Science of the Total Environment xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Airborne bacterial contaminations in typical Chinese wet market with live poultry trade

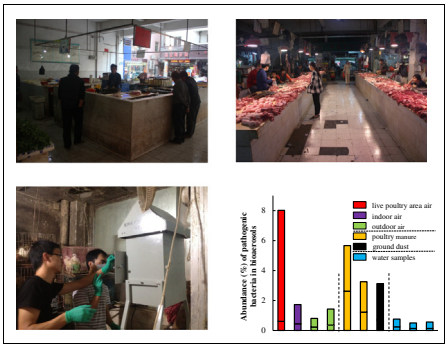
Xin-Lei Gao [a], Ming-Fei Shao [a,*], Yi Luo [b], Yu-Fang Dong [a], Feng Ouyang [a], Wen-Yi Dong [a], Ji Li [a,*]

[a] Harbin Institute of Technology, Shenzhen Graduate School, Shenzhen Key Laboratory of Water Resource Utilization and Environmental Pollution Control, Shenzhen 518055, China
[b] College of Environmental Science and Engineering, Ministry of Education Key laboratory of Pollution Processes and Environmental Criteria, Nankai University, Tianjin 300071, China

HIGHLIGHT

- Airborne biotic contaminants in wet market pose an imperative threats to customers and local residents.
- Both pathogenic bacteria and antibiotic resistance genes (ARGs) were investigated to provide quantitative information on the levels of microbial contaminations in the air.
- Poultry manures were important microbial contamination source, and bioaerosols were important route for transmissions of microbial contaminants.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 2 April 2016
Received in revised form 25 June 2016
Accepted 26 June 2016
Available online xxxx

Editor: D. Barcelo

Keywords:
Pathogenic bacteria
Bioaerosol
Antibiotic resistance gene
Wet market
Live poultry trade
Microbial contaminant

ABSTRACT

Chinese wet markets with live poultry trade have been considered as major sources of pathogen dissemination, and sites for horizontal transfer of bacterial and viral pathogens. In this study, the pathogenic bacteria and antibiotic resistant genes (ARGs) in air samples collected at a typical Chinese wet market had been analysis and quantified. *Corynebacterium minutissimum* and other pathogenic bacteria accounted for 0.81–8.02% of the whole microbial community in different air samples. The four ARGs quantified in this study showed a comparable relative concentration (copies/ng_DNA) with municipal wastewater. Poultry manures were demonstrated to be important microbial contamination source in wet market, which was supported by both microbial composition based source tracking and the quantification of airborne microbial density. A series of *Firmicutes* and *Bacteroidetes* indicators of poultry area contamination were successfully screened, which will be useful for the more convenient monitoring of airborne poultry area contamination. Our results indicate bioaerosols acted as important route for the transmissions of pathogens and ARGs. Continued surveillance of airborne microbial contamination is required in poultry trade wet market.

Practical implications: Urban live poultry markets are important sources of pathogen dissemination, and sites for horizontal transfer of viral and bacterial pathogens. In the present field-study, pathogenic bacteria and antibiotic resistance genes were focused to provide quantitative information on the levels of microbial contaminations at

* Corresponding author.
E-mail addresses: mfshao@hitsz.edu.cn (M.-F. Shao), Liji99@hitsz.edu.cn (J. Li).

http://dx.doi.org/10.1016/j.scitotenv.2016.06.208

the indoor air of wet markets. Results demonstrated that poultry manures were important microbial contamination source in wet market, and in the meanwhile bioaerosols were identified as important route for the transmissions of microbial contaminants. A series of *Firmicutes* and *Bacteroidetes* indicators of poultry area contamination were successfully screened, which will be useful for the more convenient monitoring of airborne poultry area contamination.

1. Introduction

Airborne bacteria may pose significant risks on human health, serving directly as human pathogens or triggering allergic reactions (Husman, 1996; Kash and Taubenberger, 2015; Kaushik and Balasubramanian, 2013; Wu et al., 2015). These risks will be even higher in environment with a dense crowd or the close contact between livestock, poultry and human beings. One typical case is traditional Chinese wet market, where people trade fresh food (e.g., live poultry, pork, live fish, and vegetables). Fresh food, live animals and their feces all act as important sources of bacterial hazards (Gao, 2014). Live poultry trade at local wet market has long been prevalent in China, severing more than half of the urban residents. In addition to the harmful microorganisms themselves, antibiotic resistant genes (ARGs) as emerging contaminants are attracting more and more attentions (Gao et al., 2012; Pruden et al., 2006; Zhang and Zhang, 2011).

Animal husbandry is regarded as one of the important sources of ARGs, due to the excessive uses of antibiotics (McKinney et al., 2010; Zhou et al., 2013). Comparing with the high concern over ARGs pollution in livestock or poultry farms (Just et al., 2012; Zhang et al., 2013), the contamination characterization of trading environments receive less attention. A recent survey of the colistin resistant *Enterobacteriaceae* in retail meat was alarming, in which high prevalence of plasmid-mediated colistin resistance in *Escherichia coli* isolates of retail meat origins was identified (Liu et al., 2016).

In this study, airborne microbial hazards including both pathogenic bacteria and ARGs were well quantified and characterized. Tetracycline, sulfonamides and macrolides are the most widely used antibiotics in animal husbandry (Zhang et al., 2015; Zhu et al., 2013). Thus, in the present study focusing on the ARGs contamination related with live poultry trading, *tetG*, *tetC*, *sul2* and *ermC* was chosen as the target ARGs. The objectives of this study were to provide quantitative information on the levels of microbial contaminations at the indoor and ambient air of wet markets, and to investigate the possible contamination source. To our knowledge this is the first field-study to report the occurrence of pathogens and ARGs within the atmospheric environments of the typical wet market with live poultry trade.

2. Material and methods

2.1. Air sampling and DNA extraction

Air samples were collected from June to July 2015, in and around Pingshan market Shenzhen, South China (22°35′27″N, 113°58′18″E). The market serves ca. 1000 customers daily. The layout of all sampling points in the current study was shown in Fig. S1. Air samplings were conducted by a high-volume total suspended particulates (TSP) sampler (LS2031, Qingdao, China). Air was drawn at an average rate of 1.05 m^3/min for 4–23 h resulting in 250–1500 m^3 of flow-through volume. Detail descriptions of the samples were listed in Table S1. In order to make a specific comparable, 4 coastal water (Shenzhen Bay, 5/28/2015) and 2 sea water (Shenzhen Xi-Chong, 7/8/2015) samples has add to the experiment settings.

Particulate matters were collected onto a 20.32 × 25.4 cm^2 glass fiber filters (PALL, NY, U.S.) with 99.9% typical aerosol retention. All the filters were sterilized by heating at 500 °C for 5 h before sampling. After air sampling, the filters were transported to the laboratory with ice box and kept in −20 °C until downstream analysis. Cultivable bioaerosol samples were collected using a six-stage Andersen sampler (FA-1, Liaoyang, China) in each TSP sampling site. The sampler was operated for 10 min with a 28.3 L/min airflow rate. After sampling the agar plates were transported into a thermostat incubator and cultivated at 28 °C for 48 h, before counting the colony forming units (CFU) of each stage.

A magnetic particle based microbial separation technique was utilized to enhance biomass yield before DNA extraction. Briefly, 1/8–1/2 of each glass fiber filter was placed in a clean beaker containing 40 mL buffer solution. After 30 s sonication for the biomass detachment, the glass filter changed into glass fiber slurry. Then 25 mg magnetite nanoparticles (MNPs) was added and dispersed with another 30 s sonication. The beaker was gently shaken for several times, to facilitate the attachment of MNPs onto microbial cells. Finally the biomass was recovered through magnetic separation and was ready for the subsequent DNA extraction. Total DNA extraction using the FastDNA Spin Kit for soil (MP, CA, USA) was performed following the manufacturer's instructions. DNA concentrations were determined by fluorescence spectrometer (Hitachi, Tokyo, Japan) using Hoechst 33,258 staining dye.

2.2. Barcode sequencing and sequence analysis

The bacterial DNA was amplified with following primers pair targeting the V4 region of the 16S rRNA gene (F515/R806) (Albertsen et al., 2012). Barcodes and its linker sequence that allow sample multiplexing during MiSeq sequencing were incorporated at the 5′ end of forward primer (Zhou et al., 2011). A100 μL polymerase chain reaction (PCR) reaction solution was prepared for each sample using MightyAmp polymerase (TaKaRa, Dalian, PRC). The PCR amplification was conducted in an i-Cycler (Bio-Rad, CA, USA) under the following conditions: one initial denaturation cycle at 98 °C for 2 min, 25 cycles at 98 °C for 15 s, 56 °C for 20 s, and 68 °C for 30 s, and a final extension at 68 °C for 10 min. After purification using quick-spin PCR Product Purification Kit (TaKaRa, Dalian, China), the PCR products from different samples were quantified using Nanodrop 2000 (Thermo Fisher Scientific, MA, USA), mixed accordingly to achieve the equal concentration in the final mixture, and then sent to Macrogen Inc. (Seoul, Korea) for the high throughput sequencing on Illumina MiSeq platform.

In total, 378,000 bacterial sequence reads (~18,000 reads per sample, NCBI submission number: SUB1628949) were recovered and MOTHUR software was employed for all subsequent analysis (Schloss et al., 2009). The sequences were trimmed for the removal of primers, barcodes and sequences with ambiguous nucleotides, long homopolymers or sequence length shorter than 200 bases. For the phylotype analysis, sequences were aligned, checked for chimeras, filtered, and finally classified taxonomically using the SILVA bacterial reference (Pruesse et al., 2007). For the operational taxonomic units (OTU) based analysis, aligned sequences were treated with "dist.seqs" function to generate distance matrix. Then "cluster" command was deployed to construct OTUs by the cutoff of 3% and 6% dissimilarity. One representative sequence was picked from each OTU, then treated with Local BLAST against the human pathogen database (Rusinol et al., 2013; Ye and

Please cite this article as: Gao, X.-L., et al., Airborne bacterial contaminations in typical Chinese wet market with live poultry trade, Sci Total Environ (2016), http://dx.doi.org/10.1016/j.scitotenv.2016.06.208