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Original Article

Bacterial communities in $PM_{2.5}$ and PM_{10} inside the cage broiler houses before and after disinfection

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Abstract

Background: Air in broiler houses is contaminated with considerable amounts of microbial aerosols, which affects the health of humans and birds. Thorough cleaning and disinfecting should be carried out to reduce particulate concentrations and minimize airborne microorganisms. **Aims:** To evaluate the effects of cleaning and disinfecting measures on bacterial communities in particulate matter less than 2.5 µm ($PM_{2.5}$) and particulate matter between 2.5 and 10 µm (PM_{10}) inside broiler houses. **Methods:** A mixed disinfectant (containing aldehydes, alcohol, and quaternary ammonium salt) was sprayed to decontaminate broiler cage houses. *16S rDNA* amplicon sequencing was performed in this study to compare the bacterial communities in $PM_{2.5}$ and PM_{10} before and after disinfection. **Results:** A variety of pathogens and opportunistic pathogens such as *Staphylococcus*, *Streptococcus*, *Corynebacterium*, *Bordetella*, *Pseudomonas*, and *Shewanella* were detected, the quantities of which were noticeably reduced but not eradicated after disinfection. In addition, the impacts on several pathogens and opportunistic pathogens in $PM_{2.5}$ were not significant, which may be due to bacterial resistance to this type of disinfectant or other reasons discussed in the present study. **Conclusion:** Our results suggest that disinfection measures were effective in decontaminating air and further improving the feeding environment. This finding will help develop a reasonable disinfecting scheme for broiler houses.

Key words: Bacterial communities, Broiler houses, Disinfection, Particulate matter, *16S rDNA* sequencing

Introduction

Particulate matter (PM) is a mixture of airborne particles originating from the breakdown of crustal components or from human activities (Ee-Ling *et al*., 2015). Exposure to PM is evidently responsible for public health hazards and increases deaths related to lung cancer and other respiratory diseases (Dutkiewicz *et al*., 1994; Meng *et al*., 2016). Inhalable coarse particulates between 2.5 and 10 µm (PM_{10}) can enter the respiratory tract through the nasal cavity and throat and induce various respiratory diseases. Fine particles less than 2.5 µm in diameter ($PM_{2.5}$) can enter the deep part of the respiratory tract (alveoli and bronchia) or even pass into the blood stream through the blood-gas barrier, thereby having more adverse effects on humans (Hsieh *et al*., 2008; Franck *et al*., 2011; Menichini and Monfredini, 2011). As primary components of PM, microbial aerosols are airborne microorganisms or microbial matter that can generate detrimental effects on human health by transmitting infectious diseases or triggering respiratory irritation (Grahame and Schlesinger, 2007).

The construction of modern intensified and closed poultry houses has ensured the maintenance of a stable indoor environment that is represented by constant temperature, relative humidity, and lighting. However, with the growth of broilers, the difficulty of controlling the indoor environment increases. Poor air fluidity and higher humidity create a "hotbed" for the survival and reproduction of microorganisms. Thus, indoor air is contaminated with considerable amounts of harmful microbial aerosols (Cambralopez *et al*., 2009; Lawniczek-Walczyk *et al*., 2013), causing bronchitis, pneumonia and other disorders or functional impairments to the respiratory system, and posing health risks to both poultry and farmers (Baskerville *et al*., 1992; Seedorf *et al*., 1998). In addition, microbial aerosols could be released outside poultry houses and contaminate the surrounding atmosphere through long-distance transmission (Pascual *et al*., 2003).

To minimize the number of microorganisms inside poultry houses, thorough cleaning and disinfection should be carried out. The types of disinfectants commonly used for large-scale livestock and poultry productions include ozone, glutaraldehyde, quaternary ammonium salts, available chlorine compounds, peroxyacetic acid, and mixed disinfectants (Van, 1995; Boxall *et al*., 2003). The efficacy and mechanisms of

disinfectants used for broiler houses are different (Suwa *et al*., 2013; Maertens *et al*., 2017). Among them, the mixed disinfectant (containing aldehydes, alcohol, and quaternary ammonium salt) is a type of broad-spectrum germicide that shows a strong bactericidal ability and an enhanced disinfecting effect.

In the present study, the mixed disinfectant was sprayed to decontaminate the indoor environment after removing all broilers from the house. $PM_{2.5}$ and PM_{10} concentrations were determined before and after disinfecting. The bacterial community composition in PM was also analyzed using *16S rDNA* amplicon sequencing technology (Caporaso *et al*., 2011).

Materials and Methods

Ethics approval

The research protocol was reviewed and approved by the Animal Care and Use Committee (ACUC) in the School of Life Sciences, Ludong University (SKY-ACUC-2017-04).

Locations of broiler houses

The selected sampling sites were three closed-cage broiler houses situated in different villages of Yantai (Shandong, China). The locations of these villages were as follows: village A (37°20′42.19″N, 121°23′01.77″E), village B (37°22′22.29″N, 121°23′75.97″E), and village C (37°23′91.91″N, 121°24′55.38″E). The uniform size of all three broiler houses was 85 m × 15 m × 3.5 m. Closed breeding was adopted, and the breeding scale was 1.8-2.0 × 10^4 broilers.

Cleaning and disinfection

After chickens were evicted from the house, faeces and shed feathers were removed, and the cages and floors were thoroughly flushed with clean water. The buildings were then air-dried for 48 h, and sprayed immediately with a mixed disinfectant containing aldehydes (161.8 g/L), alcohol (40 g/L), and quaternary ammonium salt (61.5 g/L) diluted at 1:1500 using a 1~10 µm diameter spray. After being enclosed for 48 h, the building was ventilated naturally for 24 h.

Sample collection

$PM_{2.5}$ and PM_{10} collection was performed using the ZR-3920 environmental air PM, using 9 cm Tissuquartz™ filters (Pall, Port Washington, NY, USA), with a typical aerosol retention of 99.9%. Briefly, the filter was cleaned and dried prior to placing it under a prescribed climate-controlled condition with constant temperature and relative humidity for 48 h. The filter was preweighed 5 times, and the average initial weight was recorded as W_0. After sampling, the filter was weighed, and the average weight was recorded as W_1. The average mass concentrations of $PM_{2.5}$ and PM_{10} [C (µg/m^3)] were calculated according to weight increase:

$$C = (W_1 - W_0)/(t \times F)$$

Where,

C: The concentration of PM
W_1: The weight of filter before sampling
W_0: The weight of filter after sampling
t: The sampling duration
F: The flow rate

Sampling was performed at a height of 1.5 m above ground level and a flow rate of 100 L/min for 48 h. Sampling processes were carried out inside the 3 broiler houses as follows:

Two days before chickens were evicted from the house, $PM_{2.5}$ (BEPM2.5) and PM_{10} (BEPM10) samples were collected from inside the house. The meteorological parameters inside the house were as follows:
WS: Wind speed 0.8 m/s
WD: Wind direction longitudinal ventilation
RH: Relative humidity 65%
P: Pressure 0.97 bar pressure
T: Environmental temperature 22°C

After cleaning and disinfecting, $PM_{2.5}$ (AFPM2.5) and PM_{10} (AFPM10) samples inside the house were obtained. The meteorological parameters inside the house were as follows:
WS: Wind speed 0.5 m/s
WD: Wind direction longitudinal ventilation
RH: Relative humidity 45%
P: Pressure 1.0 bar pressure
T: Environmental temperature 15°C

DNA extraction and pyrosequencing

Total DNA was isolated from the filters using a standard cetyltrimethylammonium bromide (CTAB) method. One microliter of DNA was used as a template in subsequent polymerase chain reaction (PCR) amplification, with specifically barcoded primers targeting the V4-V5 region of the 16S ribosome gene (515F: 5´-GTG CCA GCM GCC GCG GTA A-3´, 907R: 5´-CCG TCA ATT CCT TTG AGT TT-3´). The amplification profile started with an initial denaturation at 98°C for 1 min; followed by 30 cycles of denaturation at 98°C for 10 s, hybridization annealing at 50°C for 30 s, and extension at 72°C for 30 s, and terminated with a final extension at 72°C for 5 min. PCR products were visualized by 2.0% agarose electrophoresis and purified using a QIAquick Gel Extraction kit (QIAGEN, Dusseldorf, Germany). Samples collected from the 3 houses (12 samples) were categorized into 4 groups based on sampling period and sites, namely, BEPM2.5, BEPM10, AFPM2.5, and AFPM10.

After preparing paired-end libraries using a TruSeq DNA PCR-free sample preparation kit (Illumina, San Diego, CA, US), sequencing was performed on an Illumina HiSeq 2500 platform with rapid-mode paired-end 250 bp sequencing (PE250).

Data analysis

Paired-end reads were assigned to each specimen according to the barcode sequence before removing their barcode and primer sequences. Afterwards, the trimmed paired-end reads were merged by FLASH (V1.2.7, Baltimore, USA) software to obtain the splicing sequences termed raw tags. Quality filtering was

conducted to eliminate unqualified sequences in the raw reads, following the Quantitative Insights Into Microbial Ecology (QIIME, V1.7.0, Colorado, USA) quality control process. With this data, we acquired high quality clean tags, which were then analyzed via the Ultra-fast sequence analysis (UCHIME) algorithm by comparing sequences to the gold database to select the chimaera sequences and obtain final effective tags. Sequences of these effective tags with an average nucleotide similarity of 97% were clustered into a single operational taxonomic unit (OTU) using UPARSE (V7.0.1001, Tiburon, USA) software. For each OTU, a representative sequence was picked and subjected to an assigned taxonomic composition with a set threshold of 0.8~1, using the mother approach and a comprehensive on-line resource for quality checked and aligned ribosomal RNA sequence data (SILVA) (http://www.arb-silva.de/) SSU rRNA database. The taxon abundance of each sample was summarized at different taxonomic levels (kingdom, phylum, class, order, family, genus, and species). Alpha and beta diversity indices were analyzed using QIIME (V1.7.0, Colorado, USA) software.

Statistical analysis

Differences in the average PM concentration among groups were determined using GraphPad Prism 5 (GraphPad, La Jolla, CA). Comparisons between the two groups were carried out using Student's t-test. A P-value of less than 0.05 was considered statistically significant.

Results

Alterations in $PM_{2.5}$ and PM_{10} concentrations

As shown in Fig. 1, average concentrations of both indoor $PM_{2.5}$ and PM_{10} dramatically decreased after carrying out the thorough disinfection (P<0.05, from 314.5 to 78.1 μg/m^3 and from 412.3 to 115.2 μg/m^3, respectively).

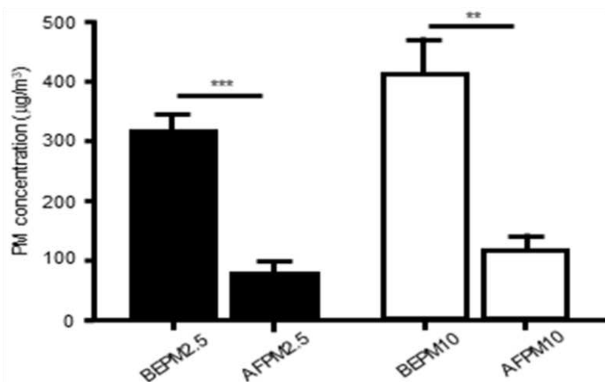

Fig. 1: Alterations in the average concentrations of PMs. Data are expressed as the means±SD of at least three independent experiments. *** P<0.001, ** P<0.01, and *P<0.05, compared two groups (BEPM2.5: $PM_{2.5}$ before disinfection; AFPM2.5: $PM_{2.5}$ after disinfection; BEPM10: PM_{10} before disinfection; AFPM10: PM_{10} after disinfection)

Basic statistics of *16S rDNA* gene sequences

High-throughput sequencing with the Illumina HiSeq platform was used to study the microbial communities in broiler houses. From all assayed samples, a total of 832,584 raw tags was obtained from 12 samples, while an average of 59,482 effective tags was obtained for each sample (ranging from 48,167 to 70,514), with an average length of 373 bp. These effective tags from all specimens were analyzed with the OTU picking protocol to assess the diversity of the bacterial communities. At a sequence similarity cutoff of 97%, effective tags from each specimen contained an average of 148 unique OTUs. There were 180 genera of bacteria detected. Rarefaction curves for all samples increased gradually with augmented sample sequence numbers, eventually approaching the saturation plateau. This result demonstrated that the species richness of the *16S rDNA* gene sequence database was high enough, thus covering an overwhelming majority of microbial sequences and ensuring the reliability of further diversity analysis.

Diversities of bacterial communities

Alpha diversity was assessed to analyze the complexity of the species' diversity for the samples. In this analysis, rarefaction curves were generated based on 97% similarity (Li *et al*., 2013). The rarefaction curve (Fig. 2) analysis of the observed number of species indicated that the number of species found in $PM_{2.5}$ (BEPM2.5) and PM_{10} (BEPM10) before disinfection was greater than that found after disinfection (AFPM2.5 and AFPM10).

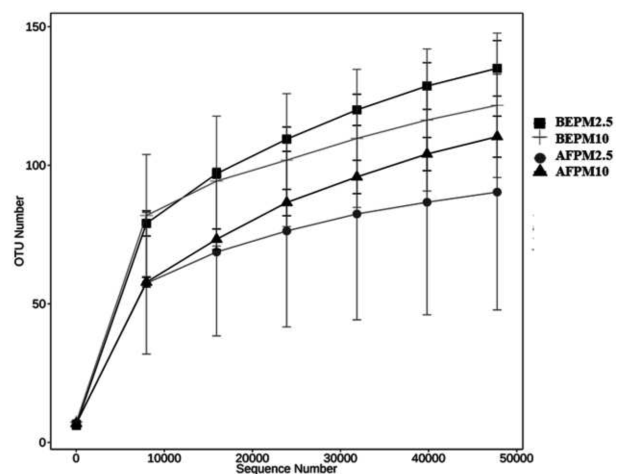

Fig. 2: Rarefaction curves of *16S rDNA* sequences for bacterial diversity (BEPM2.5: $PM_{2.5}$ before disinfection, AFPM2.5: $PM_{2.5}$ after disinfection, BEPM10: PM_{10} before disinfection, and AFPM10: PM_{10} after disinfection)

Nonmetric multidimensional scaling (NMDS) is a nonlinear model that is designed to overcome the shortcomings of the linear model (such as principal component analysis) to better reflect the nonlinear structure of ecological data. The closer the distance is between the samples, the more similar the species' composition. The combined results of NMDS (Fig. 3) and multiresponse permutation procedure analysis (MRPP) demonstrated that the between-group differences in the microbial community structure were

greater than the within-group differences in all samples. The PMs before disinfection were clustered together and separated from the PMs after disinfection.

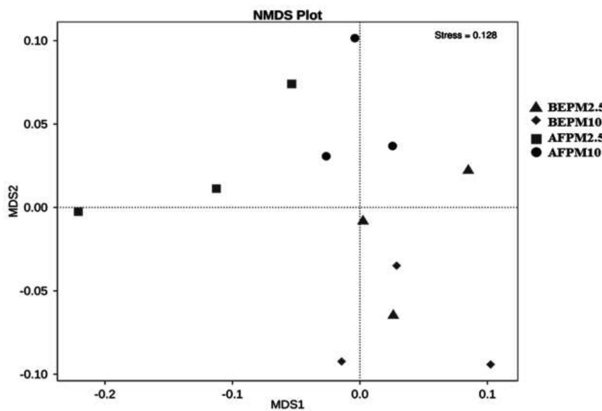

Fig. 3: Non-metric multi-dimensional scaling (NMDS) bacterial communities in the PMs before and after disinfection. Each dot in the plot represents one sample, and all samples in the same group are represented by the same shape (BEPM2.5: $PM_{2.5}$ before disinfection, AFPM2.5: $PM_{2.5}$ after disinfection, BEPM10: PM_{10} before disinfection, and AFPM10: PM_{10} after disinfection)

Bacterial communities in $PM_{2.5}$

Based on species annotation, the taxon abundance of each specimen was generated into five taxonomical levels, including phylum, class, order, family and genus. At the phylum level, the taxa of the bacterial communities in $PM_{2.5}$ before disinfection (BEPM2.5) were dominated by three phyla, namely, *Proteobacteria* (50.89%), *Firmicutes* (31.87%), and *Actinobacteria* (10.71%). Additionally, the predominant phyla in $PM_{2.5}$ after disinfection (AFPM2.5) were *Proteobacteria* (72.47%), *Firmicutes* (5.35%), and *Actinobacteria* (4.89%). The 10 most abundant bacteria at the genus level in each specimen are shown in Fig. 4. Among all samples, based on the richness of the microbial communities and diversity, the distribution in abundance of the dominant 35 genera at the genus level is demonstrated in the heat map of Fig. 5. As shown in the figure, the composition of bacterial communities in different PMs was not the same.

At the genus level, the data showed that bacteria in $PM_{2.5}$ before disinfection (BEPM2.5) contained mainly *Halomonas* (27.61%), *Lactobacillus* (20.17%), *Sphingomonas* (4.44%), *Shewanella* (4.19%), *Streptococcus* (4.08%), *Staphylococcus* (4.02%), *Corynebacterium* (3.57%), *Bordetella* (2.54%), *etc*. After disinfection, the $PM_{2.5}$ (AFPM2.5) carried bacteria including *Halomonas* (16.05%), *Shewanella* (5.40%), *Serratia marcescens* (4.28%), *Sphingomonas* (3.84%), *Pseudomonas* (3.62%), *Staphylococcus* (2.85%), *etc*.

Bacterial communities in PM_{10}

At the phylum level, the taxa of the bacterial communities in PM_{10} samples inside broiler houses before and after disinfection (BEPM10 and AFPM10, respectively) were dominated by three phyla, *Proteobacteria*, *Firmicutes*, and *Actinobacteria*. Based on relative abundance, *Proteobacteria* accounted for 72.15% of the bacteria in BEPM10, while *Firmicutes* and *Actinobacteria* had an average relative abundance of 8.59% and 6.62%, respectively. *Proteobacteria* occupied 75.71% of AFPM10, whereas *Firmicutes* and *Actinobacteria* accounted for 11.38% and 5.08%, respectively.

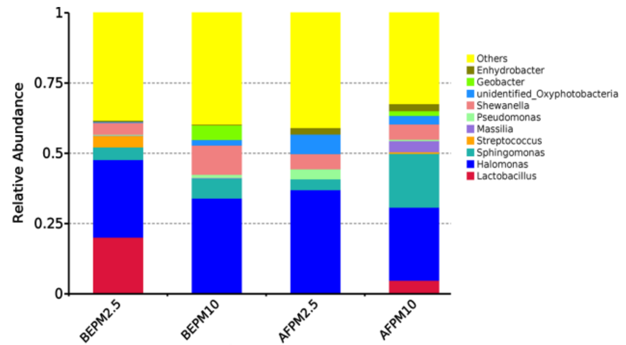

Fig. 4: Relative abundance of the dominant bacteria in different PMs at the genus level. Each colour represents a particular bacterial family. The top 10 abundant taxa are shown. Each bar represents the relative abundance of a group (BEPM2.5: $PM_{2.5}$ before disinfection, AFPM2.5: $PM_{2.5}$ after disinfection, BEPM10: PM_{10} before disinfection, and AFPM10: PM_{10} after disinfection)

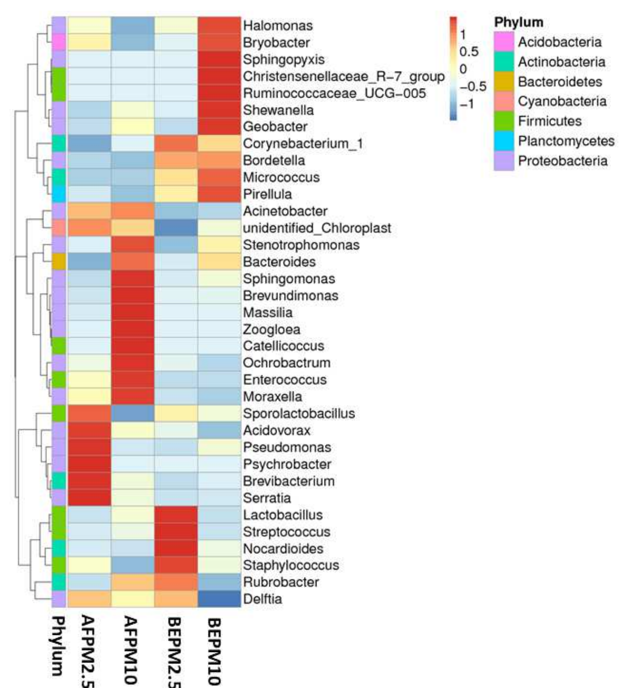

Fig. 5: Hierarchical clustering of the highly representative bacterial taxa (at the genus level) of each group is presented as a heat map. Values presented are the standardized Z values signifying the relative abundance of each species (BEPM2.5: $PM_{2.5}$ before disinfection, AFPM2.5: $PM_{2.5}$ after disinfection, BEPM10: PM_{10} before disinfection, and AFPM10: PM_{10} after disinfection)

According to the information shown in Figs. 4 and 5, bacterial genera in PM_{10} before disinfection (BEPM10)

were dominated by *Lactobacillus* (11.31%), *Shewanella* (10.48%), *Sphingomonas* (7.02%), *Bordetella* (2.68%), *Corynebacterium* (2.66%), *etc*. After disinfection, the genera composition in PM_{10} (AFPM10) was characterized by a relatively high abundance of *Sphingomonas* (19.07%), *Halomonas* (9.57%), *Shewanella* (5.37%), *Lactobacillus* (4.82%), *Massilia* (3.91%), *Moraxella* (2.52%), and *Brevundimonas* (2.30%).

Discussion

Data in this study demonstrated that decontaminating the house dramatically decreased concentrations of both PM_{10} and $PM_{2.5}$ in broiler houses compared to the period before disinfection, indicating that disinfecting measures play a great role in decreasing particulate pollution produced by broiler breeding. In addition, because organic and inorganic substances in the poultry house can reduce disinfection efficacy, faeces and dust should be thoroughly removed before disinfection (Henao *et al*., 2018).

By exploiting *16S rDNA* amplicon sequencing, we identified 131 genera in $PM_{2.5}$ before disinfection, including 39 pathogens and opportunists. Additionally, 132 genera were detected in PM_{10}, of which 36 were pathogens and opportunists. On the other hand, 108 bacterial genera were found in disinfected $PM_{2.5}$, including 29 pathogens and opportunists, while 112 genera were found in disinfected PM_{10}, including 25 pathogens and opportunists. These data suggested that disinfecting measures were effective in decreasing pathogens, opportunistic pathogens and nonpathogenic bacteria in $PM_{2.5}$ and PM_{10}. An analysis of microbial diversity indicated that airborne microbial community structure in PM before and after disinfection underwent some change. The results of this study showed that the mixed disinfectant has a strong bactericidal effect, especially for some pathogens and opportunists.

Pathogens and opportunists with high abundance detected in PMs included *Staphylococcus*, *Streptococcus*, *Corynebacterium*, *Bordetella*, *Pseudomonas*, *Shewanella*, *etc*. Among them, *Staphylococcus* is a common isolate that causes a variety of diseases in humans and animals, such as septicemia, pneumonia, and encephalomeningitis originating from pyogenic infection (Amal and Zamri-Saad, 2011; Wang *et al*., 2012). The most famous human infection caused by *Corynebacterium* is diphtheria, which is characterized by the production of pseudomembranes and exotoxins that enter the blood, causing generalized poisoning symptoms (Freney *et al*., 1991). *Bordetella* has been identified as the causative agent of many contagious infections of the respiratory system, such as pertussis (Guiso, 2009). *Pseudomonas* is recognized as a serious opportunist that may cause large-scale infection outbreaks in animals and humans under certain circumstances (Rostamzadeh, 2016; Rasooli *et al*., 2018). *Shewanella* is responsible for clinical infectious diseases and, in particular, chronic pancreatitis in humans (Pagani *et al*., 2003).

We also noticed that the means of air decontamination in this study had remarkable inhibitory effects on the bacteria with high abundance in PM_{10}, whereas the impact on some pathogens and opportunists in $PM_{2.5}$ was not significant. For example, before and after decontamination, the proportion of *Shewanella* in $PM_{2.5}$ was 4.18% and 5.40%, respectively, and *Pseudomonas* accounted for 0.31% and 3.62% of the bacterial community in $PM_{2.5}$. This result may be due to the fact that the diameter of some $PM_{2.5}$ particles was smaller than the disinfectant aerosol or because the houses were closed for a short period of time, which could have resulted in the insufficient interaction between the disinfectant aerosols and $PM_{2.5}$. Some studies have indicated that the disinfection effect is closely related to the concentration of disinfectants, action time and ambient temperature (Bauerfeld, 2014). Additionally, this finding may have resulted from bacterial resistance to this type of disinfectant. For example, *Pseudomonas* has shown resistance to some disinfectants such as chlorhexidine acetate, quaternary ammonium salt, phenol and iodine (Brozel and Cloete, 1993). The mechanisms of bacterial resistance to disinfectants include cellular impermeability, biofilm formation, efflux and mutation at the target sites (Russell, 1999; Cabrera *et al*., 2007). Some research has indicated that the resistance of bacteria will gradually increase with frequent use of the same disinfectant (Johnson, 2008). Our results suggest that a combination of different types of disinfectants can overcome the resistance of bacteria to a single disinfectant during house disinfection.

To summarize, thorough disinfection measures applied in this study considerably decreased the concentrations of PM and controlled a majority of pathogens and opportunists with high abundance in PM_{10}. Nevertheless, for $PM_{2.5}$, disinfectant aerosols with smaller diameters and prolonged disinfection times should be employed. Although mixed disinfectants (containing aldehydes, alcohol, and quaternary ammonium salt) are commonly used in poultry breeding, disinfection methods should be explored based on the conditions of the poultry house. The results of our study suggest that new mixed disinfectants should be continuously developed to improve the antimicrobial spectrum, reduce the dosage, and enhance safety for the staff and animals.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial

relationships that could be construed as a potential conflict of interest.

References

Amal, MNA and Zamri-Saad, M (2011). Streptococcosis in Tilapia (*Oreochromis niloticus*): a review. Pertanika J. Trop. Agric. Sci., 34: 195-206.

Baskerville, A; Humphrey, TJ; Fitzgeorge, RB; Cook, RW; Chart, H; Rowe, B and Whitehead, A (1992). Airborne infection of laying hens with *Salmonella enteritidis* phage type 4. Vet. Rec., 130: 395-398.

Bauerfeld, K (2014). Effect of ambient temperatures on disinfection efficiency of various sludge treatment technologies. Water Sci. Technol., 69: 15-24.

Boxall, NS; Perkins, NR; Marks, D; Jones, B; Fenwick, SG and Davies, PR (2003). Free available chlorine in commercial broiler chicken drinking water in New Zealand. J. Food Prot., 66: 2164-2167.

Brozel, VS and Cloete, TE (1993). Adaptation of *Pseudomonas aeruginosa* to 2,20-methylenebis (4-chlorophenol). J. Appl. Bacteriol., 74: 94-99.

Cabrera, CE; Gómez, RF and Zúñiga, AE (2007). Resistance to bacterial antibiotics, antiseptics and disinfectants a manifestation of the survival and adaptation mechanisms. Colomb. Medica. 38: 149-158.

Cambralopez, M; Aarnink, AJA; Yang, Z; Calvet, S and Torres, AG (2009). Airborne particulate matter livestock production systems: a review of an air pollution problem. Environ. Pollut., 158: 1-17.

Caporaso, JG; Lauberb, CL; Waltersc, WA; Berg-Lyons, D; Lozupone, CA; Turnbaugh, PJ; Fierer, N and Knight, R (2011). Global patterns of *16S rRNA* diversity at a depth of millions of sequences per sample. P. Natl. Acad. Sci. USA., 108: 4516-4522.

Dutkiewicz, J; Pomorski, ZJH; Sitkowska, J; Krysińska-Traczyk, E; Skórska, C; Prazmo, Z; Cholewa, G and Wójtowicz, H (1994). Airborne microorganisms and endotoxin in animal houses. Grana. 33: 85-90.

Ee-Ling, O; Mustaffa, NI; Amil, N; Khan, MF and Latif, MT (2015). Source contribution of $PM_{2.5}$ at different locations on the Malaysian Peninsula. B. Environ. Contam. Tox., 94: 537-542.

Franck, U; Odeh, S; Wiedensohler, A; Wehner, B and Herbarth, O (2011). The effect of particle size on cardiovascular disorders-the smaller the worse. Sci. Total Environ., 409: 4217-4221.

Freney, J; Duperron, MT; Courlier, C; Hansen, W; Allard, F; Boeufgras, JM; Monget, D and Fleurette, J (1991). Evaluation of API coryne in comparison with conventional methods for identifying coryneform bacteria. J. Clin. Microbiol., 29: 38-41.

Grahame, TJ and Schlesinger, RB (2007). Health effects of airborne particulate matter: do we know enough to consider regulating specific particle types or sources? Inhal. Toxicol., 19: 457-481.

Guiso, N (2009). *Bordetella pertussis* and *pertussis* vaccines. Clin. Infect. Dis., 49: 1565-1569.

Henao, LD; Compagni, RD; Turolla, A and Antonelli, M (2018). Influence of inorganic and organic compounds on the decay of peracetic acid in wastewater disinfection. Chem. Eng. J., 337: 133-142.

Hsieh, LY; Chen, CL; Wan, MW; Tsai, CH and Ying, IT (2008). Speciation and temporal characterization of dicarboxylic acids in pm 2.5 during a pm episode and a period of non-episodic pollution. Atmos. Environ., 42: 6836-6850.

Johnson, LR (2008). Microcolony and biofilm formation as a survival strategy for bacteria. J. Theor. Biol., 251: 24-34.

Lawniczek-Walczyk, A; Gorny, RL; Golefit-Szymczak, M; Niesler, A and Wlazlo, A (2013). Occupational exposure to airborne microorganisms, endotoxins and β-glucans in poultry houses at different stages of the production cycle. Ann. Agr. Env. Med., 20: 259-268.

Li, B; Zhang, X; Guo, F; Wu, W and Zhang, T (2013). Characterization of tetracycline resistant bacterial community in saline activated sludge using batch stress incubation with high-throughput sequencing analysis. Water Res., 47: 4207-4216.

Maertens, H; De, RK; Van, WS; Van, CE; Meyer, E; Van, MH; Van, IF; Vandenbroucke, V; Vanrobaeys, M and Dewulf, J (2017). Evaluation of the hygienogram scores and related data obtained after cleaning and disinfection of poultry houses in Flanders during the period 2007 to 2014. Poult. Sci., 97: 620-627.

Meng, K; Wu, B; Gao, J; Cai, Y; Yao, M; Wei, L and Chai, T (2016). Immunity related protein expression and pathological lung damage in mice post-stimulation with ambient particulate matter from live bird markets. Front Immunol., 7: 252.

Menichini, E and Monfredini, F (2011). Relationships between concentrations of particle-bound carcinogenic PAHs and PM10 particulate matter in urban air. Fresen. Environ. Bull., 10: 533-538.

Pagani, L; Lang, A; Vedovelli, C; Moling, O; Rimenti, G; Pristera, R and Mian, P (2003). Soft tissue infection and bacteremia caused by *Shewanella putrefaciens*. J. Clin. Microbiol., 41: 2240-2241.

Pascual, L; Pérez-Luz, S; Yánez, MA; Santamaria, A; Gibert, K; Salgot, M; Apraiz, D and Catalan, V (2003). Bioaerosol emission from waste water treatment plants. Aerobiologia. 19: 261-270.

Rasooli, A; Nouri, M; Esmaeilzadeh, S; Ghadiri, A; Gharibi, D; Javaheri Koupaei, M and Moazeni, M (2018). Occurrence of purulent mandibular and maxillary osteomyelitis associated with *Pseudomonas aeruginosa* in a sheep flock in south-west of Iran. Iran. J. Vet. Res., 19: 133-136.

Rostamzadeh, Z; Mohammadian, M and Rostamzade, A (2016). Investigation of *Pseudomonas aeruginosa* resistance pattern against antibiotics in clinical samples from Iranian educational hospital. Adv. Appl. Microbiol., 6: 190-194.

Russell, AD (1999). Bacterial resistance to disinfectants: present knowledge and future problems. J. Hosp. Infect., 43: S57-S68.

Seedorf, J; Hartung, J; Schröder, M; Linkert, KH; Phillips, VR; Holden, MR; Sneath, RW; Short, JL; White, RP; Pedersen, S; Takai, H; Johnsen, JO; Metz, JHM; Groot Koerkamp, PWG; Uenk, GH and Wathes, CM (1998). Concentrations and emissions of airborne endotoxinsand microorganisms in livestock buildings in Northern Europe. J. Agric. Engng. Res., 70: 97-109.

Suwa, M; Oie, S and Furukawa, H (2013). Efficacy of disinfectants against naturally occurring and artificially cultivated bacteria. Biol. Pharm. Bull., 36: 360-363.

Van, KB (1995). Disinfectant testing on surfaces. J. Hosp. Infect., 30: 397-408.

Wang, Y; Zhang, W; Wang, J; Wu, C; Shen, Z; Fu, X; Yan, Y; Zhang, Q; Schwarz, S and Shen, J (2012). Distribution of the multidrug resistance gene *cfr* in *Staphylococcus* species isolates from swine farms in China. Antimicrob. Agents Ch., 56: 1485-1490.