Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Comparison of culturable antibiotic-resistant bacteria in polluted and non-polluted air in Beijing, China

Yixin Mao, Pei Ding, Youbin Wang, Cheng Ding, Liping Wu, Ping Zheng, Xiao Zhang, Xia Li, Leyao Wang, Zongke Sun

*Department of Environmental Microbiology, National Institute of Environmental Health, Chinese Center for Disease Control and Prevention, Beijing 100021, China

**Department of Women’s, Children’s, and Adolescents’ Environmental Health, National Institute of Environmental Health, Chinese Center for Disease Control and Prevention, Beijing 100050, China

School of Medicine, Institute of Public Health, Washington University, St. Louis, MO 63110, USA

ARTICLE INFO

Handling Editor: Yong-Guan Zhu

Keywords:
Antibiotic resistance
Atmosphere
Culturable bacteria
Polluted air
Beijing smog
Multidrug-resistant

ABSTRACT

Background: Air pollution has been a serious health issue in Beijing for years. Airborne antibiotic-resistant bacteria could be a potential health crisis as reserve of antibiotic resistance transmission in environment. The composition and antibiotic resistance pattern of culturable bacterial community and how these are affected by air pollution remain unclear.

Objectives: This study aimed to compare the compositions and antibiotic resistance patterns of culturable bacteria in polluted and non-polluted weather conditions in Beijing.

Methods: Air samples were collected indoors and outdoors during polluted and non-polluted weather using six-stage Andersen Samplers. For each isolated bacterium, the 16S ribosomal RNA gene was amplified, sequenced, and blasted against the National Center for Biotechnology Information database. Antibiotic resistance was conducted by antimicrobial susceptibility testing.

Results: Bacterial concentration in polluted weather was significantly higher than in non-polluted weather, both indoors and outdoors (P<0.05). Gram-positive bacteria (GPB) were dominant in both weathers but gram-negative bacteria (GNB) were more abundant in polluted weather than non-polluted weather both indoors and outdoors. Multidrug-resistant (MDR) bacteria occupied 23.7% of all bacterial isolates, 22.4% of isolates from polluted weather and 27.8% of isolates from non-polluted weather. Penicillins were resisted by 72.4% and 83.3% of isolates from polluted and non-polluted weather, respectively.

Conclusions: The bacterial concentration was significantly higher in polluted weather, compared to non-polluted weather. Polluted weather is correlated with changes in the bacterial composition in the air, with a greater abundance of GNB. Penicillins was resisted by over 70% of bacterial isolates. The abundance of MDR bacteria suggested potential risks for human health.

1. Introduction

Air pollution is a serious global health issue. Exposure to particulate matter (PM) pollutants in the atmosphere increases risks for diseases in the respiratory and nervous systems and decreases life expectancy (Allen et al., 2013, 2014; Hunt et al., 2003; Maher et al., 2016; Pope 3rd et al., 2009; Weuve et al., 2012). As one of the major components and pathogenic agents, airborne microorganisms, such as bacteria, are considered to be closely related to air pollution that causes human allergic responses and respiratory diseases (Kim et al., 2018; Wu et al., 2016). Many studies have been carried out on airborne bacteria in environment (Fang et al., 2007). And it has been well documented that the air within hospitals or stock farms is highly contaminated with bacteria (Clark et al., 1983; Park et al., 2013; Solomon et al., 2017). Microorganisms are believed to have varying concentration in the ambient air depending on the pollution state (Liu et al., 2018; Zhai et al., 2018).

Bacterial infections are becoming increasingly difficult to treat due to the emergence of antibiotic resistance (AR or ABR), when bacteria evolve in response to antibiotics and make them ineffective. ABR is a

* Corresponding author.

E-mail addresses: maoyixin@nieh.chinacdc.cn (Y. Mao), dingpei@nieh.chinacdc.cn (P. Ding), wangyoubin@nieh.chinacdc.cn (Y. Wang), dingcheng@nieh.chinacdc.cn (C. Ding), wuliping@nieh.chinacdc.cn (L. Wu), zhengping@nieh.chinacdc.cn (P. Zheng), zhangxiao@nieh.chinacdc.cn (X. Zhang), lixia@nieh.chinacdc.cn (X. Li), leyao.wang@wustl.edu (L. Wang), sunzongke@nieh.chinacdc.cn (Z. Sun).

https://doi.org/10.1016/j.envint.2019.104936

Received 15 February 2019; Received in revised form 15 June 2019; Accepted 15 June 2019

Available online 05 July 2019

0160-4120/ © 2019 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/BY-NC-ND/4.0/).
global health crisis that leads to increased morbidity, mortality, and medical costs. In China, 92,700 tons of 36 frequently detected antibiotic biotics were used, according to Zhang's investigation, and 53,800 tons of which eventually entered the environment (Zhang et al., 2015). The development of antibiotic resistance among bacteria has been facilitated by the misuse and abuse of antibiotics in humans and animals. Moreover, antibiotic resistance can be transferred between bacteria in environment (Allen et al., 2016; Hiltunen et al., 2017; Xie et al., 2019). Large amounts of antibiotic resistant genes were found in air samples from Beijing smog (Pal et al., 2016), causing great public concern and panic. The existence of antibiotic resistant genes indicates the potential for resistance to be transferred between bacteria in air (Xie et al., 2019). Antibiotic-resistant bacteria in the air, particularly pathogenic bacteria, may cause respiratory diseases of a longer duration due to their resistance to antibiotic treatment. There have been studies on ABR in food or other environments, such as soil, waste water, or hospital settings (Gao et al., 2018; Uhrbrand et al., 2017; Zhu et al., 2017). However, few studies have evaluated the changes in the percentage of antibiotic-resistant bacteria in polluted and non-polluted air.

The aim of this study was to compare the compositions and antibiotic-resistant patterns of bacterial communities in ambient air samples collected under polluted and non-polluted weather conditions in a typical site of Beijing. We focused on culturable airborne bacteria through culture and antimicrobial susceptibility testing (AST) method, as the target bacteria is a prompt start of airborne AMR bacteria study and these live airborne bacteria could be obtained for further research.

2. Material and methods

2.1. Study site and air sampling

Air samples were collected from the sixth floor of the Experiment Building at the National Institute of Environmental Health (39°52′48″N, 116°27′26″E; ~18 m above the ground). This site is located in the southeast of Beijing between the 2nd and 3rd Ring Roads, with Long Tan Lake Park and Tian Tan Park to the west, Beijing Railway Station to the northwest, Cancer Hospital Chinese Academy of Medical Sciences to the southwest, Chui Yang Liu Hospital to the southeast, Fen Zhong Temple to the south and Beijing University of Technology to the east. Besides, this sampling site is surrounded by over 40 residential communities within 2 km.

Two six-stage Andersen Samplers (Thermo-Andersen, USA) were used to collect air samples from inside and outside the Experiment Building separately at the same time, with an average flow rate of 28.3 L/min for 10 min. The indoor samples were collected in a laboratory that was adjacent to the outdoor sampling site, with a single closed door between the two sites. The Andersen sampler has six stages with different cut-off sizes that represent the human respiratory system (Table S1), with stages 3–6 representing the hazardous range where particles have lung penetrability (Andersen, 1958). Nutrient agar (NA) formula (OXOID, England) were added to distilled water and mixed well according to manufacturer’s instructions. After sterilized by autoclaveng, culture medium was distributed into plastic culture plates. After solidification, the culture plates were directly placed on all six stages of the sampler to collect air samples. Both the indoor and outdoor samples were collected on 7 days chosen randomly of polluted and non-polluted weather, respectively, during heating season of Beijing (through November 2017 to March 2018) (Table S3).

The ‘polluted weather’ and ‘non-polluted weather’ in this study were defined based on the AQI (air quality index) level obtained from China’s Ambient Air Quality Standards (GB 3095-2012) (China MEE, 2012a) and Technical Regulation on Ambient Air Quality Index (HJ 633-2012) (China MEE, 2012b). Polluted weather (from slightly polluted to severe polluted level in Table S2) was defined as AQI of > 101, and non-polluted weather (excellent weather level in Table S2) was defined as AQI of < 50 in this study. Environmental parameters were recorded when sampling, including temperature, relative humidity, SO2, NO2, CO, O3, PM10, and PM2.5. Real time environmental data were obtained from the National Urban Air Quality Real-time Distribution website (http://106.37.208.233:20035/), published by China National Environmental Monitoring Centre.

2.2. Sample culture and counting

Each of the sample plate collected at each stage of the sampler was immediately incubated at 37 °C for 48 h, allowing the bacterial aerosols to grow on the NA plates. The number of colony-forming units (CFUs) was counted manually after 48 h. And concentrations were expressed as CFU per cubic meter of air (CFU/m³). Because during sampling, there existed a superposition when microbial particles impact the same spot through the same pore, counting results of each sample were statistically corrected according to Andersen (Andersen, 1958). Samples from 2017/12/29 and 2018/3/1 were selected to represent polluted and non-polluted weather, respectively, for further analysis.

2.3. Bacterial isolation and identification

Each culturable colony from the original sample plates of selected days (2017/12/29 and 2018/3/1) was picked using inoculation loop or needle onto a Columbia Agar plate adding 5% sheep blood (CO; BioMerieux) to isolate individual bacteria. These COS plates were incubated at 37 °C for 48 h. The above isolation step was repeated at least twice to ensure that the colonies growing on each of the final culture plates had been purified without any other mixed bacteria.

Total genomic DNA was extracted from each purified culturable bacterium using a prepGEM® Bacteria DNA Extraction Kit (ZyGEM, New Zealand) following the manufacturer’s instructions. The 16S ribosomal RNA (rRNA) gene was then amplified by polymerase chain reaction with the universal primer pair 27F/1492R and the amplified gene was cloned and sequenced (ABI3730XL, Majorbio, China). Each purified culturable bacterium was identified by performing Nucleotide Basic Local Alignment Search Tool (BLAST) searches against the National Center for Biotechnology Information’s (NCBI’s) 16S rRNA gene sequence reference database (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and taxonomic information for each identified culturable bacterium was obtained from the NCBI Taxonomy database (https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi). The database also provides references of Gram information.

2.4. Antimicrobial susceptibility testing (AST) of bacterial isolates

AST was conducted using the minimal inhibitory concentration (MIC) agar dilution method with AST plates (Biofosun, China). The wells of each AST plate were covered with specific antibiotics that included nearly all common antibiotic classes for gram-positive bacteria (GPB) and gram-negative bacteria (GNB) (CLSI, 2017; Magiorakos et al., 2012) in a gradient concentration (Tables S4–S6). Negative and positive control well did not cover with antibiotics. Antibiotic classes included penicillins, β-Lactam/β-Lactamase inhibitor combinations, cephalosporins, lipopeptides, glycopeptides, penems, macrolides, lincosamides, Macrolides/Lincosamides combinations, pseudomonic acid, fluoroquinolones, folate pathway inhibitors, tetracyclines, phenicol, and aminoglycosides (Table S6).

Single colonies of bacterial isolates were suspended in Nutrient Broth (NB) and diluted to a concentration of 1.5 × 10^6 CFU/ml according to the McFarland standard. Each bacterial suspension was added to one AST plate except for the negative control well, adding NB without bacterial suspension. The AST plates were then incubated at 37 °C for 18–20 h. The plates were read manually according to the manufacturer’s instructions and analyzed based on the MIC values published in the guidelines of the U.S. Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2017). Each bacterial isolate was
categorized as susceptible, intermediate, or resistant to a specific antibiotic using MIC breakpoints. We set some breakpoints of antibiotics where they were not included in the guideline, such as erythromycin (ERY) for GNB (Tables S4–S5). The AST results were analyzed using the software provided by the manufacturer.

3. Results

3.1. Bacterial concentration in different weathers

The total numbers of CFUs collected during polluted and non-polluted weather conditions are shown in Fig. 1A. Real time environmental parameters are shown in Fig. S3. CFUs of the polluted weather samples were significantly greater than those of the non-polluted weather samples, both indoors and outdoors ($P < 0.05$).

Numbers of CFUs collected by 6 stages of the Andersen Sampler are shown in Fig. 1B. Plates on stage 4 of the Andersen Sampler had the highest and second to highest CFUs of outdoor samples in polluted and non-polluted weather, respectively. Plates on stage 5 had the highest number of indoor samples in both polluted and non-polluted weather.

3.2. Composition of culturable bacteria

A total of 249 and 32 colonies were picked from the original plates on the samplers of selected days under polluted and non-polluted weather conditions (2017/12/29 and 2018/3/1), respectively. Of these, 133 isolates (53.4%) from the polluted weather samples and 25 isolates (78.1%) from the non-polluted weather samples were successfully cultured. All culturable bacterial isolates were then sequenced and identified through 16S rRNA sequencing. GPB were dominant in both weather conditions, indoors and outdoors. However, GNB were more abundant in polluted than non-polluted weather in both the indoor and outdoor samples (Fig. 2).

The culturable bacteria collected in non-polluted weather were distributed across 10 genera, with Bacillus, Streptomyces, and Rhodococcus being most abundant (Fig. 3). By contrast, in polluted
weather, the bacterial isolates were distributed across 43 genera, with *Bacillus*, *Kocuria*, and *Streptomyces* being the top three most dominant genera. *Streptomyces*, *Bacillus*, *Micrococcus*, and *Microbacterium* were in the top 10 most abundant genera in both weathers. Five genera detected in non-polluted weather (*Rhodococcus*, *Curtobacterium*, *Isoptericola*, *Planococcus*, and *Variovorax*) were not detected in polluted weather samples.

At the phylum level, Actinobacteria were dominant (68.4% in polluted weather and 68.0% in non-polluted weather). At the order level, Micrococcales, Streptomycetales, and Corynebacteriales in Actinobacteria, Bacillales in Firmicutes, and Burkholderiales in Proteobacteria were all detected in both weathers and were most abundant in their corresponding classes (Fig. S1).

### 3.3. Culturable multidrug-resistant (MDR) bacteria

In total, 76 bacterial isolates out of 158 (48.1%) were successfully tested through AST, including 18 isolates from the non-polluted weather samples and 58 isolates from the polluted weather samples. MDR bacteria were detected in 23.7% (18/76) of all cultivable bacteria, 22.4% (13/58) of polluted weather and 27.8% (5/18) of non-polluted weather (Fig. 4A). In non-polluted weather group, 16.67% (3/18) of the MDR bacterial isolates were resistant to three antibiotic classes and 11.11% (2/18) were resistant to four classes, whereas in polluted weather group, 8.62% (5/58) of MDR bacterial isolates were resistant to three antibiotic classes and 6.90% (4/58) were resistant to five classes.

In GPB group, 16.7% (11/66) MDR bacteria were detected, while in GNB group, 70% (7/10) were detected (Fig. 4B). Among GPB MDR bacterial isolates, 63.64% (7/11) were resistant to three antibiotic classes, 27.27% (3/11) were resistant to four classes, and 9.09% (1/11) were resistant to five classes. By contrast, 42.86% (3/7) of the GNB MDR bacterial isolates were resistant to five antibiotic classes and 28.57% (2/7) were resistant to six classes.

### 3.4. Antibiotic drug resistance in different weathers

Percentage of all tested antibiotics resisted by cultivable bacteria in different sample groups were calculated and shown in Fig. 5. Penicillins were resisted by most of the cultivable bacterial isolates in polluted (72.4%, 42/58) and non-polluted (83.3%, 15/18) weather. Furthermore, penicillins were resisted by 100% (11/11) and 85.7% (6/7) culturable bacterial isolates in MDR of GPB and GNB group, respectively.

Cephems were resisted by 29.3% (17/58) and 33.3% (6/18) culturable bacterial isolates in polluted and non-polluted weather, respectively. 90.0% (10/11) and 100.0% (10/10) culturable MDR bacterial isolates resisted cephems in GPB and GNB group, respectively.

### 4. Discussion

Here, the quantity and composition of cultivable airborne bacteria in the ambient air along with their antibiotic resistance patterns in polluted and non-polluted weathers were surveyed in Beijing. Many studies including airborne bacteria have been carried out in different environments in the past (Cao et al., 2014; de Rooij et al., 2019; Fang et al., 2006, 2007; Gao et al., 2018; He et al., 2017; Xie et al., 2019). Yet this is the first observation of the existence and composition analysis of cultivable antibiotic-resistant bacteria in the ambient air from polluted and non-polluted weather conditions in Beijing using bacterial culture samples.
positive bacteria (GPB) and gram-negative bacteria (GNB) groups.

**Results**

Results revealed that bacterial concentrations were significantly higher in polluted weather compared to non-polluted weather, regardless of whether they were collected indoors or outdoors. This could be attributed to the environmental conditions, sampling time, and bacteria's capability of adhering to PM in the ambient air. Air sampling using different samplers could bring various differences to bacterial concentration. The limitation of using Andersen Samplers is that it could damage cells during sampling process which may cause missing of a fraction of culturable bacteria. Still, it is a convenient and efficient method for air sample collection. Bacteria concentrations in indoor air were lower than in outdoor air under both weather conditions. Similar findings have been reported (Shelton et al., 2002; Emerson et al., 2017). In addition, results suggested that in polluted weather, most bacteria collected outdoor and indoor are likely to influence the secondary bronchi and terminal bronchi, respectively, indicating that people who remain inside during polluted weather may still be at risk of bacterial infection, particularly of the lower respiratory tract. Therefore, residents should better use health protective equipment (e.g., an air purifier) if they stay at home in polluted weather. It has previously been demonstrated that microbes can become attached to ambient particles and have significant effects on climate change (Brodie et al., 2007), increasing the risk to human health.

Biological agents in both indoor and outdoor environments have been associated with reduced health, including allergies, asthma, infectious diseases, such as severe acute respiratory syndrome, and even cancer (Douwes et al., 2003; Kim et al., 2018; Yoo et al., 2017), which may have high socio-economic impacts (Ghosh et al., 2015).

Bacteria from environment, especially from ambient air, can be vulnerable through medium culture process. After several generations, those bacteria may become too weak to grow continually. In this case, a total of 56.2% bacterial isolates (53.4% from the polluted weather samples and 78.1% from the non-polluted weather samples) were found to be culturable airborne bacteria. GNB were dominant under both weather conditions, which was in agreement with other studies (Brandl et al., 2014; Fang et al., 2006; Görny and Dutkiewicz, 2002; Görny et al., 1999; Liang et al., 2013). However, in both the indoor and outdoor samples, GNB had a higher relative abundance in polluted weather than in non-polluted weather, which indicated a potential correlation between pollutants and GNB. Actinobacteria were most abundant at the phylum level, and multiple genera within Actinobacteria were soil inhabitants (e.g., *Streptomyces* (Ventura et al., 2007), indicating that soil inhabitants may be one of the key components of the airborne bacterial community, which was also found by Cao et al. during a severe smog event (Cao et al., 2014). Bacteria has correlation with the ground disturbance caused by human activities, which can stir up the dust and small soil particles from the ground. The relationship between air pollutants and GNB need further research to explore.

To the best of our knowledge, this is also the first observation of the existence of culturable MDR bacteria in the ambient air in Beijing using bacterial culture methods. MDR was defined as acquired non-susceptibility to at least one agent in ≥ 3 antimicrobial categories (Magiorakos et al., 2012). And ≥ 10 categories of antibiotics and their combinations were used to detect MDR bacteria in this study. A total of 23.7% of all tested culturable bacteria were MDR, which raises the question as to whether these bacteria could serve as reservoirs of resistant genes in the air and could pass on this resistance to more bacteria, including pathogenic species. MDR bacteria occupied 70% in GNB, compared to 17% in GPB. It is worth noticing that compared to GNB, GPB dominant much more in ambient air, which we believe may influence this difference of MDR occupation percentage to a certain extent. Still, the situation of MDR bacteria occupation in the ambient air was serious, approaching the percentage of airborne MDR bacteria that have been isolated from swine feeding operations (Chapin et al., 2005) or wastewater treatment plants (Teixeira et al., 2016). MDR bacteria occupied approximately one forth in both polluted or non-polluted weather, which also suggest the serious situation of airborne MDR bacteria. And interestingly, the distribution of MDR bacteria is not dependent on the weather conditions.

However, it should be noticed that the difference in the proportion of MDR bacteria between different groups may influenced by the...
relatively small sample size of non-polluted weather and GNB. Also, some bacteria were unable to survive the entire AST process, which we believe was due to the following reasons: a) some airborne bacteria cannot be cultured in NB; b) environmental bacteria can be vulnerable during bacterial culture, purification, isolation, and the AST process; c) the bacterial suspension used during the AST process was diluted to a certain concentration according to the manufacturer’s instructions, which may not be suitable for certain bacteria; and d) there were fewer original bacteria in the non-polluted weather samples than in the polluted weather samples, and fewer original GNB in the air than GPB. Nevertheless, MDR bacteria were detected in both non-polluted and polluted weather, and the actual proportion of MDR bacteria in the atmosphere would be no less than we detected in the present study.

Penicillins were found being resisted by >70% airborne culturable bacteria in both weathers in this study, followed by cephems being resisted by approximately 30% bacteria. Two these classes of antibiotics were also resisted by >85% MDR bacteria. These findings are consistent with β-lactams (including penicillins and cephems) being one of the most highly used antibiotics in China. And Li et al. have also found the β-lactams resistance gene being most abundant in air of a city in China (Li et al., 2018). The main groups of antibiotics for human use in China are similar to those used in other countries (Zhang et al., 2015); moreover, penicillins, along with macrolides and fluoroquinolones, are the main antibiotic active ingredients sold in veterinary medicinal products in the US. People should be aware of this alarming phenomenon and reduce the unnecessary usage of antibiotics.

This study evaluated the airborne antibiotic-resistant bacteria patterns in different weather conditions in Beijing. Airborne AMR bacteria could be causing health problems by contributing to the conservation or expansion of the antibiotic resistance and resistant gene pool in the environment, thereby increasing the risk of resistance transfer to pathogenic microorganisms. It has been proven that resistance genes are capable of moving from animals to the environment and from the environment to the clinic (Cabello, 2006; Forsberg et al., 2012), with the latter representing a great threat to human health. Furthermore, transmission from the clinic to the environment can also occur, causing resistance to rise in environmental microorganisms, which may cause them to emerge as pathogens that continue to transfer resistance genes to other microorganisms. The antibiotic/antibiotic resistance phenomenon and ARGs have been present in the environment since long before their discovery by humans, because microorganisms produce antibiotics to defend themselves against competing organisms. It is widely known that resistant bacteria can be selected when high concentrations of antibiotic drugs are used therapeutically, particularly when these are misused or abused. Subtherapeutic application of antibiotics can also promote the development of antibiotic resistance (Lu et al., 2005). However, it has also been shown that even very low concentrations of antibiotics are sufficient to provide a selective advantage for resistant microorganisms (Gullberg et al., 2011). Furthermore, the presence of heavy metals and biocides could co-select antibiotic resistance in the environment. Resistance genes from this increasing environmental reservoir can then be transferred to pathogenic bacteria (Allen et al., 2010). While the results of this study are essential for public health, regretfully, it cannot be confirmed whether this bacterial resistance originated from the clinic or the environment. Therefore, further research is required to expand our understanding of MDR in the air around China and the world.

In conclusion, an investigation of bacterial composition and AMR pattern of culturable bacteria in the ambient air in polluted and non-polluted weathers was conducted in Beijing. Results indicated that (1) bacterial concentration was significantly higher in polluted weather, compared to non-polluted weather. (2) Polluted weather is correlated with changes in the bacterial composition in the air, with a greater abundance of GNB. (3) Approximately one-fourth of bacteria in the ambient air were MDR, regardless of the weather conditions, which may serve as a potential pathway for antibiotic resistance transfer between bacteria. (4) Penicillins was resisted by over 70% of bacterial isolates from the ambient air. These findings emphasized the importance of studying the antibiotic resistance of bacteria in the air. Future studies may focus on the pathway of AMR and MDR in the human respiratory tract by obtaining a larger number of air samples more frequently and by designing cross-sectional or case-control studies to determine whether the isolation and virulence of pathogens from the air are associated with human or clinical diseases.

Funding

This work was supported by the National Key Research and Development Program of China (No. 2017YFC0702800); and the Health Impact of Air Pollution Program of Chinese Center for Disease Control and Prevention.

Declaration of Competing Interest

The authors declare they have no actual or potential competing financial interests.

Acknowledgments

We thank all participants. We thank professor S Tong who provided help and assistance during our research and manuscript writing. We thank J Ban and Y Hu who provided suggestions during our manuscript writing. We also thank the editor and reviewers for improving the quality of our work.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2019.104936.

References

Allen, H.K., Donato, J., Wang, H.H., Cloud-Hansen, K.A., Davies, J., Handelsman, J., 2010. Call of the wild: antibiotic resistance genes in natural environments. Nat. Rev. Microbiol. 8, 251–259. PMID: 20190823. https://doi.org/10.1038/nrmicro2312.

Allen, J.L., Conrad, K., Oberdörster, G., Johnston, C.J., Sleezer, B., Cory-Slechta, D.A., 2013. Developmental exposure to concentrated ambient particles and preference for immediate reward in mice. Environ. Health Perspect. 121, 32–38. PMID: 23063827. https://doi.org/10.1289/ehp.1205505.

Allen, J.L., Liu, X., Pelkowski, S., Palmer, B., Conrad, K., Oberdörster, G., et al., 2014. Early postnatal exposure to ultrafine particulate matter air pollution: persistent ventriculomegaly, neurochemical disruption, and glial activation preferentially in male mice. Environ. Health Perspect. 122, 935–945. PMID: 24901756. https://doi.org/10.1289/ehp.1307984.

Andersen, A.A., 1958. New sampler for the collection, sizing, and enumeration of viable airborne particles. J. Bacteriol. 76, 471–484 (PMID: 13598704).

Brandl, H., Friicker-Feer, C., Ziegler, D., Mandal, J., Stephan, R., Lehner, A., 2014. Distribution and identification of culturable airborne microorganisms in a Swiss milk processing facility. J. Dairy Sci. 97, 240–246. PMID: 24210492. https://doi.org/3168/j.dts.2013-7028.

Brodie, E.L., DeSantis, T.Z., Parker, J.P.M., Zubieta, I.X., Piceno, Y.M., Andersen, G.L., 2014. Distribution and identification of culturable airborne microorganisms in a Swiss milk processing facility. J. Dairy Sci. 97, 240–246. PMID: 24210492. https://doi.org/3168/j.dts.2013-7028.

Brodie, E.L., DeSantis, T.Z., Parker, J.P.M., Zubieta, I.X., Piceno, Y.M., Andersen, G.L., 2007. Urban aerosols harbor diverse and dynamic bacterial populations. Proc. Nat. Acad. Sci. 104, 299–304. https://doi.org/10.1073/pnas.0608251104.

Cabello, F.C., 2006. Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. Environ. Microbiol. 8, 1137–1144. PMID: 16817922. https://doi.org/10.1111/j.1462-2920.2006.01054.x.

Cao, C., Jiang, W., Wang, B., Fang, J., Lang, J., Tian, G., et al., 2014. Inhalable microorganisms in Beijing’s pm2.5 and pm10 pollutants during a severe smog event. Environ. Sci. Technol. 48, 1499–1507. PMID: 24456276. https://doi.org/10.1021/es4048472.

Chapin, A., Rule, A., Gibson, K., Buckley, T., Schwab, K., 2005. Airborne multidrug-resistant bacteria isolated from a concentrated swine feeding operation. Environ. Health Perspect. 113, 137–142. PMID: 15667049. https://doi.org/10.1289/ehp.7473.

China MEE, 2012a. Ambient Air Quality Standards (GB 3095-2012). Ministry of Ecology and Environment of The People’s Republic of China, Beijing, China.

China MEE, 2012b. Technical Regulation on Ambient Air Quality Index (on Trial) (hj 633–2012). Ministry of Ecology and Environment of The People’s Republic of China, Beijing, China.

Clark, S., Rylander, R., Larsson, L., 1983. Airborne bacteria, endotoxin and fungi in dust
in poultry and swine confinement buildings. Am. Ind. Hyg. Assoc. J. 44, 537–541. PMID: 6613856. https://doi.org/10.1080/15299668391405265. CLSI (Clinical and Laboratory Standards Institute), 2017. Performance standards for antituberculosis susceptibility testing. In: CLSI supplement M100, 27th ed. Clinical and Laboratory Standards Institute. Wayne, PA, USA.
derooij, M.M.T., Hooi, G., Schmitt, H., Janse, J., Swart, A., Maassen, C.R.M., et al., 2019. Insights into livestock-related microbial concentrations in air at residential level in a livestock dense area. Environ. Sci. Technol. https://doi.org/10.1021/acs.est.8b07629. PMID: 31081619.
douwes, J., Thorne, P., Pearce, N., Heederik, D., 2003. Bioaerosol health effects and exposure assessment: progress and prospects. Ann. Occup. Hyg. 47, 187–200 (PMID: 12695832).
deresson, J.B., Oedla, I., M.Ø., Dants, G., 2012. The selection of resistant bacteria at very low antibiotic concentrations. PLoS Pathog. 7, e1002039. https://doi.org/10.1371/journal.ppat.1002039.
ferguson, K.J., Reyes, A., Wang, B., Selleck, E.M., Sommer, M.O., Dantas, G., 2012. The genomics of Actinobacteria: an international expert proposal for interim standard definitions. Environ. Sci. Technol. 46, 18 (PMID: 2296105). 2012.01.043.
ghosh, B., Lai, H., Srivastava, A., 2015. Review of bioaerosols in indoor environment with special reference to sampling, analysis and control mechanisms. Environ. Res. 85, 254–272. PMID: 26436919. https://doi.org/10.1016/j.envres.2015.09.018.
Gorny, R., Dutkiewicz, J., 2002. Bacterial and fungal aerosols in indoor environment in central and eastern European countries. Ann. Agric. Environ. Med. 9, 17–23 (PMID: 12088392).
Gorny, R., Dutkiewicz, J., Kryński-Traczyk, E., 1999. Size distribution of bacterial and fungal bioaerosols in indoor air. Ann. Agric. Environ. Med. 6, 105–113 (PMID: 10607991).
Gullberg, E., Cao, S., Berg, O.G., Ilbäck, C., Sandegren, L., Hughes, D., et al., 2011. Antibiotic resistance in the wild: an eco-evolutionary perspective. Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci. 372https://doi.org/10.1098/rstb.2016.0039. PMID: 27920384.
Hiltunen, T., Virta, M., Laine, A.L., 2017. Antibiotic resistance in the wild: an eco-evolutionary perspective. Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci. 372https://doi.org/10.1098/rstb.2016.0039. PMID: 27920384.