High concentration of coagulase-negative staphylococci carriage among bioaerosols of henhouses in Central China

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Abstract

Background: Coagulase-negative staphylococci (CoNS) are a group of opportunistic pathogens, which are widely spread in the environment. Animal breeding is an important source of pathogen spreading. However, the concentration and characteristics of CoNS in the bioaerosols of henhouses are unclear.

Results: In this study, we showed that CoNS were significantly increased in bioaerosols of henhouses during the first 60 days, and reached 2.0 × 10^6 CFU/m^3, which account for 75.4% of total bacteria. One hundred and two CoNS isolates from bioaerosols and nasal swabs of farmers were further identified, covering seven species. Among these, 41.2% isolates were Staphylococcus sciuri, which was the predominant species, followed by S. equorum, S. saprophyticus, S. haemolyticus, S. xylosus, S. arlettae and S. gallinarum. There were high rates of resistance to oxacillin in CoNS (49.0%), which were defined as Methicillin-Resistant CoNS (MRCoNS), and 36.3% isolates contained resistance gene meca. Bioaerosol infection models showed that, chickens exposed to aerosolized S. sciuri had significant induction of inflammatory cytokines interleukin (IL)-1β, IL-6, IL-8 and IL-10 at 5 days post-infection (dpi) in lungs and at 7 dpi in spleens.

Conclusions: We reported a high concentration of CoNS in henhouses, and S. sciuri was the preponderant CoNS species. Antibiotic resistance analysis and bioaerosols infection of CoNS further highlighted its hazards on resistance and immunological challenge. These results suggested that, CoNS in bioaerosols could be one serious factor in the henhouses for not only poultry industry but also public health.

Keywords: Bioaerosol, Coagulase-negative staphylococci, Antibiotic resistance, meca, Inflammatory cytokine

Background

Coagulase-negative staphylococci (CoNS) are a group of opportunistic pathogens, which are not only in animals and humans but also widely spread in the environment, such as dust, soil, water and air [1–3]. CoNS can cause human and animal infections. In humans, CoNS are associated with endocarditis, septicaemia and blood stream infection, and they have become one of the most important sources of hospital infection [4, 5]. In chickens, CoNS infection can cause arthritis, fibrinopurulent blepharitis and even systemic infection [6–8]. In addition, methicillin-resistant CoNS (MRCoNS)-contaminated chicken meat is frequently reported, suggesting foodborne transmission of the bacteria [9, 10].

Recently, the reports on multi-resistant CoNS were increasing, with the increase in antibiotic usage [11]. The increasing antibiotic resistance of CoNS also limits the drug choices for treatment of CoNS infections [12]. What is more, CoNS exist in the places where antibiotics are widely used, such as hospitals and animal farms [13, 14], which accelerate the spread of resistance genes. Methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant S. aureus (VRSA) have been frequently reported [15, 16], and show similar resistance genes to CoNS, such as the methicillin-resistance gene meca [17]. As a widespread bacterium in the environment, CoNS has been considered as a reservoir of resistance genes, which highlights its threat to public health.
Bioaerosols, mainly including bacteria, viruses and fungi, are potential environmental sources of animals and human infection. For animals, piglets exposed to 10^6 cfu/m^3 MRSA in the air were persistent colonized and 10^4 cfu/m^3 were transient [18]. MRCoNS carriage analysis suggested potential transmission of MRCoNS from livestock to humans by occupational livestock contact [19]. In poultry houses, bioaerosol is one important route of transmission of viral as well as bacterial pathogens. In Switzerland, an analysis on 12 poultry houses showed that the mean bacterial exposure level to poultry farmers was 53 × 10^7 cells/m^3, among them, 62 × 10^6 cells/m^3 were staphylococci [20]. In Canada, Enterococcus spp., Escherichia coli, and Staphylococcus spp. spread widely in bioaerosols of poultry houses, and high levels of zinc bacitracin, erythromycin and tetracycline resistance genes have been found in bioaerosols [21]. Therefore, bioaerosols in animal houses are potentially harmful to the health of both animals and farmers. In addition, besides livestock manure and waste water, bioaerosol is another important pathway for diffusing pathogens and resistance genes to the outside environment.

The goal of this study was to investigate the concentration and antibiotic resistance of CoNS in bioaerosols from henhouses in China, and further examine its effect on immune response of chickens. This study will provide important information for the poultry industry and public health.

**Results**

**CoNS in bioaerosols and farmers**

Bioaerosol samples were collected from nine henhouses covered the first 90 days of the growing period before changing their cage. As shown in Fig. 1, at day 2, the mean total bacterial count was 7.8 × 10^4 cfu/m^3 in the bioaerosols of henhouses, and among them, the mean of CoNS was 1.7 × 10^3 cfu/m^3, which accounted for approximately 2.2% of total bacteria. As time progressed, the total bacterial count, especially the total CoNS count, was significantly increased during the first 60 days. At day 60, the mean total bacterial count was 2.6 × 10^6 cfu/m^3, which was approximately 34-fold of that at day 2, and among them, 75.4% (2.0 × 10^6 cfu/m^3) of bacteria were CoNS. At day 90, the bacterial count was similar to that at day 60 (p > 0.05). These results suggested that CoNS was the primary genus in the bioaerosols of henhouses. In addition, nasal swabs from 14 poultry farmers were also evaluated, and among them, eight (57.1%) samples were CoNS-positive.

To characterize further the CoNS in bioaerosols and farmers, 102 CoNS were randomly picked and further identified. As shown in Table 1, 102 isolates covered seven species. Forty-two (41.2%) isolates were S. sciuri, which was the predominant species, followed by S. equorum (21.6%), S. saprophyticus (18.6%) and S. haemolyticus (10.8%). The others included S. xylosus (5.9%), S. arlettae (1.0%) and S. gallinarum (1.0%). Among them, five S. sciuri, two S. equorum and one S. haemolyticus isolates were from nasal swabs of farmers.

**Presence of mecA and antibiotic resistance rates in CoNS**

The susceptibility of CoNS isolates to nine antibiotics was tested using disk diffusion assays (Fig. 2). The isolates showed high resistance rates to antibiotics that are widely used in animal breeding, including penicillin (69.61%), ampicillin (58.82%), ciprofloxacin (66.67%), chloramphenicol (93.14%), erythromycin (48.04%), tetracycline (91.18%) and clindamycin (45.10%). In contrast, lower resistance rates were seen for amikacin (6.86%) and rifampicin (20.59%), which are less used in breeding. Susceptibility to oxacillin and vancomycin, which are two important antibiotics used in human, were tested using MIC assays (Table 2). The MICs of oxacillin in 49.0% isolates were >
2 μg/ml, including all of the mecA-positive isolates and 20% (13/65) mecA-negative isolates. The MICs of vancomycin in 40.2% isolates were between 4 and 8 μg/ml, which were intermediate. Compared with mecA-negative isolates, mecA-positive isolates showed higher resistance rates to oxacillin, penicillin, ampicillin and ciprofloxacin, but lower resistance rates to erythromycin and clindamycin (p < 0.05).

The presence of mecA was screened by PCR in all the CoNS isolates. As shown in Table 1, 36.3% isolates (34 isolates from bioaerosols and three from nasal swabs of farmers) were found to contain mecA gene. Among these mecA-positive CoNS isolates, 30 (two from nasal swabs of farmers) were S. sciuri, accounting for 71.4% of total S. sciuri isolates, followed by S. equorum (four isolates, one from nasal swab of farmer), S. xylosus (two isolates) and S. gallinarum (one isolate). In contrast, mecA was not found in S. saprophyticus, S. haemolyticus and S. arlettae isolates. The results suggested that mecA was wide spread in CoNS, especially S. sciuri, in henhouses.

### Table 1 The distribution of mecA in different species of CoNS strains

| Species            | No. of strains | Proportions in CoNS | No. of mecA positive strains | mecA Positive rates |
|--------------------|----------------|---------------------|------------------------------|---------------------|
| S. equorum         | 22 (2)         | 21.57%              | 4 (1)                        | 18.18%              |
| S. haemolyticus    | 11 (1)         | 10.78%              | 0                            | 0.00%               |
| S. saprophyticus   | 19             | 18.63%              | 0                            | 0.00%               |
| S. sciuri          | 42 (5)         | 41.18%              | 30 (2)                       | 71.43%              |
| S. xylosus         | 6              | 5.88%               | 2                            | 33.33%              |
| S. arlettae        | 1              | 0.98%               | 0                            | 0.00%               |
| S. gallinarum      | 1              | 0.98%               | 1                            | 100.00%             |
| Total              | 102 (8)        | 100.00%             | 37 (3)                       | 36.27%              |

*aThe No. in the brackets indicate the number of isolates from the nasal swabs of farmers*

Inflammatory response induced by aerosolized MRCoNS in chickens

As above described, the concentration of CoNS could reach 10^6 magnitudes cfu/m^3 in henhouses, so we chose approximately 1.0 × 10^6 cfu/m^3 as the concentration of exposure with aerosolized MRCoNS (mecA-positive S. sciuri). As shown in Fig. 3, although there were no typical symptoms after infection, the expression of proinflammatory cytokines IL-1β, IL-6 and IL-8 were significantly induced at 5 dpi in lungs and at 7 dpi in spleen, and at the same time, the expression of anti-inflammatory cytokine IL-10 was also induced. Then, IL-1β, IL-6, IL-8 and IL-10 were reduced at 14 dpi. The expression of TNF-α was not greatly induced by aerosolized MRCoNS. These results suggested that aerosolized MRCoNS induced inflammatory cytokines in chickens.

**Discussion**

In this study, we showed that CoNS were highly prevalent, and they were the dominant cultivable bacterial group under aerobic condition in bioaerosols in the later period of chicken breeding. In addition, CoNS were isolated from bioaerosols and farmers, and further characterized by species, antibiotic resistance, and poultry infection.

In recent decades, intensive animal production has become common, but it followed environmental concerns...
and public health, and bioaerosols contamination is one of the important risk factors [22]. Various bacteria have been identified in bioaerosols from animal houses, such as Pseudomonas, Bacillus, Salmonella, E. coli, Streptococcus and Staphylococcus [23]. The composition of bioaerosols in different animal houses shows different features. For example, the mean total concentration of bacteria inside swine barns was $6.6 \times 10^4$ cfu/m$^3$, mainly including Staphylococcus, Pseudomonas and Bacillus [24]. During cattle breeding, bioaerosols are the most important route of transmission for Mycoplasma bovis [25]. In hospitals, Staphylococcus, including S. aureus and CoNS, is one of the most serious bacteria in bioaerosols. Several studies on the bioaerosols of poultry houses have been done. In Switzerland, the mean bacterial exposure level to poultry farmers was $53 \times 10^7$ cells/m$^3$, and among them, $62 \times 10^6$ cells/m$^3$ were staphylococci [20]. In Australia and Poland, staphylococci was also one of the dominant bacteria in the poultry houses [26, 27]. Even in some broiler houses of Germany, staphylococci concentrations were higher than between $1 \times 10^6$ cfu/m$^3$, and affected by the wind in the barn [28]. Besides to staphylococci, potential pathogens, such as Enterococcus spp., Escherichia coli and Salmonella

| No. of isolates | MICs of oxacillin | MICs of vancomycin |
|----------------|------------------|-------------------|
| meCA-positive CoNS ($n = 37$) | ≤0.25 | 0.5 | 1 | ≥2 | ≥4-8 | ≥16 |
|              | 0 | 0 | 0 | 37 | 25 | 12 | 0 |
| meCA-negative CoNS ($n = 65$) | 8 | 31 | 13 | 13 | 36 | 29 | 0 |

**Table 2** Distribution of CoNS isolates in different MICs

**Fig. 3** The mRNA expression of inflammatory cytokines induced by aerosolized CoNS. The different normal letters indicate significant difference among different dpi, a, b means there is no significant difference between a, b and a, b, and a.
spp., were also found in poultry houses [21, 29]. However, the knowledge on the concentration and characterization of CoNS in henhouses was still limited.

We investigated the bacteria in bioaerosol began with breeding of 1 day old chicken. Compared with membrane filtration and impaction, we could see different sampling procedures had obvious influences on the results, and two samplers, the Andersen 6-stage sampler and the All glass impinger-30 (AGI-30), were recommended as standards for sampling of microbiological aerosols: [24, 30]. In this study, we chose SKC BioSampler, which is All-Glass-Impinger for sampling. Our results showed that the total bacterial counts, especially the CoNS counts, were significantly increased during breeding. Especially in the later period in our tests, 75.4% of bacteria in bioaerosols were CoNS. These results suggested that CoNS were the dominant bacterial group in the bioaerosols of henhouses. CoNS can colonize well on the surface of animal skin and have biofilm forming ability to resist environmental stresses [31], and these phenotypes help them to survive and spread. Compared with swine houses, that and these phenotypes help them to survive and spread film forming ability to resist environmental stresses [31], colonize well on the surface of animal skin and have biofilm [31].

Seven species of CoNS were identified in bioaerosols and nasal swabs of farmers, and S. sciuri was the predominant species, which was consistent with the incidence in other environments of chicken breeding, like bedding and litter [32, 33]. Among our identified species, S. saprophyticus and S. haemolyticus were frequently recovered from humans. In the previous study, the CoNS were recovered from chicken and their carcasses, minced meat, and the contact persons, suggesting its potential transmission from animal to humans [34]. Most human infections caused by CoNS are subacute and chronic [35], but sometimes, foreign-body-related infections of the bloodstream by CoNS can be fatal [36, 37]. As opportunistic pathogens, the presence of high concentrations of CoNS in bioaerosols have a potential risk of disease for chickens as well as poultry farmers.

Antibiotic resistance and resistance transmission of CoNS in bioaerosols is another important risk factor, for not only poultry industry but also public health. According to previous reports, hospital-acquired MRSA is often multidrug resistant, while community-acquired MRSA strains are usually limited to beta-lactam resistance and are susceptible to fluoroquinolones, aminoglycosides, erythromycin, and clindamycin [38, 39]. In contrast, the staphylococci from animal breeding have different resistance patterns [40]. Our CoNS isolates showed high resistance rates to penicillin, ampicillin, ciprofloxacin, chloramphenicol and tetracycline, which were used in animal breeding, suggesting the importance of selection stress from antibiotics. In addition, 49.0% CoNS isolates were MRCoNS (MIC 2), and among them, 74% contains mecA, which was responsible for its resistance. S. aureus is one of the most important pathogens for humans and animals and widely exist in poultry breeding [41]. MRSA, which contains the horizontally transferable methicillin-resistance gene mecA, was a global risk to human health [17]. Because CoNS is a closely related staphylococci of S. aureus, it is considered as an important resistance determinants provider of S. aureus. In this study, high percentage of CoNS isolates (36.3%) contained mecA gene, and this is of concern of potential spread of mecA to S. aureus in the henhouses. What was more serious, lots of MRCoNS isolates were not susceptible to vancomycin, which was the gold standard for treating the infections of methicillin-resistant staphylococci [42]. Therefore, these isolates had more broad-spectrum resistance, that suggested the emergence of multi-drug resistant CoNS in the bioaerosols of poultry houses. In addition, it has been proved that bacteria, such as E. coli carrying plasmid-mediated quinolone resistance genes, could spread from farms to the external environment via air [43]. In this study, although we did not test the CoNS around the henhouses, we suppose that the multi-drug resistant CoNS can spread out from the inside of henhouses to the outside through the air, which further highlight its serious threat to public health.

CoNS are the predominant pathogens causing intramammary infections in dairy cows [14], while in chickens, the economic burden of staphylococcal infections includes decreased weight gain, drop in egg production, mortality, condemnation at slaughter and lameness [33]. Although obvious symptoms caused by CoNS did not occur frequently, immunological challenge from CoNS bioaerosol was one of the important negative effects in chickens. To simulate the CoNS bioaerosol infection, we chose mecA-positive S. sciuri, which was the predominant species in the henhouses, as the model of aerosolized bacteria, and we chose 1.0 × 10⁶ cfu/m³, which was the detected concentration in this study, as the concentration of exposure. As shown in Fig. 3, although there were no typical symptoms caused by independent CoNS infection, proinflammatory cytokines including IL-1β, IL-6 and IL-8 were significantly induced at 5 or 7 dpi, and at the same time anti-inflammatory cytokine IL-10 was also up-regulated. Both of them were reduced at 14 dpi to maintain a homeostatic state. These results suggest that exposure in aerosolized CoNS could induce obvious inflammatory response in chickens. It is evident that the inflammatory process is a life-saving response to microbial challenge, however, it is supported
by available nutrients diverted from productive purposes [44, 45]. As previously reported, equalized for feed intake, a vigorous acute phase immune response in chickens has been estimated to account for around 10% of nutrient use [46], and the threonine requirement increase by 2 to 10% [47]. A general model for predicting animal performance during pathogen challenges suggested that, subclinical infections, even with no visible symptoms but immune responses, caused a reduction in feed intake, and greater reduction was seen during clinical disease [48]. Therefore, the induction of inflammatory cytokines by CoNS suggested that, continuous exposure to high concentration of CoNS had caused subclinical infection of chickens, which could lead to reduced production performance. In addition, CoNS were also isolated in the nasal swabs of poultry farmers, which suggested that the presence of CoNS in bioaerosols might represent a significant immunological challenge to chickens as well as farmers.

Conclusions
In this study, we showed high concentration of CoNS in henhouses, and S. sciuri was the preponderent CoNS species. Antibiotic resistance analysis and bioaerosols infection of CoNS further highlighted its hazards on resistance and immunological challenge. These results suggested that, CoNS in bioaerosols could be a serious factor in the henhouses for not only poultry industry but also public health.

Methods
Sampling from the henhouses
Bioaerosol samples were collected from nine henhouses for the growing period of caged Hy-Line Layers in Central China during April to July 2017 and 2018. The sampling times covered the first 90 days of the growing period before changing their cage (chickens age 2, 20, 40, 60 and 90 days). All the houses were strictly disinfected before breeding. The used sampler was SKC BioSampler (SKC, Pittsburgh, PA, USA) and the flow rate was 12.5 L/min. According to the indoor air quality standard in China (GB/T18883–2002), bioaerosols were collected at five sampling sites, which were evenly distributed in the henhouses. At each sampling site, the sampling time was 20 min and a total of 250 L bioaerosols were collected into 20 ml phosphate-buffered saline (PBS). The collected samples were kept in 50 ml sterile centrifuge tubes in an ice bath and then transferred to the laboratory for analysis immediately. Nasal swabs of farmers in each henhouse were also collected and transported to the laboratory for bacterial isolation.

Enumeration, isolation and identification of staphylococci in bioaerosols
To count the total culturable bacteria and staphylococci from bioaerosols, the samples in PBS were 10-fold serial diluted, and spread onto Tryptic Soy Agar (TSA) (BD, Franklin Lakes, NJ, USA) plates and Baird Parker plates (BD) at 37 °C for 24 h. The suspect staphylococcal colonies on the Baird Parker plates were confirmed by Gram stain and PCR targeting the Staphylococcus-specific 16S rRNA fragment as previously described [49]. The bacterial count in each henhouse was calculated as the mean from five sampling sites. To further isolate and identify the species of staphylococci, three to four suspect colonies from each plate were picked randomly and identified using 16S rRNA sequence analysis and Microbiology Identification System Phoenix-100 (BD). To isolate and identify the staphylococci from poultry farmers, the collected nasal swabs were streaked onto Baird Parker plates and cultured at 37 °C for 24 h, and the suspect staphylococcal colonies were identified as described above.

Antibiotic susceptibility testing
CoNS isolates were tested for susceptibility to antimicrobial drugs using disk diffusion assay or minimum inhibitory concentration (MIC) assay according to the Clinical and Laboratory Standards Institute (CLSI) [50]. Disks were placed on the surfaces of CoNS inoculated Mueller Hinton agar plates (Oxoid, Basingstoke, UK). These antimicrobial disks (Oxoid) included penicillin (Pen, 10 U), ampicillin (Amp, 10 μg), ciprofloxacin (Cip, 5 μg), chloramphenicol (Chl, 30 μg), erythromycin (Ery, 15 μg), tetracycline (Tet, 30 μg), amikacin (Ami, 30 μg), rifampicin (Rif, 5 μg) and clindamycin (Cli, 2 μg). Inoculated plates were incubated at 37 °C for 24 h. The inhibition zone diameters were measured and interpreted following the CLSI guidelines. MIC assays were carried out to determine the susceptibility of CoNS to oxacillin (used for confirmation of methicillin resistance in CoNS, CLSI) and vancomycin, and the evaluated concentrations were 0.125–128 μg/ml for oxacillin and 0.125–16 μg/ml for vancomycin. E. coli ATCC 25922 and S. aureus ATCC 25923 strains were included in the test for quality control.

Detection of meca in CoNS
Genomic DNA was extracted using MiniBEST Universal Genomic DNA Extraction Kit (TaKaRa). The presence of meca was detected using PCR targeting the fragment of meca (163 bp), as previously reported [51]. PCR was performed in a GeneAmp PCR System 9700 (ABI, Darmstadt, Germany). The primers were mecAF, 5′-ACTGCTATCC ACCCTCAAAC-3′ and mecAR, 5′-CTGGTGAAGT TGTAATCTG-3′. The reaction conditions were as
followed: initial denaturation temperature of 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, renaturation at 55°C for 1 min, elongation at 72°C for 30 s and final elongation at 72°C for 5 min. The PCR products were subject to agarose gel electrophoresis. The DNA bands were stained with ethidium bromide and visualized using a GelDoc XR System (Bio-Rad, Shanghai, China).

**Immune responses of chickens infected with aerosolized CoNS**

To investigate the effects of chickens exposed to aerosolized CoNS, bioaerosol infection was carried out. Embryonated eggs from SPF Leghorn chickens were purchased from Merital-Vital, Beijing, China. SPF chickens were hatched in a contained environment, and raised in negative pressure isolators for animal work. A total of 40 1-day-old SPF chickens were randomly divided into two groups (n = 20). Chickens in the infection group were exposed to bioaerosols containing approximately $10^6$ cfu/m³ $\text{mecA}$-positive $S. \text{sciuri}$ for 1 h (range from $6 \times 10^5$ to $2.5 \times 10^6$ cfu/m³ during this hour), and were transferred into individually ventilated cages for breeding. Aerosolized bacteria were exported by Atomizer Aerosol Generator Model 3079A (TSI Incorporated, Shoreview, MN, USA) as previously reported [52], and the particle diameter was 0.2–0.3 μm. The concentration of bacteria in the aerosol (20 cm above the ground of the cages) was assessed every 15 mins using plate sedimentation method, and a proper amount of aerosolized bacteria was replenished every 15 mins. Chickens exposed in exported PBS without bacteria were used as a control group (n = 20). At 1, 3, 5, 7 and 14 days post infection (dpi), four chickens in each group were randomly selected, and the total mRNA of lungs and spleens was collected, and the total mRNA of lungs and spleens was isolated using MiniBEST Universal RNA Extraction Kit (TaKaRa, Dalian, China). The expression levels of inflammatory cytokines were detected by real-time reverse transcription (RT)-PCR. The $\text{gapdh}$ gene was used as the internal control [53]. The primers were as follows: $\text{gapdh-f}$, 5′-TCTCCATGGTGTTGAAGACA-3′; $\text{gapdh-r}$, 5′-GACGTGCAGCAGGAACTA-3′; $\text{IL-1β-f}$, 5′-GGATTCTGAGCACCACACGT-3′; $\text{IL-1β-r}$, 5′-TCTGGTGATGTGAGATGTC-3′; $\text{IL-6-f}$, 5′-ATCCGGCAGATGGTAAA-3′; $\text{IL-6-r}$, 5′-CCCTCAGGGTCTTCCCTCA-3′; $\text{IL-8-f}$, 5′-GAAGGTAGCTGGTATAA-3′; $\text{IL-8-r}$, 5′-CCAGGCACACCTCTTCTTCCA-3′; $\text{TNF-α-f}$, 5′-CCTCTGAGGGCATTTGGAAGC-3′; $\text{TNF-α-r}$, 5′-ACTGGGGCGTCTAGAAACAG-3′. Each assay was carried out with at least three biological replicates. After the experiment, the chickens were euthanized and underwent harmless treatment according to the regulations from Hubei Provincial Animal Care and Use Committee.

**Statistical analysis**

To test the bacterial loads in the bioaerosols, the bacteria count in each henhouse was calculated as the mean from five sampling sites, and the bacteria counts at different times were compared using Student’s $t$-test. To evaluate the inflammatory responses induced by aerosolized CoNS, the relative transcript abundance levels of inflammatory cytokines were calculated using the $2^{-\Delta \Delta Ct}$ method and the expression levels at different times were compared using Student’s $t$-test. A $p$-value < 0.05 was considered statistically significant.

**Abbreviations**

CLSI: Clinical and Laboratory Standards Institute; CoNS: Coagulase-negative staphylococci; dpi: Days post-infection; IL: Interleukin; MIC: Minimum inhibitory concentration; MRCoNS: Methicillin-resistant CoNS; MRSA: Methicillin-resistant $S. \text{ aureus}$; PBS: Phosphate-buffered saline; TNF: Tumor necrosis factor; VRS: Vancomycin-resistant $S. \text{ aureus}$

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**Authors’ contributions**

YL, GW, and TZ participated in the conception and design of the study. YL, QLuo, and YC performed the farm and laboratory work. YC, HS, and TZ analyzed the data and wrote the manuscript. QLuo and HS contributed to the analysis and helped in the manuscript discussion. All authors read and approved the final manuscript.

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**Availability of data and materials**

The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

**Ethics approval and consent to participate**

The protocols of experimentation on animals were approved by the Hubei Provincial Animal Care and Use Committee (approval number SCXK 2018-0022). The animals used in this study were derived from commercial sources, and the owner consent was not required. In this study, the collections of bioaerosol samples were approved by the owners of these henhouses. Nasal swabs were from the farmers, and written informed consent was obtained from these poultry farmers.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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