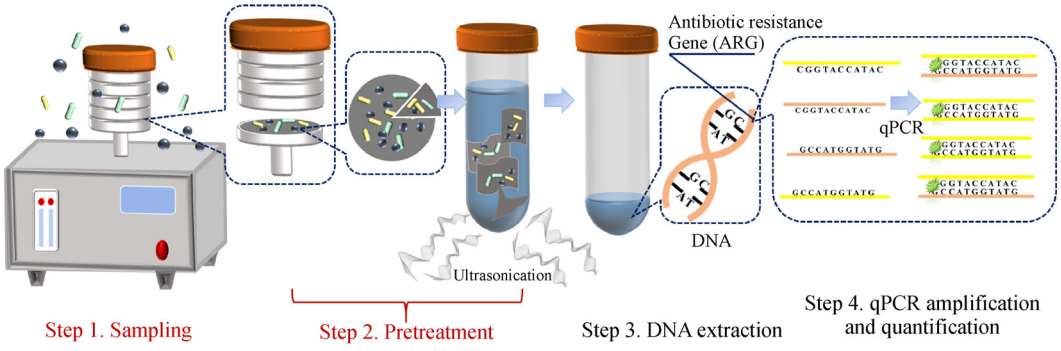

Fig. 1 The schematic depicting experimental approach.

where α is the buffer wash (μL/mm^2); V is the protective buffer (μL); S is the area of the glass microfiber filter (mm^2); n is the different proportions of the glass microfiber filter (1/4, 1/8, and 1/16); and r is the radius of the glass microfiber filter (mm).

(2) Ultrasonication pretreatment

Ultrasonication not only separates a sample and a glass fiber membrane but also breaks cells to extract DNA (Kyllönen et al., 2005). In the study, the Lysing Matrix E tubes were placed in the ultrasonic cleaner (PS-20A) and sonicated for 5, 10, and 15 min at 120 W.

Step 3. DNA extraction

The FastDNA® SPIN Kit (MP Biomedicals, USA) was used to extract the genomic DNA from individual samples. The final purified DNA was passed through a filter bucket and stored in a sterilized microfuge tube. The concentration of DNA was detected with the UV-Vis spectrophotometer (Q5000; Quawell, USA). Then, the extracted 20°C until□genomic DNA was prepared for quantitative PCR and stored at extended periods.

Step 4. qPCR amplification and quantification

The abundance of airborne tetR was determined by the Bio-Rad iQ5 (Bio-Rad Company, CA, USA). The total volume of the reaction mixtures was 25 μL, which contains the 12.5 μL SYBR Premix Ex *Taq*II (Takara), 1 μL DNA sample, 9.5 μL ddH_2O, and 1 μL forward and reverse primers (10 μM). Amplification was 95°C (30 s); 40 cycles of 95°C (5 s) and 60°C (30 s); and fluorescence acquisition at the end of each 60°C elongation step. The qPCR was performed in triplicate. Extraction negative controls were below qPCR detection limits.

2.3 Sampling site description

Two types of laboratories, located in Tianjin University (North of China, 117° N, 39° E), were selected as sampling sites. A biological laboratory (Laboratory 1) and a chemical laboratory (Laboratory 2) were utilized. To collect airborne tetracycline resistance genes samples, we recommended locations without the major interference of sources nearby. The samples were collected in the two laboratories (1.5 m above ground) by using medium-volume samplers with glass microfiber filters (Φ90 mm, 0.22 μm). And they were collected at 0:00–6:00, 6:00–12:00, 12:00–18:00, and 18:00–24:00 according to the researchers' long-term experimental schedule in the same day, which was repeated for three times every three days.

2.4 Data analysis

(1) Recovery efficiency

A standard sample at a concentration of approximately 8.0×10^5 copies/m^3 was used to test the recovery efficiency of the protocol. The recovery efficiency was calculated with Eq. (2):

$$R = \frac{c_t}{c}, \tag{2}$$

where R is the recovery efficiency (%), c is the theoretical concentration of the sample (8×10^5 copies/m^3), and c_t is the experimental concentration of the sample (copies/m^3).

(2) Filter capture loading

Capture loading (L) on a membrane was introduced to evaluate the sampling efficiency. L refers to tetracycline airborne resistance genes retained per unit area of a glass fiber membrane [Eq. (3)]:

$$L = \frac{c \times Q \times T}{S} = \frac{c \times Q \times T}{\pi \times r^2}, \tag{3}$$

where L is the capture loading on the membrane (copies/mm^2); c is the detection concentration of the standard sample (copies/m^3); Q is the sampling flow (5 L/min), T is the sampling time (min); S is the glass microfiber filter area (mm^2); and r is the radius of the glass microfiber filter (Φ25 mm).

(3) Shannon–Wiener index and dissimilarity index

The dissimilarity index (D) (Hiraishi et al., 1991) and Shannon–Wiener index (H) (He et al., 1998) were used to compare the characteristics of four *tet*R in the two laboratories: these parameters calculated using Eqs. (4) and (5), respectively:

$$D(Lab1,Lab2) = \frac{1}{2}\sum_{i=1}^{4}|f_{Lab1,i} - f_{Lab2,i}|, \tag{4}$$

$$H = -\Sigma f_i \ln f_i, \tag{5}$$

where f_i is the concentration fraction of the i-th tetracycline airborne resistance gene; $f_{Lab1,i}$ and $f_{Lab2,i}$ and are the concentration fractions of the i-th tetracycline airborne resistance gene respectively in laboratory 1 and 2. The more uniform the concentration among various airborne tetracycline resistance genes was, the greater the value of H would be. D can be used to quantitatively compare the differences in the size of various resistance gene compositions in the two laboratory samples. The larger the value of D was, the greater the difference between the two laboratories would be. When D is equal to 0, the two laboratories were identical.

3 Results and discussion

3.1 Optimization of the collection and detection of airborne resistance genes

3.1.1 Collection through filter sampling

Sampling duration is an important parameter of airborne ARG detection. This parameter is dependent on initial ARG concentrations. High initial ARG concentrations

often require short sampling duration, whereas low initial ARG concentrations need long sampling duration. Therefore, the index of capture loading (Eq. (3)) was calculated in this study. The samples collected under various conditions (initial ARG concentrations and sampling durations) were analyzed in accordance with the proposed protocol to determine the concentrations of airborne tetracycline resistance genes. The relationship between recovery efficiency (Eq. (2)) and capture loading is presented in Fig. 2.

Our data followed Gaussian distribution. Recovery efficiency initially increased and then declined as capture loading increased, suggesting that an optimal capture loading should be used for airborne ARG sampling. When the capture loading was too high, the membrane surface became saturated, thereby exceeding the maximum capture capacity. Under low capture loading conditions (less than 550 copies/mm^2), the amount of captured ARGs was so small that they were easily influenced by the impacting and shearing force during sampling. The fitted curve indicated that the capture loading should be 550–780 copies/mm^2 when the recovery efficiency was greater than 75% (recommended in this study).

In addition, the filtering sampling can achieve the enrichment of low concentration airborne ARGs by extending the sampling time. Figure 3 shows the surface plots of recovery efficiency under different sampling conditions and the recovery efficiency under various initial ARG concentrations and sampling volumes or sampling durations. The red area corresponds to a recovery efficiency of higher than 75%, which can provide ranges of sampling volume for each ARGs concentration. According to the detection concentration and gas volume, if the recovery efficiency is out of the 75% area in Fig. 3, collection parameters (sampling duration and sampling flow) in this filtering sampling should be changed. For example, when the detection concentration is 4.1 × 10^6 copies/m^3, it can be found from Fig. 3 that the gas volume range should be above 0.65 m^3. However, the actual gas volume (0.45 m^3) is too low, so that we can increase collection parameters. This time, again detection concentration and gas volume could find the recovery efficiency in Fig. 3, which determines whether the sample parameters need to be changed. The efficient sampling parameters of the environment are obtained by analogy. Therefore, this study could provide a theoretical basis for the efficient filter collection of airborne ARGs in different environments.

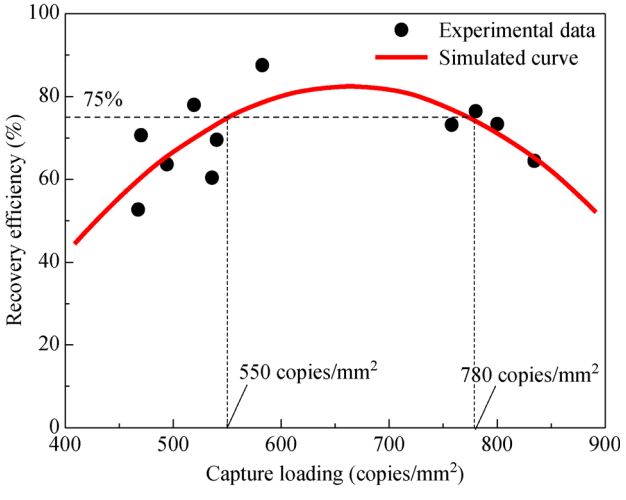

Fig. 2 The relationship between recovery efficiency and capture loading.

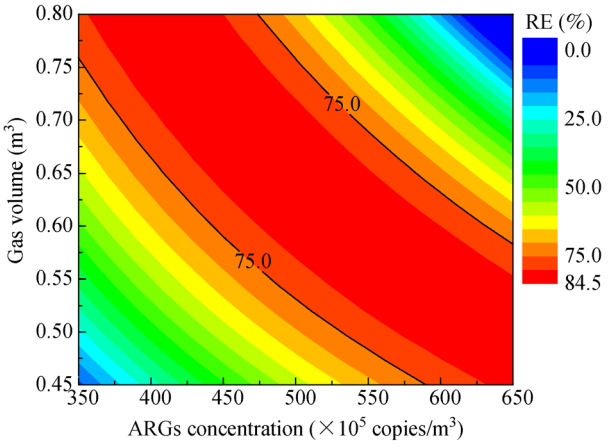

Fig. 3 Surface plots of recovery efficiency under different conditions. RE: Recovery efficiency.

3.1.2 Optimal ratio of buffer volume to membrane area

In buffer washing, the ratio of the buffer volume to the membrane area (α, Eq. (1)) is important to the detection of airborne ARGs. Figure 4 shows the results of various airborne tetracycline resistance genes under different ratios of buffer volume to membrane area.

α [Eq. (1)] was calculated to be 0.7, 1.4, 2.8 μL/mm^2. The highest concentrations of airborne tetracycline resistance genes were detected with 1.4 μL/mm^2 buffer wash. When α decreased to 0.7 μL/mm^2, the buffer was sufficient to completely wash off the cells and DNA from the membrane. Conversely, when α increased to 2.8 μL/mm^2, the concentration of airborne tetracycline resistance genes in the buffer solution was so low that adsorption by lysing matrix particles could be observed. Jiang et al. (2015) shred the filter (Supor 200 PES Membrane Disc Filter, 0.2 μm) into small pieces before DNA extraction for improved extraction efficiency. The portion of the filter sample (QMA, 203 × 254 mm, Whatman 1851-65, UK) in air from industrial and urban sites was extracted with sterilized 1 × PBS (Xie et al., 2018). In these previous studies, it was found that it was feasible to shear the filter

membrane, but the best cutting ratio was not determined. According to the experimental data in Eq. (1), cutting and breaking 1/8 filter membrane was the optimal filter membrane for airborne ARG detection efficiency in this study.

3.1.3 Ultrasonic pretreatment

Figure 5 shows the concentrations of tetracycline resistance genes under different ultrasonic pretreatment durations. The concentration of airborne tetracycline resistance genes in the ultrasonic-pretreated solution was higher than that in the untreated solution. Furthermore, 5 min was the optimal ultrasonication time because it positively affected ultrasonic lysing. When ultrasonication time was further extended, the concentrations of the tetracycline resistance gene decreased.

Ultrasonication can disrupt cells by generating microbubbles and introducing extreme power to the solution (Foladori et al., 2007; Wen et al., 2017). These microbubbles vibrate and implode, producing mechanical shear stress and turmoil (Liang et al., 2012). Shear stress agitates the solution, preventing *Zoogloea* formation and AGR adsorption on lysing matrix particles (Duquenne et al., 2013). In the study, Gram-negative bacterial cells were washed off and broken within the short ultrasonication time of 5 min. The tetracycline resistance gene was

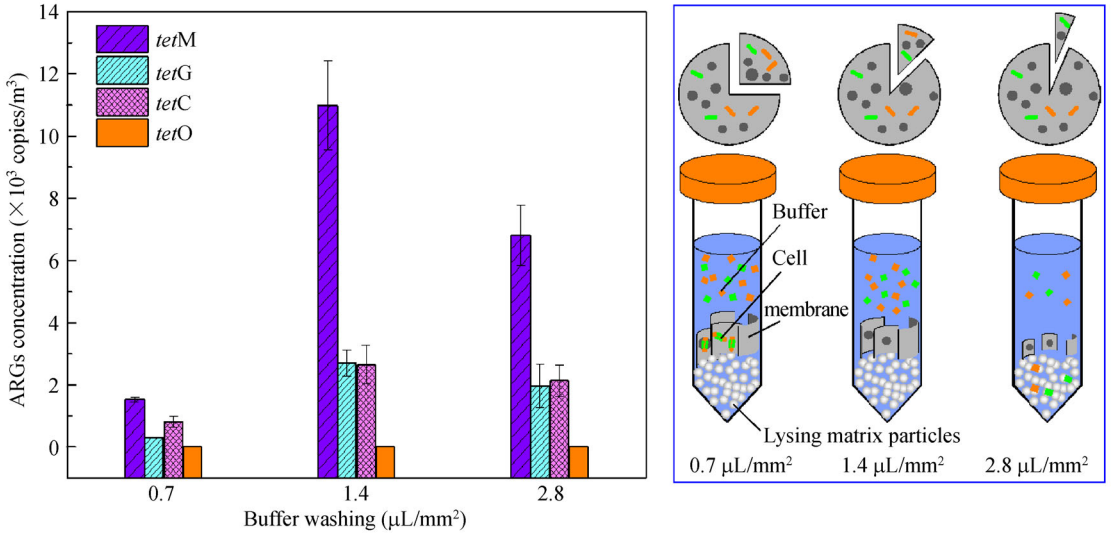

Fig. 4 Airborne tetracycline resistance gene concentrations under different ratios of buffer volume to membrane area during buffer washing procedure (Error bar presents standard deviation, *n* present numbers of samples, *n* = 3 in each buffer wash).

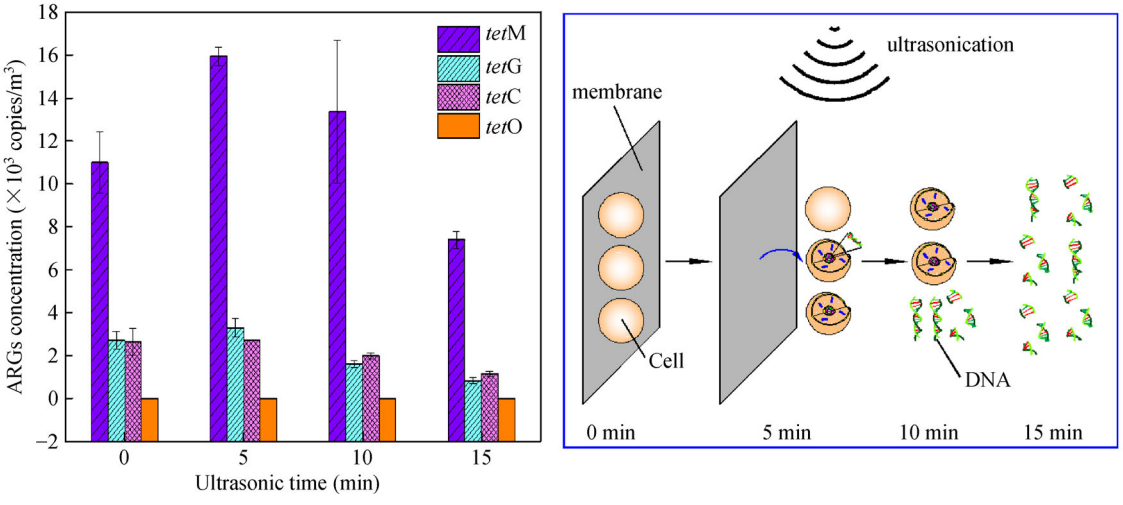

Fig. 5 Airborne tetracycline resistance gene concentrations under different ultrasonic pretreatment time. (Error bar presents standard deviation, *n* present numbers of samples, *n* = 3 in each ultrasonic pretreatment time)

liberated from the membrane or the broken cells and quantified through qPCR.

Further extending the ultrasonication time may negatively affect ARGs. Cui et al. (2011) demonstrated that *E. coli* cells are severely damaged after they are ultrasonically disinfected (20 kHz, 64 kJ/L), indicating that ultrasonication can disrupt cell structures. In our study, as ultrasonication was continuously prolonged from 5 min to 15 min, more energy was introduced to the solution. The effective gene sequence (tetracycline resistance genes) was broken down into fragments. Consequently, they could not be detected.

3.2 Airborne tetracycline resistance genes in laboratories

In accordance with the proposed protocol of airborne ARG collection and detection, the samples from different laboratories (Laboratory 1 and 2) were examined. Each measurement was conducted in 6 h at the same day.

3.2.1 Exposure concentrations of airborne tetracycline resistance genes

The concentrations of airborne *tet*R in the two laboratories are presented in Fig. 6. The concentrations of these genes were distinct at different sampling sites. *tet*M in both laboratories yielded the highest concentration. A range of 9.7×10^3–1.3×10^4 copies/m^3*tet*M was detected in Laboratory 1 (Table 2). By comparison, the concentration of *tet*M in Laboratory 2 was 6.0×10^3–7.4×10^3 copies/m^3, which was much lower than that in Laboratory 1 (Table 3). The bioreactor device continuously operated in Laboratory 1 that produced abundance microorganisms in these areas (Cui et al., 2011). This phenomenon probably led to the production of high concentrations of airborne ARGs.

Few studies have yet to measure airborne ARGs levels in laboratories. The airborne ARGs level (1.5×10^4–1.7×10^4 copies/m^3) in our indoor laboratory atmosphere was significantly higher than previously reported indoor levels in Table 1 (1.6×10^{-3}–4.0×10^2 copies/m^3), suggesting that the airborne ARGs of an indoor laboratory environment could pose the remarkable health risk to inhabitants. To ensure the health of laboratory researchers, airborne ARGs in the biological laboratory cannot be ignored. For quality laboratory atmosphere, the fresh outdoor air is conditioned and supplied into the laboratory by the air conditioning system, in order to dilute the airborne ARGs (Cheng et al., 2019).

3.2.2 Changes in airborne ARG diversity and dissimilarity

D and *H* of airborne tetracycline resistance genes in two laboratories were calculated on the basis of the concentration fraction of airborne tetracycline resistance genes by using Eqs. (4) and (5). Figure 7 shows the comparison of the diversity and dissimilarity of airborne tetracycline resistance genes in the two laboratories. In Laboratory 1, the increase of *H* indicates the more uniform concentration distribution of the four tetracycline resistance genes over time. In Laboratory 2, the uniformity of the concentration distribution initially increased and then decreased.

After midnight (0:00–6:00), the air qualities in the two laboratories were close to the background level because of low occupancy. Therefore, the dissimilarity between the two laboratories was small in this period. After 6:00, the

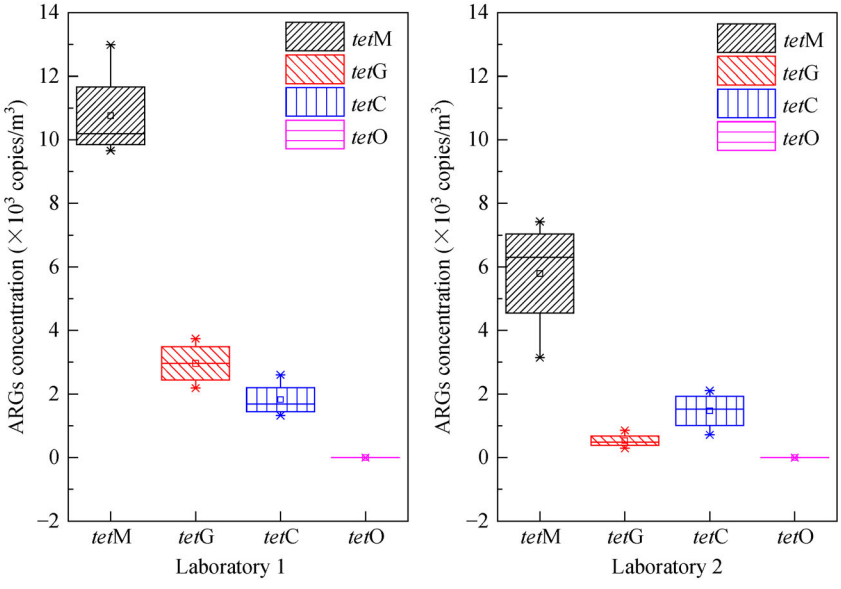

Fig. 6 The airborne tetracycline resistance gene concentration in laboratory (Error bar presents standard deviation, n presents numbers of samples in the same day, $n = 4$ in each laboratory).

Table 2 The concentrations of airborne tetracycline resistance genes in laboratory 1

Target gene	ARGs concentrations in Lab 1 (copies/m^3)							
	0:00–6:00		6:00–12:00		12:00–18:00		18:00–24:00	
	Mean	Min–Max	Mean	Min–Max	Mean	Min–Max	Mean	Min–Max
*tet*M ($n = 4 \times 3$)	1.3×10^4	1.2×10^4–1.4×10^4	1.0×10^4	8.8×10^3–1.3×10^4	1.0×10^4	7.5×10^3–1.3×10^4	9.7×10^3	9.1×10^3–1.1×10^4
*tet*G ($n = 4 \times 3$)	2.2×10^3	1.8×10^3–2.9×10^3	3.2×10^3	2.9×10^3–3.7×10^3	3.7×10^3	3.0×10^3–4.3×10^3	2.7×10^3	2.5×10^3–3.1×10^3
*tet*C ($n = 4 \times 3$)	1.8×10^3	1.7×10^3–2.0×10^3	1.3×10^3	7.6×10^2–2.1×10^3	1.6×10^3	1.4×10^3–1.9×10^3	2.6×10^3	2.1×10^3–3.0×10^3
*tet*O ($n = 4 \times 3$)	2.1×10^{-1}	1.8×10^{-1}–2.5×10^{-1}	1.4×10^{-1}	8.0×10^{-2}–1.9×10^{-1}	1.2×10^{-1}	7.0×10^{-2}–2.0×10^{-1}	8.0×10^{-2}	3.0×10^{-2}–1.2×10^{-1}

Note: The data for analysis in this study represent the mean of triplicates.

Table 3 The concentrations of airborne tetracycline resistance genes in laboratory 2

Target gene	ARGs concentrations in Lab 2 (copies/m^3)							
	0:00–6:00		6:00–12:00		12:00–18:00		18:00–24:00	
	Mean	Min–Max	Mean	Min–Max	Mean	Min–Max	Mean	Min–Max
*tet*M ($n = 4 \times 3$)	7.4×10^3	6.9×10^3–8.3×10^3	6.1×10^3	5.6×10^3–6.8×10^3	6.0×10^3	5.8×10^3–6.1×10^3	6.7×10^3	6.2×10^3–7.1×10^3
*tet*G ($n = 4 \times 3$)	5.8×10^2	5.7×10^2–6.0×10^2	5.1×10^2	4.6×10^2–5.9×10^2	4.8×10^2	4.2×10^2–5.1×10^2	2.9×10^2	2.8×10^2–3.1×10^2
*tet*C ($n = 4 \times 3$)	1.7×10^3	1.5×10^3–2.1×10^3	1.3×10^3	1.0×10^3–1.7×10^3	1.7×10^3	1.1×10^3–2.3×10^3	2.1×10^3	1.8×10^3–2.7×10^3
*tet*O ($n = 4 \times 3$)	1.3×10^{-1}	8.0×10^{-2}–2.0×10^{-1}	1.3×10^{-1}	6.0×10^{-2}–2.4×10^{-1}	7.7×10^{-2}	5.1×10^{-2}–1.1×10^{-1}	7.4×10^{-2}	2.0×10^{-2}–1.3×10^{-1}

Note: The data for analysis in this study represent the mean of triplicates.

dissimilarity of the two laboratories increased because of high occupancy and distinct experimental operations. ARGs could transmit between a variety of bacterial species (Li et al., 2018). High concentrations of microorganisms may contribute to increase the abundance of ARGs. The bioreactor devices continuously operated (The long-term anaerobic digestion of sludge with high concentration of tetracycline resistance gene is in these reactors.) in Laboratory 1 may produce abundance microorganisms in these areas, thereby causing the high uniformity of airborne ARGs. In Laboratory 2, the usage of a chemical reagent probably reduced the concentrations and microbial species of microorganisms, resulting in the low uniformity of airborne ARGs.

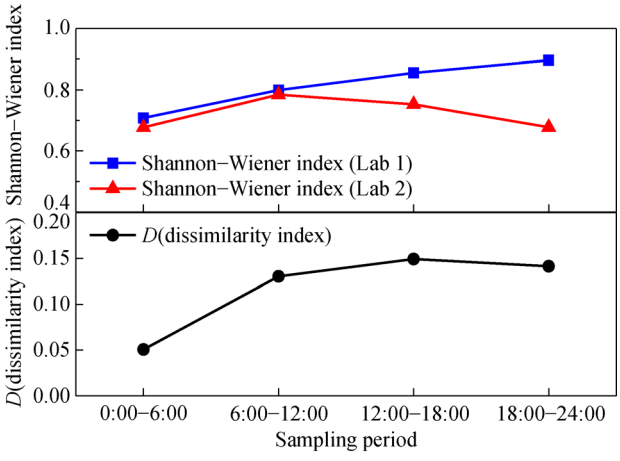

Fig. 7 The Shannon–Wiener index and dissimilarity index in two laboratories.

3.2.3 Temporal variation of *tet*M abundance in the two laboratories

The "*H*-*f*(*tet*M) plots" (*f*(*tet*M): concentration fraction of dominant ARGs) in both laboratories are illustrated in Fig. 8 to quantify the changes in the airborne ARG diversities in the two laboratories. The plots in Laboratory 1 moved from the upper left to the lower right as the time progressed. Simultaneously, the fraction of the dominant airborne ARG (*tet*M) decreased, and *H* increased, suggesting that an increase of *H* could be mainly caused by an increase of other airborne ARGs (*tet*G and *tet*C). Similarly, in Laboratory 2, *tet*M was the dominant species

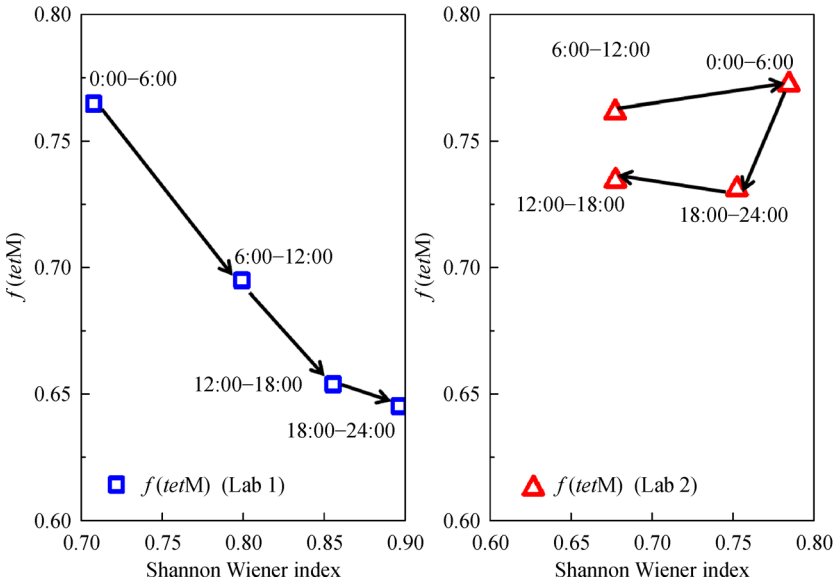

Fig. 8 Temporal variation of *tet*M abundance in the two laboratories.

of airborne ARGs. The samples from Laboratory 2 were clustered together, suggesting the slight changes in the dominant species and diversity of airborne ARGs, which were quite different from those in Laboratory 1. The changes in the airborne ARGs were clearly observed by using "*H*-*f*(*tetM*) plots." The changes in microbial communities and airborne ARGs in the two laboratories showed different behaviors. Therefore, we should pay attention to some complicated indoor air environment and adopt some treatment technologies to control it when necessary.

4 Conclusions

In this study, an optimized protocol for the collection and detection of airborne ARGs is presented and used to analyze airborne ARGs in indoor laboratory atmosphere.

The relationship between recovery efficiency and capture loading follows Gaussian distribution in this analysis. When recovery efficiency is greater than 75%, capture loading should be 550–780 copies/mm^2, and a range of optimum sampling volume can be provided for a given initial ARG concentrations. As the cell walls were mechanically disrupted and nucleic acids were released, the buffer wash protects ARGs dissolution. Furthermore, the majority of the cells were disrupted by an ultrasonication pretreatment, allowing the efficiency ARGs detection of airborne samples. An optimized sampling method was proposed that the buffer wash was 1.4 μL/mm^2 and the ultrasonication duration was 5 min.

Airborne tetracycline ARGs (*tet*M, *tet*G, *tet*C, and *tet*O) were analyzed in terms of their spatial and temporal patterns in laboratories. The *tet*M in two laboratories was found to be dominant. The *tet*M level in the chemical laboratory was 6.0×10^3–7.4×10^3 copies/m^3, which was much lower than 9.7×10^3–1.3×10^4 copies/m^3 in the biological laboratory. The dissimilarity (*H*) between the two laboratories ranged from 0.03 to 0.22 due to the distinct occupancy and experimental operations. Therefore, we should pay attention to some complicated indoor air environment and adopt some treatment technologies to control it when necessary.

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