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A tracing method of airborne bacteria transmission across built environments

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ABSTRACT

Disease transmission across built environments has been found to be a serious health risk. Airborne transmission is a vital route of disease infection caused by bacteria and virus. However, tracing methods of airborne bacteria in both lab and field research failed to veritably express the transporting process of microorganism in the air. A new tracing method of airborne bacteria used for airborne transmission was put forward and demonstrated its feasibility by conducting a field evaluation on the basis of genetic modification and bioaerosol technology. A specific gene fragment (pFPV-mCherry fluorescent protein plasmid) was introduced into nonpathogenic *E. coli DH5α* as tracer bacteria by high-voltage electroporation. Gel electrophoresis and DNA sequencing proved the success of the synthesis. Genetic stability, effect of aerosolization on the survival rate of tracer bacteria, and the application of the tracer bacteria to the airborne bacteria transmission were examined in both lab and field. Both the introduced plasmid stability rates of tracer *E. coli* in pre-aerosolization and post-aerosolization were above 95% in five test days. Survival rate of tracer *E. coli* at 97.5% ± 1.2% through aerosolization was obtained by an air-atomizer operated at an air pressure of 30 Psi. In the field experiment, the airborne transmission of *E. coli* between poultry houses was proved and emitted *E. coli* was more easily transmitted into self-house than adjacent house due to the ventilation design and weather condition. Our results suggested that the tracing method of airborne bacteria was available for the investigation of airborne microbial transmission across built environments.

1. Introduction

Airborne transmission which can occur between individuals, buildings and regions has been known as a vital route of disease infection caused by aerosol or respiratory with bacteria and virus. Severe acute respiratory syndrome (SARS) caused worldwide severe epidemic outbreak in 2003 [1]. Air was verified as one of the SARS transmission pathways across built environments by the epidemiological proof and numerical simulation [2]. Airborne transmission was also proved as an important mode of Influenza A virus spread [3,4]. For animal production, above 50 million chickens and turkeys in USA died of highly pathogenic avian influenza (HPAI) or were destroyed to prevent the disease spread between December 2014 and June 2015, involving Iowa and Minnesota and other adjacent states [5–7]. Evidences obtained from both laboratory-simulated process and field investigation supported the existence of airborne transmission of HPAI virus [8,9].

A complete process of airborne disease transmission included: 1) aerosolization of microorganism, 2) movement and decay of airborne microorganism, 3) pathogen infection. Laboratory-scale manual controlled experiments had proved that short distance airborne transmission of several infectious pathogens could occur from animal to animal [8,10–12]. Field studies, mostly focused on human cases and presented as evidences of airborne transmission between individuals, rooms or residential buildings, had also demonstrated the existence of airborne transmission of virus and bacteria [2,13–20]. Homology detection using ERIC-PCR and REP-PCR was applied in animal farm to conduct the source identification of airborne *E. coli* surrounding swine house [21]. The airborne transmission of porcine reproductive and respiratory

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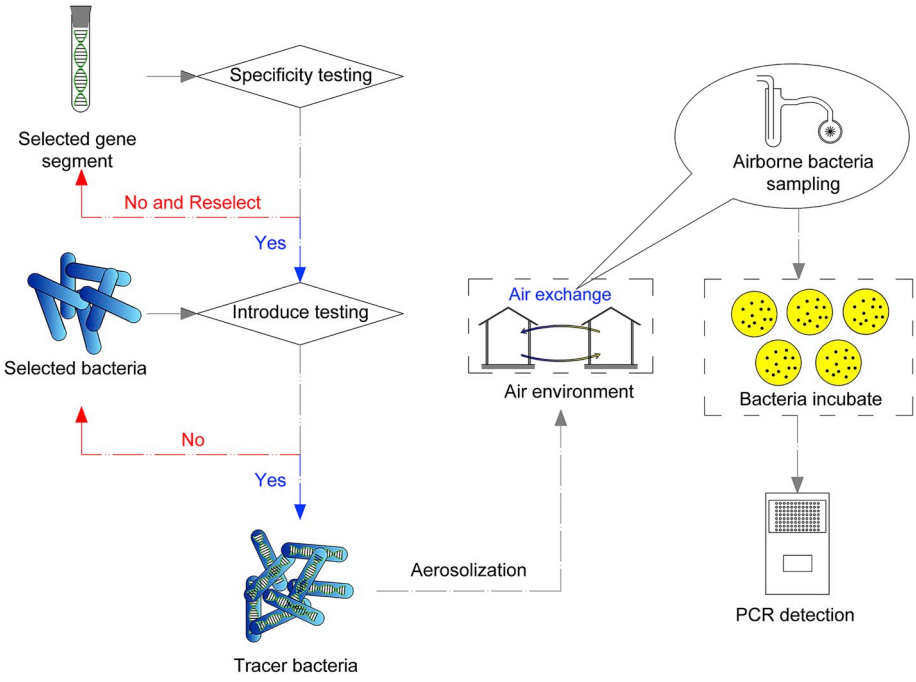

Fig. 1. Flowchart of bacterial tracing method for airborne transmission.

syndrome virus (PRRS) between buildings and via long spatial distance were supported based on virus homology between sampling points [22,23]. Still, it was challenged to directly certify the air transmission of microorganism by homology detection due to its inability to suppress the interference of surrounding microorganism. Results-orientated research of airborne transmission neglected the transporting process of the microorganism in the air and was lack of quantitative description of airborne pathogen concentration. Tracer was required to express the movement and decay of airborne microorganism in both experiment and numerical simulation.

To comprehend the route of airborne transmission, tracer had been applied into both lab and field experiment. Tracer gases such as N_2O, SF_6, R134a and He were widely used to investigate the spread of airborne microorganism in indoor and outdoor environment by means of computational fluid dynamics (CFD) for its quantitative evaluation of the cross-infection risks and low-budget [24–30]. However, airborne microorganism transporting process across built environments was unable to be interpreted thoroughly by tracer gases for their distinct absence of physical and biological characteristics of airborne microorganism. In CFD, the tracer gases were generally represented by passive scalars, which were just following the air movement. Particles (e.g., fluorescent particles) and some microbial surrogate were also considered as tracers to investigate the airborne transmission of microorganism [31–37]. DNA barcoded aerosol, as a novel test particle overcoming the effect of test material from pre-existing environmental or background contaminants, was developed to track the fate of the airborne particle in built environment [38,39]. However, airborne microorganism tracing was still a big challenge for its reliability on environment factor, complicated detection, and species-dependence. The decay of airborne microorganism was indispensable factor in both empirical and theoretical models of airborne transmission, which was difficult to be quantified [2,40,41]. Inlet air filter to remove the effect of background concentration was indispensable in the use of tracer bacteria without specificity in UVGI studies [42–44]. Whereas, the lack of biological characteristics for particles or narrow application of microbial surrogate in clean or isolated room prevented it to be an ideal substitution of airborne microorganism. Totally, current tracers of airborne microorganism failed to veritably express the airborne transporting process in atmosphere. Both physical and biological characteristics of bacterial tracer made it a more reasonable substitution of current tracers of airborne microorganism.

Gene modification of bacteria could make it a unique existence in particular environment. Specific gene strain tracing had already been applied in many food safety-related studies to monitor their fate in complex environment or in laboratory animal medicine to explore the distribution of target bacteria in internal organs [45,46]. However, there was still a knowledge gap of gene-modified bacteria in the trace of airborne microorganism. Aerosolization of bacteria, which was susceptible to the suspending medium and shear stress, was widely performed in lab-experiment such as air sterilization and air infection [47–55]. Aerosolization and gene modification of bacteria could be jointly used for tracing airborne microorganism. The new method which could fulfill the following three conditions was essential to set up for airborne bacteria tracing across built environments.

i) The modified gene should be specific to make it distinguished in surrounding environment.
ii) Selected bacteria could be genetic-modified as a bacterial tracer.
iii) Aerosolization should have little impact on the survival of the bacteria and the stability of the modified gene.

E. coli was selected in this method due to its universality, culturability and non-pathogenicity of certain category. pFPV-mCherry fluorescent protein plasmid was the selected gene, which was introduced into the *E. coli* by high-voltage electroporation. The objective of this article is to establish a tracing method of airborne bacteria transmission and demonstrate its feasibility by conducting a field evaluation across built environments.

2. Materials and methods

2.1. Establishment of airborne bacterial tracing method

The tracing method of airborne bacteria transmission was summarized in Fig. 1. Selected gene fragment should be specific so that it could not be found in the air environment of the place where the research was conducted. Besides, the selected gene fragment should be compatible so that it could be introduced into the selected

nonpathogenic bacteria without affecting its reproduction. The tracer bacteria were synthetized successfully when both of the two above conditions were accomplished, or new gene fragment needed to be selected and tested again. The finished tracer bacteria could be aerosolized into the air environment at a source point using a nebulizer, and airborne bacteria sampling was conducted at the target points. Taking the activity of the airborne bacteria into consideration, the bacteria sampled at the target points should be incubated on solid selective medium, followed by PCR assay and Gel electrophoresis which were used for the detection of the tracer bacteria. Finally, the concentrations of the tracer bacteria and the ratios of the tracer bacteria to the total selected bacteria at the source and different target points could be used for analyzing the transporting process of the microorganism in the air.

Serval considerations needed to be taken for the application of this bacterial tracing method. Firstly, specificity tests to confirm the absence of the selected gene fragment in the air environment should be performed. Liquid collision sampling technology of airborne bacteria could be used for the airborne bacteria sampling, followed by PCR assay and Gel electrophoresis analysis to determine whether the selected gene fragment existed or not. Secondly, a successful synthesis of the tracer bacteria should satisfy three simultaneous conditions: natural reproduction capacity, positive reaction of PCR assay and Gel electrophoresis, correct genetic sequencing. Thirdly, the airborne bacteria sampled after the aerosolization needed to be incubated on the solid medium since only the active bacteria which could be cultured on the solid medium was considered.

2.2. Synthesis of the tracer bacteria

Non-pathogenic *E. coli DH5α* and pFPV-mCherry fluorescent protein plasmid were chosen as the selected bacteria species and the selected gene fragment in this experiment, respectively. High-voltage electroporation was used to introduce the pFPV-mCherry fluorescent protein plasmid into the *E. coli DH5α*. The isolated and identified tracer *E. coli* was kept in −48 °C for backup. A primer was designed to amplify the 550bp region of pFPV-mCherry fluorescent protein plasmid.

Forward primer: CACGAGTTCGAGATCGAGGG
Reverse primer: GGTGTAGTCCTCGTTGTGGG

E. coli DH5α is a nonpathogenic strain, which is developed for the use of laboratory clone. The cell of *E. coli DH5α* is Gram-negative and its colony present circular pink and opaque on McConkay agar medium plate.

2.3. DNA extraction and PCR amplification

Single *E. coli* colony was randomly isolated and cultured in liquid media at 37 °C for 6 h. Afterwards, the liquid media with the *E. coli* was centrifugated at 4 °C and 8000 rpm (5724 g) for 2 min. Supernatant was discarded and 2 mL sterilized deionized water was poured into the centrifugal tube and resuspended, which was repeated for 3 times. Water bath at 100 °C for 5 min was used to extract the bacteria gene. The extracted gene was amplified by PCR assay (95 °C for 5 min, followed by 35 cycles at 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s and a final extension at 72 °C for 10 min) using the primer described above. PCR reaction was performed in triplicate 25 μL mixture containing 22 μL Mix, 1 μL of each primer and 1 μL sample.

2.4. Tracer bacteria aerosolization and sampling

Frozen beads containing tracer *E. coli* were rolled on a sterilized solid selective medium of *E. coli* before being incubated at 37 °C for 24 h. Single colony of tracer *E. coli* was scraped off and transferred into sterilized liquid nutrient broth (Qingdao Hope Bio-Technology Co., Ltd, Qingdao, China) before being incubated at 37 °C for 24 h.

An air-atomize spraying nozzle or nebulizer could be used to aerosolize the liquid nutrient broth with tracer *E. coli*. Airborne bacteria sampler (e.g. liquid collision sampler or Anderson sampler) could be used to sample airborne *E. coli*. In this study, an air-atomize spraying nozzle (Jieao Industrial Spraying Co. Ltd. Guangzhou, China) was used to aerosolize the liquid nutrient broth with tracer *E. coli*. The flow rate of the air-atomize spraying nozzle was set at 100 mL/min and the air pressures were set at 30, 45, 60 Psi. The Dv(50) and Span of the droplets produced by the air-atomize spraying nozzle operated at a flow rate of 100 mL/min and air pressures of 30, 45, 60 Psi were (48.12 ± 5.13 μm, 1.43 ± 0.08), (42.83 ± 3.49 μm, 1.61 ± 0.11), (35.27 ± 4.71 μm, 1.32 ± 0.06), respectively. All-glass airborne bacteria impingers (AGI-30, Ace Glass, Inc., Vineland, N.J., USA) were used to collect the airborne *E. coli*.

2.5. Genetic stability of plasmid

The genetic stability of tracer bacteria (Gene-modified *E. coli DH5α* in this experiment) was accessed by continuous passage method. The conjugant (pFPV-mCherry fluorescent protein plasmid + *E. coli DH5α*) was cultured in liquid culture medium and 100 μL of the bacteria solution was transferred to new liquid culture medium every 12 h for five days. Duplicate 0.1 mL aliquots of the diluted sample were transferred on four Petri dishes with sterilized tryptic soy agar every 24 h and incubated for 24 h. Ninety-six colonies on the Petri dishes (twenty-four colonies from each Petri dish) were randomly selected to conduct DNA extraction, PCR assay and Gel electrophoresis analysis. Stability rate of the plasmid is the ratio of the quantity of the positive reacted bacteria to ninety-six.

2.6. Effect of aerosolization on the tracer bacteria

Both the survival of tracer *E. coli* and genetic stability of plasmid after aerosolization were measured and quantified by survival rate and stability rate. The tracer *E. coli* solutions in pre-aerosolization and post-aerosolization were diluted and transferred on five Petri dishes with selective medium of *E. coli*, respectively. The survival rate of tracer *E. coli* after aerosolization is the ratio of the *E. coli* concentration in pre-aerosolized solution to that in post-aerosolized solution Stability rate of the plasmid was defined above.

2.7. Field validation of the tracing method in layer hen farm

Field experimental site: The field experiment of airborne bacteria transmission was carried out between two side-by-side layer hen houses with the approval of a farm located in Sichuan Province of China from December 2017 to January 2018 (Fig. 2a and b). Each experimental house (12.0 m W × 96.0 m L) had a capacity of 65,000 Roman hens (28 week of hen age at the start of the field experiment) and was equipped with manure belts to remove manure out of the house every three days. Both layer hen housing facilities and the environment control system were supplied by Big Dutchman (Vechta, Germany). The distance between the two experimental houses was 11 m and the height of the side-wall inlet (0.8 m × 0.5 m) was 6.1 m. The twenty-eight inlets and three exhaust fans evenly distributed in each side-wall were used for the air exchange in winter. Inside and outside temperature & relative humidity were monitored using HOBO data loggers (Onset Computer Corp., Bourne, MA, USA) with a 5-min interval. Wind speed and direction were recorded by the weather station (BX-5, Jingge Co., Ltd, Beijing, China) installed in the farm.

The specificity test before each release indicated that pFPV-mCherry fluorescent protein plasmid was not found in the surrounding environment of the two houses. An air-atomize spraying nozzle was used to aerosolize the liquid nutrient broth with tracer *E. coli* at a flow rate of 100 mL/min and an air pressure of 30 Psi. As shown in Fig. 2, the nozzle was installed at the height of the fan axis and 70 cm away from the fan

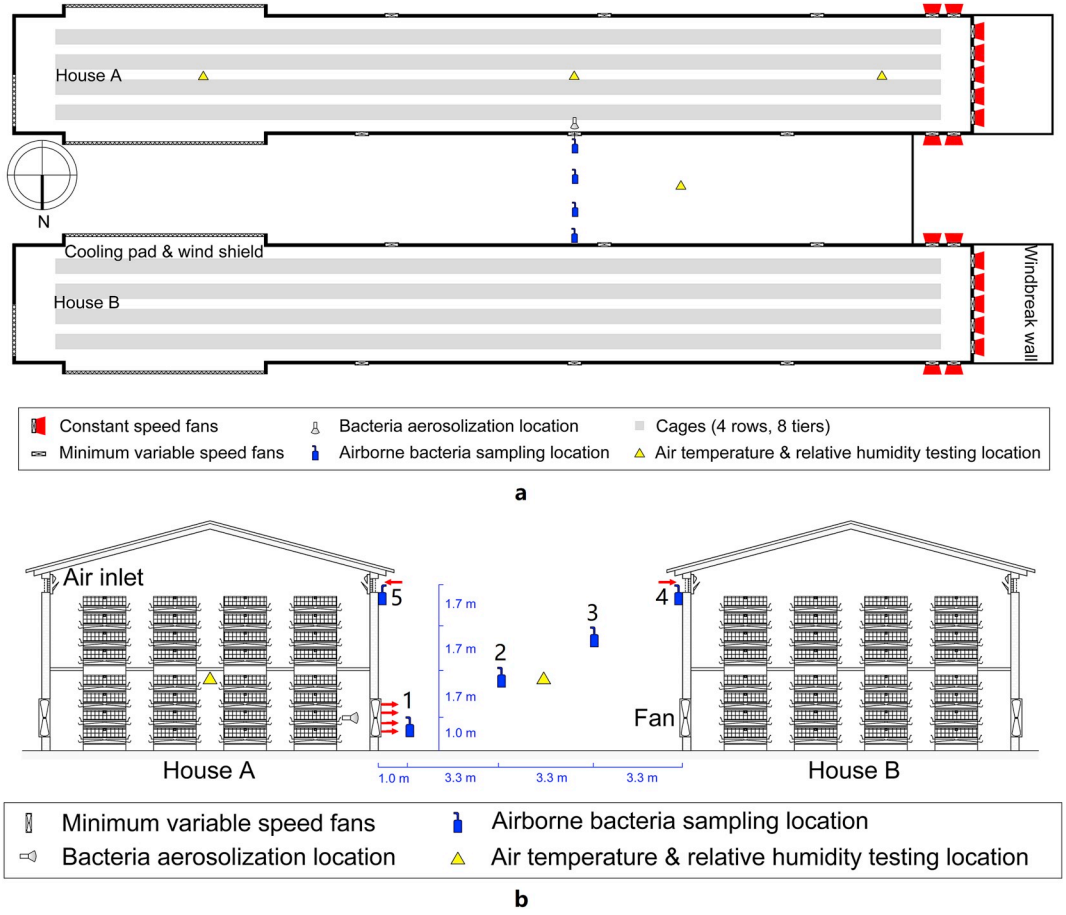

Fig. 2. Layer hen houses in field experiment site (a: Top view of the experimental layer hen house; b: Cross-sectional view of the experimental layer hen house).

inside the layer hen house. All-glass airborne bacteria impingers (AGI-30, Ace Glass, Inc., Vineland, N.J., USA) were used to collect the airborne *E. coli* between two houses (points 1-5). The impingers were sterilized in an autoclave at 121 °C and 100 kPa for 15 min before each sampling. Each airborne *E. coli* sampling lasted 15 min.

The airborne *E. coli* were collected in 15 mL of sterilized 0.9% physiological saline solution in the impinger at a stable flow rate of 12.5 L/min. Duplicate 0.1 mL aliquots of the concentrated sample were transferred on five petri dishes with selective medium of *E. coli*. Then the Petri dishes were incubated at 37 °C for 24–48 h. Total *E. coli* concentration was calculated according to the colony count on the petri dishes. 90 colonies of all the *E. coli* colonies (or total *E. coli* colonies if the total *E. coli* colonies were less than 90) were randomly isolated and cultured in liquid media at 37 °C for 6 h for DNA extraction and PCR assay. The proportion of tracer *E. coli* account for the total *E. coli* could be used to calculate the tracer *E. coli* concentration.

2.8. Experimental operation

Each trial, including tracer *E. coli* aerosolization and airborne *E. coli* sampling at points 1-5, was carried out from 16:00 to 17:00 once a week. Specificity test was conducted to determine the specificity of tracer *E. coli* before each trial. Five experimental trials were conducted in this study. The pFPV-mCherry fluorescent protein plasmid was not found by PCR assay and Gel electrophoresis analysis before each experiment trial.

3. Results

3.1. Synthesis and genetic stability of tracer bacteria

Result of Gel electrophoresis indicated that the pFPV-mCherry fluorescent protein plasmid was successfully introduced into *E. coli DH5α* (Fig. 3). Gene sequencing analysis (Conducted by Tsingke Biological Technology Ltd., Beijing, China) also proved the successful introducing of the pFPV-mCherry fluorescent protein plasmid accurately in molecular level. Stability rate of the introduced pFPV-mCherry fluorescent protein plasmid was tested in five days to cater for application duration of the tracer *E. coli*. Results showed that stability rate of introduced plasmid was above 95% in all five test days with little fluctuation (Fig. 4).

3.2. Assessment of the effect of aerosolization on the tracer bacteria

The survival rate of tracer *E. coli* through aerosolization decreased with the increase of the operated pressure of the air-atomize spraying nozzle. The survival rate of tracer *E. coli* was 97.5%, 70.8%, and 61.3% at the operated pressure of 30, 45, and 60 Psi, respectively (Fig. 5). 30

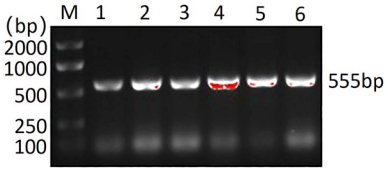

Fig. 3. Gel electrophoresis map of the pFPV-mCherry fluorescent protein plasmid.

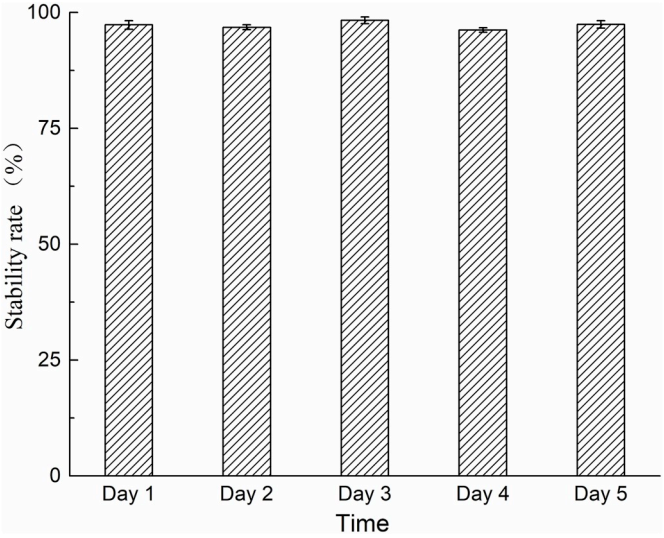

Fig. 4. Stability rate of introduced plasmid before aerosolization.

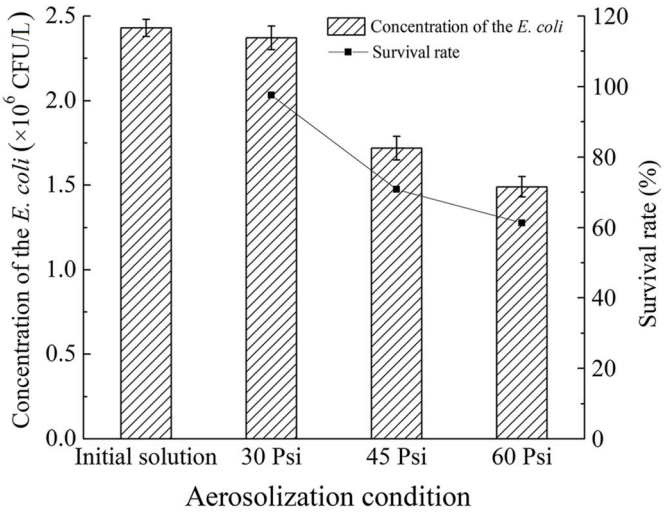

Fig. 5. Survival rate of tracer bacteria after aerosolization.

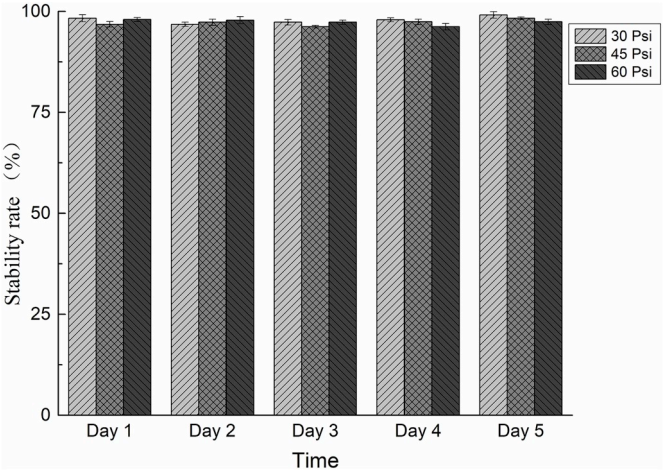

Fig. 6. Stability rate of introduced plasmid after aerosolization.

Psi was determined as the optimal operated pressure of the selected air-atomize spraying nozzle in field application of tracer bacteria. However, there was no difference at stability rate of the pFPV-mCherry fluorescent protein plasmid among different operated pressure of the air-atomize spraying nozzle (Fig. 6). Results showed that stability rate of introduced plasmid was above 95% in all five test days with little fluctuation.

Table 1
Environmental condition in the field site.

Parameter	Room	Ambient
Temperature (°C)	20.1–23.0	0.2–5.7
Humidity (%)	58.3–92.5	70.3–97.6
Wind (speed, direction)	—	<0.8 m/s, astatic

3.3. Airborne bacteria transmission between layer hen houses based on tracer bacteria

Environment parameters (temperature, relative humidity, and wind) were shown in Table 1. The room temperature of the experimental house ranged 20.1–23.0 °C, which was higher than ambient temperature 0.2–8.7 °C. Room relative humidity (58.3–92.5%) was more fluctuant than the ambient relative humidity. The wind speed between the two houses was <0.8 m/s with an astatic direction for the windbreak of the houses.

The tracer *E. coli* concentrations measured at the five sampling points were shown in Fig. 7. Original *E. coli* concentration existed in the air environment of the field site was 18 ± 6 CFU/m^3 and the ratios of the tracer *E. coli* to total *E. coli* were larger than 98% at all five sampling points. The concentrations of tracer *E. coli* decreased from sampling point 1 to 4 with the increase of the distance from the source point. The concentration of tracer *E. coli* at sampling point 5 was higher than sampling point 4, which indicated emitted *E. coli* was more easily transmitted into self-house than adjacent house (48 ± 7 vs 21 ± 5 CFU/m^3 at source intensity of 828 ± 173 CFU/m^3).

4. Discussion

The present trace study of bacteria across built environments was the first attempt to explore airborne bacteria transmission with the consideration of biological characteristics of airborne microorganism. Specifically, Gene-modification technology was utilized to labelled *E.*

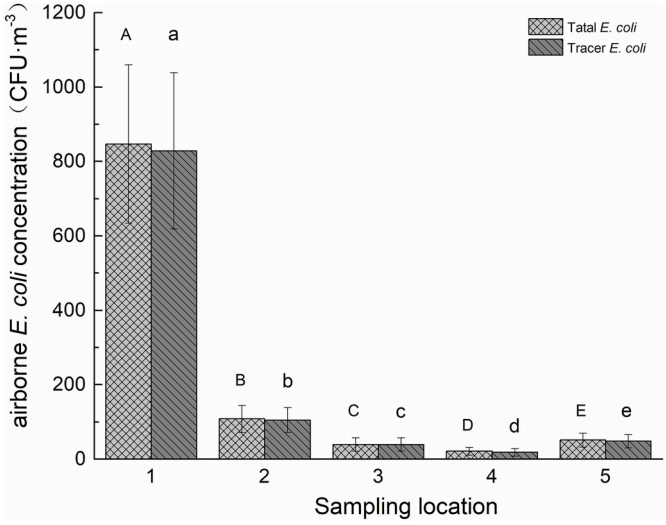

Fig. 7. Concentration of total airborne *E. coli* and tracer *E. coli* at different sampling locations. Different capital letters mean the concentrations of total airborne *E. coli* at different sampling locations are different ($P < 0.05$); Different lowercase letters mean the concentrations of tracer airborne *E. coli* at different sampling locations are different ($P < 0.05$).

coli DH5α with pFPV-mCherry fluorescent protein plasmid, which distinguished the tracer bacteria with the homogeneous background bacteria. Feasibility of *E. coli DH5α* gene-modification and high stability rate of pFPV-mCherry fluorescent protein plasmid in the passage of *E. coli DH5α* made it practicable for the trace of airborne bacteria. Assessment result of the effect of aerosolization on the survival rate of tracer *E. coli* and stability rate of introduced plasmid supported its application in airborne transmission. Additionally, its field application directly demonstrated the existence of airborne transmission between animal houses at the current mode of ventilation system design.

The three hypotheses we proposed to validate this new airborne bacterial tracing method were all tested. As a foreign gene, the specificity of pFPV-mCherry fluorescent protein plasmid was verified at the first time. However, tracer bacteria were also imported into the experimental site by aerosolization, which urged us to verify the specificity of introduced plasmid before each trial. Five specificity tests conducted before each trial indicated no existence of tracer *E. coli* or pFPV-mCherry fluorescent protein plasmid in field validation of the tracing method between lay hen houses, which could be interpreted by low imported concentration and unsuitability of survival for airborne microorganism. Above 95% stability rate of the introduced plasmid in *E. coli DH5α* proved that the selected bacteria could be genetic-modified as a bacterial tracer. *E. coli O157:H7* labeled with green fluorescent protein (GFP) applied for tracking of introduced foodborne pathogens to monitor their fate in complex environments also supported its feasibility [45]. Culturability and cell membrane integrity of bacteria could be affected by aerosolization resulting in free DNA release [56,57], which was agreed with our study. Rational operating pressure used by aerosolization reduced its impact on the survival rate of the bacteria. Totally, the feasibility of this new tracing method based on gene-modification and bioaerosol aerosolization was confirmed rationally and practically.

The decay of airborne microorganism (performed as particles) and particle was a non-ignorable parameter in empirical and theoretical models of airborne transmission [2,40,41]. With no consideration of the biological decay of airborne microorganism, some spread pattern of virus particles needs further validation in future [2,30]. Both physical and biological characteristics of airborne microorganism determined the difference of tracing method between airborne microorganism with particulate matter and gas. The tracing method mentioned in this article would contribute to the quantitative description in the dispersion of airborne microorganism and the validation of empirical and theoretical airborne transmission models in atmosphere.

In field validation of the method, the results proved that the airborne transmission of *E. coli* happened between two layer hen houses and more tracer *E. coli* went back to self-house than to the adjacent house in condition of present ventilation mode of the house and weather condition in winter. Exhausted air would rise and easily reach to the inlets of self-house due to the temperature difference between exhausted air and ambient air. On a broader scale, microorganism could be shed via/from activity (coughing, sneezing, talking, or breathing), skin and excrement of individuals and aerosolized into airborne microorganism [41,58–61]. Large amount of particulate matter and microorganism, including pathogens, were emitted from animal houses by the ventilation system [62–65], which resulted in higher concentration of particulate matter and airborne microorganism in their vicinity [66,67]. An airborne transmission model, which obtained from Gaussian Plume Model by incorporating the dust deposition process, pathogen decay, and a model for the infection process on exposed farms, revealed that wind-borne route alone was insufficient when compare transmission risk pattern predicted by the model with the pattern observed during the 2003 epidemic [68]. However, biological decay was seldom taken into account for its complexity and difficulty of quantification.

Additionally, the biological decay of airborne microorganism could be expressed in three ways: decay rate or death rate, survival, and half-time [41]. However, species-independence of airborne microorganism's biological decay indicated its large difference among diverse microbials. Unculturability and infectivity of certain microorganism made it unsuitable to conduct practically field research on airborne transmission. Accordingly, “relative biological decay” meaning the ratio of biological decay of the target microorganism species to that of the selected standard microorganism species at given environment conditions was proposed in this article, which could contribute to the study of airborne microbial transmission with pathogenicity in atmosphere safely and simply.

However, the present tracing method has several limitations. Firstly, wet aerosolization used in this method does imitate the fate of microbials expelled from respiratory tracts in wet aerosols. Nevertheless, the other aerosolization way of microbials (e.g. dry aerosolization) needs further study when airborne microorganisms originated from different source were considered. Secondly, bioaerosol particle with single diameter is hard to generated, which results in blend with multiple factors. In this field validation experiment, particle size was not taken into consideration. Thirdly, although precise identification is achieved by this airborne bacterial tracing method, the operation procedure was complicated for essential bacteria culture and large amount of PCR amplification. Fourthly, risk of experimental site infection by tracer bacteria was one potential obstacle for duplicate test. This airborne bacterial tracing method depends largely on the improvement of bioaerosol technology. Applicable complexity of this airborne bacterial tracing method will be reduced in the future.

In summary, this tracing method of airborne bacteria transmission across built environments provides a novel and integrated way to conduct airborne transmission research of disease. To our knowledge, this is the first bacterial tracing method to veritably express airborne transmission with the consideration of physical and biological characteristics of airborne bacteria. This method warrants further investigation and needs to be improved with the development of bioaerosol technology.

5. Conclusion

The novel airborne bacterial tracing method with the consideration of physical decay and biological decay was designed and used to explore the airborne transmission in layer hen farm. Results provide findings as following:

1) The stability rate of introduced plasmid into tracer *E. coli* was above 95% in all five test days with little fluctuation. The aerosolization of tracer *E. coli* with air-atomize spraying nozzle at operated pressure of 30 Psi could get a survival rate of 97.5%.
2) Airborne bacteria transmission between layer hen houses was proved with the novel tracing method. In the experimental duration, the released tracer *E. coli* could reach both the inlets of the self-house and adjacent house, and the intensity of its transport into self-house is higher than into adjacent house.

Declarations of interest

None.

Conflicts of interest

We declare no conflict of interest.

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References

[1] N. Lee, D. Hui, A. Wu, P. Chan, P. Cameron, G.M. Joynt, A. Ahuja, M.Y. Yung, C.B. Leung, K.F. To, S.F. Lui, C.C. Szeto, S. Chung, J.J.Y. Sung, A major outbreak of severe acute respiratory syndrome in Hong Kong, N. Engl. J. Med. 348 (20) (2003) 1986–1994.

[2] I.T.S. Yu, Y. Li, T.W. Wong, W. Tam, A.T. Chan, J.H.W. Lee, D.Y.C. Leung, T. Ho, Evidence of airborne transmission of the severe acute respiratory syndrome virus, N. Engl. J. Med. 350 (17) (2004) 1731–1739.

[3] B.J. Cowling, D.K.M. Ip, V.J. Fang, P. Suntarattiwong, S.J. Olsen, J. Levy, T.M. Uyeki, G.M. Leung, J.S. Malik Peiris, T. Chotpitayasunondh, H. Nishiura, J. Mark Simmerman, Aerosol transmission is an important mode of Influenza A virus spread, Nat. Commun. 4 (2013) 1935.

[4] R. Tellier, Aerosol transmission of Influenza A virus: a review of new studies, J. R. Soc. Interface 6 (2009) S783–S790.

[5] J.L. Greene, Update on the Highly-Pathogenic Avian Influenza Outbreak of 2014-2015, CRS, 2015.

[6] S. Ramos, M. MacLachlan, A. Melton, Impacts of the 2014-2015 Highly Pathogenic Avian Influenza Outbreak on the U.S. Poultry Sector, USDA, 2017.

[7] E. Spackman, M.J. Pantin-Jackwood, D.R. Kapczynski, D.E. Swayne, D.L. Suarez, H5N2 Highly Pathogenic Avian Influenza Viruses from the US 2014-2015 outbreak have an unusually long pre-clinical period in turkeys, BMC Vet. Res. 12 (1) (2016) 260.

[8] K. Bertran, C. Balzli, Y.K. Kwon, T.M. Tumpey, A. Clark, D.E. Swayne, Airborne transmission of highly pathogenic influenza virus during processing of infected poultry, Emerg. Infect. Dis. 23 (11) (2017) 1806–1814.

[9] M. Torremorell, C. Alonso, P.R. Davies, P.C. Raynor, D. Patnayak, M. Torchetti, B. McCluskey, Investigation into the airborne dissemination of H5N2 highly pathogenic avian influenza virus during the 2015 spring outbreaks in the midwestern United States, Avian Dis. 60 (3) (2016) 637–643.

[10] X. Li, Y. Shi, Q. Liu, Y. Wang, G. Li, Q. Teng, Y. Zhang, S. Liu, Z. Li, Airborne transmission of a novel Tembusu virus in ducks, J. Clin. Microbiol. 53 (8) (2015) 2734–2736.

[11] C.J. Oliveira, L.F. Carvalho, T.B. Garcia, Experimental airborne transmission of Salmonella Agona and Salmonella Typhimurium in weaned pigs, Epidemiol. Infect. 134 (1) (2006) 199–209.

[12] J.M. Warfel, J. Beren, T.J. Merkel, Airborne transmission of Bordetella pertussis, J. Infect. Dis. 206 (6) (2012) 902–906.

[13] B.F. Buss, A. Keyser-Metobo, J. Rother, L. Holtz, K. Gall, J. Jereb, C.N. Murphy, P.C. Iwen, S. Robbe-Austerman, M.A. Holcomb, P. Infield, Possible airborne person-to-person transmission of Mycobacterium Bovis - Nebraska 2014-2015, MMWR Morb. Mortal. Wkly. Rep. 65 (8) (2016) 197–201.

[14] D.W. Fraser, Legionellosis: evidence of airborne transmission, Ann. N. Y. Acad. Sci. 353 (1980) 61–66.

[15] S. Hara, H. Yamamoto, A. Kawabata, T. Azuma, S. Ishii, N. Okumura, Y. Ito, Airborne transmission from a neonate with Netherton syndrome during an outbreak of MRSA, Pediatr. Int. 58 (6) (2016) 518–520.

[16] J.M. Leclair, J.A. Zaia, M.J. Levin, R.G. Congdon, D.A. Goldmann, Airborne transmission of chickenpox in a hospital, N. Engl. J. Med. 302 (8) (1980) 450–453.

[17] J. Mao, N. Gao, The airborne transmission of infection between flats in high-rise residential buildings: a review, Build. Environ. 94 (2015) 516–531.

[18] P.J. Marks, I.B. Vipond, D. Carlisle, D. Deakin, R.E. Fey, E.O. Caul, Evidence for airborne transmission of Norwalk-like virus (NLV) in a hotel restaurant, Epidemiol. Infect. 124 (3) (2000) 481–487.

[19] P.J. Marks, I.B. Vipond, F.M. Regan, K. Wedgwood, R.E. Fey, E.O. Caul, A school outbreak of Norwalk-like virus: evidence for airborne transmission, Epidemiol. Infect. 131 (1) (2003) 727–736.

[20] S. Xiao, Y. Li, M. Sung, J. Wei, Z. Yang, A study of the probable transmission routes of MERS-CoV during the first hospital outbreak in the Republic of Korea, Indoor Air 28 (1) (2018) 51–63.

[21] H. Duan, T. Chai, J. Liu, X. Zhang, C. Qi, J. Gao, Y. Wang, Y. Cai, Z. Miao, M. Yao, G. Schlenker, Source identification of airborne Escherichia coli of swine house surroundings using ERIC-PCR and REP-PCR, Environ. Res. 109 (5) (2009) 511–517.

[22] S. Dee, S. Otake, S. Oliveira, J. Deen, Evidence of long distance airborne transport of porcine reproductive and respiratory syndrome virus and Mycoplasma hyopneumoniae, Vet. Res. 40 (4) (2009) 1–13.

[23] A. Pitkin, J. Deen, S. Dee, Use of a production region model to assess the airborne spread of porcine reproductive and respiratory syndrome virus, Vet. Microbiol. 136 (1–2) (2009) 1–7.

[24] Z.T. Ai, A.K. Melikov, Airborne spread of expiratory droplet nuclei between the occupants of indoor environments: a review, Indoor Air 28 (4) (2018) 500–524.

[25] N.P. Gao, J.L. Niu, M. Perino, P. Heiselberg, The airborne transmission of infection between flats in high-rise residential buildings: tracer gas simulation, Build. Environ. 43 (11) (2008) 1805–1817.

[26] N.P. Gao, J.L. Niu, M. Perino, P. Heiselberg, The airborne transmission of infection between flats in high-rise residential buildings: particle simulation, Build. Environ. 44 (2) (2009) 402–410.

[27] J. Hang, Y. Li, W.H. Ching, J. Wei, R. Jin, L. Liu, X. Xie, Potential airborne transmission between two isolation cubicles through a shared anteroom, Build. Environ. 89 (2015) 264–278.

[28] L. Liu, Y. Li, P.V. Nielsen, J. Wei, R.L. Jensen, Short-range airborne transmission of expiratory droplets between two people, Indoor Air 27 (2) (2017) 452–462.

[29] I.-H. Seo, I.-B. Lee, O.-K. Moon, N.-S. Jung, H.-J. Lee, S.-W. Hong, K.-S. Kwon, J.P. Bitog, Prediction of the spread of highly pathogenic avian influenza using a multifactor network: Part 1 – development and application of computational fluid dynamics simulations of airborne dispersion, Biosyst. Eng. 121 (2014) 160–176.

[30] J. Wei, J. Zhou, K. Cheng, J. Wu, Z. Zhong, Y. Song, C. Ke, H.-L. Yen, Y. Li, Assessing the risk of downwind spread of avian influenza virus via airborne particles from an urban wholesale poultry market, Build. Environ. 127 (2018) 120–126.

[31] N.P. Gao, J.L. Niu, Modeling particle dispersion and deposition in indoor environments, Atmos. Environ. 41 (18) (2007) 3862–3876.

[32] J. Lavoie, G. Marchand, Y. Cloutier, S. Hallé, S. Nadeau, C. Duchaine, G. Pichette, Evaluation of Bioaerosol Exposures During Hospital Bronchoscopy Examinations, 17 (2014), pp. 288–299 (2).

[33] W.T. Leung, G.N. Sze-To, C.Y. Chao, S.C. Yu, J.K. Kwan, Study on the interzonal migration of airborne infectious particles in an isolation ward using benign bacteria, Indoor Air 23 (2) (2013) 148–161.

[34] X. Li, J. Niu, N. Gao, Co-occupant's exposure to exhaled pollutants with two types of personalized ventilation strategies under mixing and displacement ventilation systems, Indoor Air 23 (2) (2013) 162–171.

[35] D. Rim, A. Novoselac, Transport of particulate and gaseous pollutants in the vicinity of a human body, Build. Environ. 44 (9) (2009) 1840–1849.

[36] Y. Yang, W.Y. Chan, C.L. Wu, R.Y. Kong, A.C. Lai, Minimizing the exposure of airborne pathogens by upper-room ultraviolet germicidal irradiation: an experimental and numerical study, J. R. Soc. Interface 9 (77) (2012) 3184–3195.

[37] P. Zhou, Y. Yang, A.C.K. Lai, G. Huang, Inactivation of airborne bacteria by cold plasma in air duct flow, Build. Environ. 106 (2016) 120–130.

[38] K.C. Dannemiller, N. Lang-Yona, N. Yamamoto, Y. Rudich, J. Peccia, Combining real-time PCR and next-generation DNA sequencing to provide quantitative comparisons of fungal aerosol populations, Atmos. Environ. 84 (2014) 113–121.

[39] R.N. Harding, C.A. Hara, S.B. Hall, E.A. Vitalis, C.B. Thomas, A.D. Jones, J.A. Day, V.R. Tur-Rojas, T. Jorgensen, E. Herchert, R. Yoder, E.K. Wheeler, G.R. Farquar, Unique DNA-barcoded aerosol test particles for studying aerosol transport, Aerosol Sci. Technol. 50 (5) (2016) 429–435.

[40] B. Lighthart, A.S. Frisch, Estimation of viable airborne microbes downwind from a point source, Appl. Environ. Microbiol. 31 (5) (1976) 700–704.

[41] Y. Zhao, A. Aarnink, M. De Jong, P.W.G. Groot Koerkamp, Airborne Microorganisms from Livestock Production Systems and Their Relation to Dust, (2014).

[42] C.B. Beggs, C.J. Noakes, P.A. Sleigh, L.A. Fletcher, K.G. Kerr, Methodology for determining the susceptibility of airborne microorganisms to irradiation by an upper-room UVGI system, J. Aerosol Sci. 37 (7) (2006) 885–902.

[43] C.F. Green, P.V. Scarpino, The use of ultraviolet germicidal irradiation (UVGI) in disinfection of airborne bacteria, Environ. Eng. Policy 3 (1) (2001) 101–107.

[44] G. Pichurov, J. Srebric, S. Zhu, R.L. Vincent, P.W. Brickner, S.N. Rudnick, A validated numerical investigation of the ceiling fan's role in the upper-room UVGI efficacy, build, Environ. Times 86 (2015) 109–119.

[45] L. Ma, G. Zhang, M.P. Doyle, Green fluorescent protein labeling of Listeria, Salmonella, and Escherichia coli O157:H7 for safety-related studies, PLoS One 6 (4) (2011) e18083e18083.

[46] J. Zeng, C. Lei, Y. Wang, Y. Chen, X. Zhang, Z. Kang, X. Zhai, X. Ye, H. Wang, Distribution of Salmonella Enteritidis in Internal Organs and Variation of Cecum Microbiota in Chicken after Oral Challenge, (2018).

[47] A. Ghandi, I.B. Powell, T. Howes, X.D. Chen, B. Adhikari, Effect of shear rate and oxygen stresses on the survival of Lactococcus lactis during the atomization and drying stages of spray drying: a laboratory and pilot scale study, J. Food Eng. 113 (2) (2012) 194–200.

[48] J.F. Heidelberg, M. Shahamat, M. Levin, I. Rahman, G. Stelma, C. Grim, R.R. Colwell, Effect of aerosolization on culturability and viability of gram-negative bacteria, Appl. Environ. Microbiol. 63 (9) (1997) 3585–3588.

[49] D.-K. Kim, D.-H. Kang, UVC LED irradiation effectively inactivates aerosolized viruses, bacteria, and fungi in a chamber-type Air disinfection system, Appl. Environ. Microbiol. 84 (17) (2018) e00944-18.

[50] K.M. Lai, H.A. Burge, M.W. First, Size and UV germicidal irradiation susceptibility of Serratia marcescens when aerosolized from different suspending media, Appl. Environ. Microbiol. 70 (4) (2004) 2021–2027.

[51] B. Marthi, V.P. Fieland, M. Walter, R.J. Seidler, Survival of bacteria during aerosolization, Appl. Environ. Microbiol. 56 (11) (1990) 3463–3467.

[52] N. Moghimi, S.-j. Kim, S.-i. Park, Assessing of flexible packaging integrity: using the aerosolization bacteria, Packag. Technol. Sci. 29 (3) (2016) 135–143.

[53] V. Ulevicius, K. Willeke, S. Grinshpun, J. Donnelly, X. Lin, G. Mainelis, Aerosolization of particles from a bubbling liquid: characteristics and generator development, Aerosol Sci. Technol. 26 (2) (1997) 175–190.

[54] W. Zheng, L. Ni, X. Hui, B. Li, J. Zhang, Optimization of slightly acidic electrolyzed water spray for airborne culturable bacteria reduction in animal housing, Int. J. Agric. Biol. Eng. 9 (9) (2016) 185–191.

[55] J. Zhou, J. Wei, K.T. Choy, S.F. Sia, D.K. Rowlands, D. Yu, C.Y. Wu, W.G. Lindsley, B.J. Cowling, J. McDevitt, M. Peiris, Y. Li, H.L. Yen, Defining the sizes of airborne particles that mediate influenza transmission in ferrets, Proc. Natl. Acad. Sci. U.S.A. 115 (10) (2018) E2386–E2392.

[56] H. Zhen, T. Han, D.E. Fennell, G. Mainelis, Release of free DNA by membrane-impaired bacterial aerosols due to aerosolization and air sampling, Appl. Environ. Microbiol. 79 (24) (2013) 7780–7789.

[57] H. Zhen, T. Han, D.E. Fennell, G. Mainelis, A systematic comparison of four bioaerosol generators: affect on culturability and cell membrane integrity when aerosolizing Escherichia coli bacteria, J. Aerosol Sci. 70 (2014) 67–79.

[58] M. Cambra-Lopez, A.J. Aarnink, Y. Zhao, S. Calvet, A.G. Torres, Airborne particulate matter from livestock production systems: a review of an air pollution problem, Environ. Pollut. 158 (1) (2010) 1–17.

[59] D. Hospodsky, J. Qian, W.W. Nazaroff, N. Yamamoto, K. Bibby, H. Rismani-Yazdi, J. Peccia, Human occupancy as a source of indoor airborne bacteria, PLoS One 7 (4)

(2012) e34867.
[60] G.R. Johnson, L. Morawska, The mechanism of breath aerosol formation, J. Aerosol Med. Pulm. Drug Deliv. 22 (3) (2009) 229–237.
[61] W.C. Noble, J.D. Habbema, R. van Furth, I. Smith, C. de Raay, Quantitative studies on the dispersal of skin bacteria into the air, J. Med. Microbiol. 9 (1) (1976) 53–61.
[62] S. Li, H. Li, H. Xin, R.T. Burns, Particulate matter concentrations and emissions of a high-rise layer house in Iowa, Trans. ASABE 54 (3) (2011) 1093–1101.
[63] J. Seedorf, J. Hartung, M. Schröder, K.H. Linkert, V.R. Phillips, M.R. Holden, R.W. Sneath, J.L. Short, R.P. White, S. Pedersen, H. Takai, J.O. Johnsen, J.H.M. Metz, P.W.G. Groot Koerkamp, G.H. Uenk, C.M. Wathes, Concentrations and emissions of airborne endotoxins and microorganisms in livestock buildings in Northern Europe, J. Agric. Eng. Res. 70 (1) (1998) 97–109.
[64] J. Skóra, K. Matusiak, P. Wojewódzki, A. Nowak, M. Sulyok, A. Ligocka, M. Okrasa, J. Hermann, B. Gutarowska, Evaluation of microbiological and chemical contaminants in poultry farms, Int. J. Environ. Res. Public Health 13 (2) (2016) 192-192.
[65] Y. Zhao, D. Zhao, H. Ma, K. Liu, A. Atilgan, H. Xin, Environmental assessment of three egg production systems - Part III: airborne bacteria concentrations and emissions, Poult. Sci. 95 (7) (2016) 1473–1481.
[66] A. Friese, J. Schulz, K. Zimmermann, B.A. Tenhagen, A. Fetsch, J. Hartung, U. Rosler, Occurrence of livestock-associated methicillin-resistant Staphylococcus aureus in Turkey and broiler barns and contamination of air and soil surfaces in their vicinity, Appl. Environ. Microbiol. 79 (8) (2013) 2759–2766.
[67] D. Hu, L. Wang-Li, O.D.I. Simmons, J.J. Classen, J.A. Osborne, Size distributions of bioaerosols in an egg production facility and its vicinity, Environ. Eng. Sci. 33 (4) (2016) 215–223.
[68] A. Ssematimba, T.J. Hagenaars, M.C. de Jong, Modelling the wind-borne spread of highly pathogenic avian influenza virus between farms, PLoS One 7 (2) (2012) e31114.