Characteristics of airborne Staphylococcus aureus (including MRSA) in Chinese public buildings

Xiaoxia Li · Yuyu Qiu · Ailian Yu · Weifeng Shi · Guomin Chen · Zhong Zhang · Dunjiang Liu

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Abstract The aim of this study was to evaluate the concentration and size distribution of airborne culturable Staphylococcus aureus (S. aureus) (including MRSA) in Chinese public buildings. Air samples were collected, using six-stage Andersen sampler from five different public buildings in one large Chinese community. The mean indoor concentrations of the total and respirable airborne S. aureus were 72 and 50 CFU/m³ in the general hospital, 72 and 49 CFU/m³ in the kindergarten, 76 and 52 CFU/m³ in the hotel, 84 and 57 CFU/m³ in the movie theater, and 55 and 40 CFU/m³ in the university classroom. Respirable S. aureus amounted to approximately 57–73% of the total S. aureus concentrations. Mean total and respirable concentrations of airborne MRSA were 32 and 20 CFU/m³ in the general hospital, 20 and 13 CFU/m³ in the kindergarten, 23 and 16 CFU/m³ in the hotel, 33 and 20 CFU/m³ in the movie theater, and 24 and 17 CFU/m³ in the university classroom. Respirable MRSA amounted to approximately 61–72% of the total MRSA concentrations. The ratios of indoor and outdoor concentration for airborne S. aureus and MRSA were more than 1.0 in all the investigated public buildings. The size distribution results showed relatively high collection rates on stage 4 (2.1–3.3 µm) for both airborne culturable S. aureus and MRSA regardless of the type of public buildings.

Keywords Airborne Staphylococcus aureus (S. aureus) · Methicillin-resistant Staphylococcus aureus (MRSA) · Size distribution · Public buildings

1 Introduction

Airborne and droplet transmission are one of the major routes for spreading human and animal bacteria, such as Escherichia coli, Staphylococcus aureus, anthrax, Klebsiella pneumonia, and Pseudomonas aeruginosa (Wagenvoort et al. 1993; CDC 2003; Prazmo et al. 2003; Jones et al. 2003). Numerous facts proved that microbial aerosol is a great threat to human and animal health, and a study on the source identification of the origins and transmission of microbial pathogens should be conducted urgently (Li and Hou 2003; Agranovski et al. 2004; Dent et al. 2008; Liu et al. 2012).

Staphylococcus aureus, especially methicillin-resistant S. aureus (MRSA), has become one of the
most predominant pathogenic bacteria in hospitals and
in the community, which causes diseases of wide
concern, e.g., skin and tissue infections, deep abscess
formation, pneumonia, endocarditis, osteomyelitis,
toxic shock syndrome, and bacteremia endocarditis
and bacteremia (Deurenberg et al. 2007; Naseer and
Jayaraj 2010; Liu et al. 2012). Although the principal
mode of \textit{S. aureus} transmission is contact-spread, the
contribution of airborne \textit{S. aureus} to the spread of
infection is likely to be greater than is currently
recognized, and MRSA is the most frequently isolated
airborne microbe (Dürmaz et al. 2005). Shiomori et al.
(2001) also indicated that airborne MRSA may play a
role in MRSA colonization in the nasal cavity or in
respiratory tract infections. Further studies will be
needed to assess the levels of \textit{S. aureus}, especially
MRSA, contamination of air and to develop more
effective means of controlling and removing airborne
MRSA in the public buildings. However, compared to
other countries, there are little extant data about
airborne \textit{S. aureus} or MRSA in China. In this study,
the concentration and size distribution of airborne \textit{S.
aureus} (including MRSA) in indoor air of different
public buildings in China were investigated. These
studies could provide information that is available to
improve the current ambient air quality standard
(AAQS) and is necessary for reducing indoor air
contamination by airborne \textit{S. aureus}.

2 Materials and methods

2.1 Public buildings studied

Measurement was taken on sunny days between
March and May in 2013 in one large community
located at the northern part of Tai’an city of Shandong
Province in China. As shown in Table 1, indoor and
outdoor air samples were collected and characterized
in the following locations: general hospital, kindergar-
ten, hotel, movie theaters, and university teaching
building. Two rooms were randomly selected in each
public building. All public rooms gave an approval to
our phone or written requests for cooperation with this
study. Because of the short-term effects of everyday
activities on indoor bioaerosol concentrations, there
were no residents or human activities in the building
during the sampling campaigns.

| Public buildings     | Area       | Sampling sites | Ventilation type |
|----------------------|------------|----------------|------------------|
| General hospital     | >300 (ward)| Surgical ward  | Mechanical       |
| Kindergarten         | >1,000 m² (area)| Classroom | Natural          |
| Hotel                | >100 (room)| Room           | Mechanical       |
| Movie theaters       | >1,000 m² (area)| Screens | Mechanical       |
| University teaching building | >50 (room) | Classroom | Natural          |

2.2 Air sample collection

Air samples were taken from indoor and outdoor air of
five public buildings with six-stage Andersen sampler
(Andersen 1958) at the flow rate of 28.3 L/min. Aerodynamic
diameter ranges for each stage in cascade impactor are as follows:
1 stage (>7.0 µm), 2 stage (4.7–7.0 µm), 3 stage (3.3–4.7 µm), 4 stage
(2.1–3.3 µm), 5 stage (1.1–2.1 µm), and 6 stage (0.65–1.1 µm) (Andersen 1958). The Andersen
samplers were located near the middle of the room about
1 m above the ground. One outdoor site, the air inlet of
each building, was investigated in comparison with the
indoor concentration (Kim and Kim 2007). Before
sampling, Andersen samplers were equipped with
Baird–Parker agar (lot number 060227; Tian He,
Hangzhou, China) plates and operated for 8–12 min
according to the environmental situation of the
measurement locations. Before sampling, the inside
of the sampler was disinfected with 70 % alcohol and
then was inserted with the agar plate according to the
collection stage. The meteorological conditions (tem-
perature, humidity, and wind speed) were monitored
in both indoors and outdoors. During the sampling
periods, temperatures varied between 21 and 28 °C
indoors, and between 14 and 24 °C outdoors, relative
humidity indoors varied between 40 and 70 %, and
outdoors between 34 and 60 %, wind speed on the
days of the monitoring varied between 0.02 and 0.1 m/s
indoors, and outdoors between 0.5 and 0.2 m/s.

Two impingers were used at each location per
sampling event and represented duplicate samples.
Three continuous samplings in each room per building
were collected on each visiting day. Each building was visited three times, once every 2 weeks. In total, 36 indoor samples and 18 outdoor samples were obtained from each public building.

2.3 Isolation, identification, and enumeration of airborne *S. aureus*

The air samples were immediately taken to the microbe laboratory and were processed within 24 h. The Baird–Parker agar plates for air samples were cultured at 37 °C for 24 h. Then, all the colonies generated in the plates were screened for their Gram reaction using the “KOH assay.” All the Gram-positive colonies were subcultured in mannitol salt agar medium, and their species were then identified by using the API 20 E system (BioMerieux, Marcy-l’Etoile, France). The colonies were counted after 24 and 48 h to determine whether the plates were overgrown. The positive-hole correction was applied (Andersen 1958). Concentration of air samples was expressed as colony-forming unit (CFU/m³ air). Finally, *S. aureus* cultures were frozen at −20 °C in LB broth containing 20 % glycerol.

2.4 MRSA isolation and identification

Methicillin resistance was initially confirmed by using acefoxitin disk (30 µg) on Mueller–Hinton agar (CLSI 2011). The meca gene was detected by polymerase chain reaction and primers as previously described (Vannuffel et al. 1998). The forward and reverse primers for meca gene were 5′-AAAATCGATGGT AAA GGTTTGGC-3′ and 5′-AGTTCAGTACC GGATTTGC-3′. *S. aureus* ATCC 43300 (MRSA) and *S. aureus* ATCC 25923 (MSSA) were used as meca-positive controls and meca-negative controls, respectively. The presence of a 533-bp amplimer was taken as an indication of the presence of the meca gene.

3 Results

3.1 Concentrations of airborne *S. aureus* and MRSA in public buildings

As shown in Table 2, the mean concentrations of the total and respirable airborne *S. aureus* were 72 and 50 CFU/m³ in the general hospital, 72 and 49 CFU/m³ in the kindergarten, 76 and 52 CFU/m³ in the hotel, 84 and 57 CFU/m³ in the movie theaters, and 55 and 40 CFU/m³ in the university classrooms, respectively. As a result, both the concentrations of total and respirable airborne *S. aureus* were the highest in the movie theaters and it was the lowest in the university classrooms (p < 0.05). The mean ratios of respirable *S. aureus* to total airborne *S. aureus* concentrations were 57–73 %, and no significant differences were found among the public buildings (p > 0.05). The mean ratios of indoor to outdoor concentrations for total and respirable *S. aureus* were 1.7–2.6 and 1.8–3.0, respectively. The ratios of indoor to outdoor concentrations were the highest in the university classroom (p < 0.05). However, the differences between the rest of the public buildings were not statistically significant (p > 0.05).

As shown in Fig. 1, the 533-bp desired product was obtained. The concentrations of total and respirable airborne MRSA in public rooms are also shown in Table 2 and were 32 and 20 CFU/m³ in the general hospital, 20 and 13 CFU/m³ in the kindergarten, 23 and 16 CFU/m³ in the hotel, 33 and 20 CFU/m³ in the movie theaters, and 24 and 17 CFU/m³ in the university classrooms, respectively. As a result, both the concentrations of total and respirable airborne MRSA were the highest in the movie theaters and it was the lowest in kindergarten (p < 0.05). The mean ratios of respirable MRSA to total airborne MRSA concentrations were 61–72 %, and no significant differences were found among the public buildings (p > 0.05). The mean ratios of indoor to outdoor concentrations for total and respirable MRSA were 2.0 and 1.9 in the hospitals, 2.0 and 0.9 in the kindergarten, 2.4 and 1.1 in the hotel, and 3.5 and 1.5 in the movie theaters, and 4.8 and 1.7 in the university classrooms, respectively.
**Table 2** Concentrations of total and respirable airborne *S. aureus* and MRSA in Chinese public rooms

|                      | General hospital | Kindergarten | Hotel | Movie theaters | University classroom |
|----------------------|------------------|--------------|-------|---------------|----------------------|
| **Airborne *S. aureus*** |                  |              |       |               |                      |
| Concentration (CFU/m³) |                  |              |       |               |                      |
| **Total**            |                  |              |       |               |                      |
| Max.                 | 102              | 88           | 97    | 119           | 66                   |
| Min.                 | 49               | 49           | 53    | 57            | 40                   |
| Median               | 68               | 77           | 80    | 84            | 55                   |
| Mean                 | 72a***           | 72c          | 76ab  | 84b           | 55d                  |
| **Respirable***      |                  |              |       |               |                      |
| Max.                 | 71               | 66           | 66    | 80            | 53                   |
| Min.                 | 35               | 31           | 35    | 40            | 27                   |
| Median               | 49               | 51           | 55    | 57            | 40                   |
| Mean                 | 50a              | 49a          | 52a   | 57a           | 40b                  |
| **Ratio (%)***       |                  |              |       |               |                      |
| Max.                 | 85               | 75           | 76    | 79            | 40                   |
| Min.                 | 58               | 61           | 59    | 61            | 86                   |
| Median               | 71               | 70           | 68    | 67            | 62                   |
| Mean                 | 71a              | 68a          | 69a   | 57a           | 73a                  |
| **I/O ratio****     |                  |              |       |               |                      |
| **Total**            |                  |              |       |               |                      |
| Max.                 | 2                | 2.5          | 2.9   | 3.6           | 3.6                  |
| Min.                 | 1.2              | 1.4          | 1.6   | 1.7           | 1.7                  |
| Median               | 1.7              | 2.1          | 2.4   | 2.5           | 2.5                  |
| Mean                 | 1.7              | 2.0a         | 2.3b  | 2.5a          | 2.6b                 |
| **Respirable**       |                  |              |       |               |                      |
| Max.                 | 2.4              | 3.6          | 3.5   | 4.2           | 4.0                  |
| Min.                 | 1.2              | 1.7          | 1.9   | 2.1           | 2.0                  |
| Median               | 1.8              | 2.7          | 2.9   | 3.0           | 2.7                  |
| Mean                 | 1.8a             | 2.7a         | 2.8a  | 3.0a          | 2.8b                 |
| **Airborne MRSA**    |                  |              |       |               |                      |
| Concentration (CFU/m³) |                  |              |       |               |                      |
| **Total**            |                  |              |       |               |                      |
| Max.                 | 44               | 35           | 44    | 49            | 31                   |
| Min.                 | 18               | 9            | 13    | 22            | 13                   |
| Median               | 31               | 20           | 22    | 33            | 29                   |
| Mean                 | 32a              | 20b          | 23b   | 33a           | 24b                  |
| **Respirable**       |                  |              |       |               |                      |
| Max.                 | 35               | 18           | 27    | 35            | 27                   |
| Min.                 | 9                | 4            | 4     | 9             | 9                    |
| Median               | 18               | 15           | 13    | 18            | 18                   |
| Mean                 | 20b              | 13b          | 16b   | 20a           | 17ab                 |
| **Ratio (%)**        |                  |              |       |               |                      |
| Max.                 | 83               | 100          | 100   | 100           | 100                  |
| Min.                 | 40               | 33           | 33    | 40            | 50                   |
| Median               | 59               | 63           | 73    | 59            | 71                   |
| Mean                 | 62a              | 63a          | 69a   | 61a           | 72a                  |
The ratios of indoor to outdoor concentrations for total MRSA were the lowest in kindergarten ($p < 0.05$).

### 3.2 Size distribution of culturable airborne *S. aureus* and MRSA in public buildings

Figures 2 and 3 show the size distribution, determined by the six-stage Andersen sampler, of culturable airborne *S. aureus* and MRSA in different public buildings. Both the distribution ratios of indoor and outdoor airborne *S. aureus* were highest for a particle diameter of 2.1–3.3 μm (4th stage) in the general hospital, hotel, and movie theaters and show the highest collection rate at stage 3 (3.3–4.7 μm) in the university classrooms. The distribution ratio was generally highest on stage 2 (4.7–7.0 μm) for airborne *S. aureus* in indoor air of kindergarten (Figs. 2a, 3a).

The size distribution of airborne MRSA showed relatively high collection rates at third stage in indoor air of the hotel, movie theaters rooms, and university classrooms, at second stage in indoor air of the general hospital and the kindergarten (Figs. 2b, 3b). It showed equally high collection rates at the second and fourth stages in outdoor air of general hospital and kindergarten, respectively (Figs. 2b, 3b).

Regardless of the kind of the public buildings, isolates of airborne *S. aureus* and MRSA were detected in all stages [from stage 1 (>$7 \mu m$) to stage 6 (0.65–1.1 μm)], and the size distribution in indoor air is showing the highest collection rate at stage 4 (2.1–3.3 μm).

### 4 Discussion

Although emergence of respiratory viral pathogens such as SARS, avian influenza viruses, and 2009 H1N1...
influenza virus had attracted widespread public attention in China, systematic investigations of airborne pathogenic microorganisms in public buildings have not be performed. Airborne *S. aureus* (including MRSA), known as the commonly isolated airborne microbe (Dürmaz et al. 2005), is investigated in the present study. Our field results demonstrate that both the concentrations of total and respirable airborne *S. aureus* were the highest in movie theaters and it was the lowest in the university classrooms (Table 2). Previous study showed that the range of mean total concentration of *Staphylococcus* spp. was 16–58 CFU/m³ in Korea public buildings (Kim and Kim 2007). Gandara et al. (2006) reported that the average recovered concentration of the respirable *S. aureus* in Texas homes was only 15 CFU/m³. The *Staphylococcus* spp. counts ranged from 55 to 84 CFU/m³ in the present study. Based on these results, the concentration of airborne *S. aureus* showed inconsistency among different countries. Therefore, it may not be appropriate to apply foreign data directly to hygienic management guideline of public buildings in China. Therefore, the present study is very necessary. The ratio of respirable to total airborne *S. aureus* inside the building ranged from 57–73 % in the present study, which was higher than the foreign reports that showed a range of 30–60 % (DeKoster and Thorne 1995; Pastuszka et al. 2000; Kim and Kim 2007; Kim et al. 2010).

Since the methicillin-resistant *S. aureus* (MRSA) was discovered in 1961, it has become one of the most
predominant pathogenic bacteria in nosocomial and community-acquired infections and extensive research of MRSA had emerged worldwide (Jevons 1996; Hsueh et al. 2004; Cimolai 2010; Deleo et al. 2010; Otter and French 2010). The studies of S. aureus (including MRSA) strains isolated from clinical specimens, such as sputum, urine, pus or secretions, and blood, have already been reported frequently in China and abroad. Airborne MRSA may play a role in respiratory tract MRSA infections, and measures should be taken to prevent the spread of airborne MRSA (Shiomori et al. 2001). Therefore, the concentration of airborne MRSA was also evaluated in this study. The results showed that mean total concentrations of airborne MRSA were 20–33 CFU/m³ in the investigated buildings (Table 2). Considering the previous reports, the mean total of airborne MRSA was 59–73 CFU/m³ in bedsheets in surgery unit (Shiomori et al. 2001).

Over the last decade, community-acquired MRSA (CA-MRSA) causing primarily skin and soft tissue infections has emerged as a major cause of disease in the general population in day cares, schools, prisons, and sport teams around the world (King et al. 2006; Roberts et al. 2011, 2013). MRSA has been isolated from recreational beaches, high-touch surfaces in homes, universities, and other community environmental surfaces (Roberts et al. 2013). However, there have been few recent studies demonstrating the detection and isolation of airborne MRSA in different
public buildings. The results of the present study demonstrated that airborne MRSA was present in all buildings studied, which is clearly worth considering.

The ratios of indoor to outdoor concentrations of airborne *S. aureus* and MRSA in all the investigated public buildings exceeded 1.0 (Table 2), which demonstrated that the indoor air has been contaminated with airborne *S. aureus* and MRSA (Li and Kuo 1993; Kim and Kim 2007; Kim et al. 2010).

The size of airborne bacteria determines the location in the respiratory system where the inhaled airborne bacteria are deposited (Kim and Kim 2007). In humans, aerosol particles ≤5 μm are capable of reaching deep into the respiratory tract and being deposited in alveolar tissues. Aerosols of this size, as well as larger particles, can be deposited via inertial impaction in the upper airway, where airflow velocity is greatest, at points in the respiratory tract of airflow directional (Kortney et al. 2011). Regardless of the kind of the public buildings, the size distribution of airborne *S. aureus* (including MRSA) in indoor air is showing the highest collection rate at stage 4 and stage 3, respectively (Figs. 2, 3). The results showed that most of airborne *S. aureus* in indoor air could deposit in the lower regions of the human respiratory tract. However, the previous study indicated that *Staphylococcus spp.* showed relatively high collection rates at first (≥7.0 μm) and second (4.7–7.0 μm) stages (Kim and Kim 2007). The size distribution of airborne *S. aureus* showed inconsistency in the public buildings (Figs. 2, 3), which appears to be due to the indoor environmental condition like everyday indoor activities or the discharge mechanism related to the velocity of air flow near the microbial habitat in the indoor structures (Li and Hou 2003; Kim and Kim 2007). Further research is needed to determine the cause of inconsistency. The distribution of outdoor airborne *S. aureus* was considerably similar to the indoor distribution and was consistent with the previous results (Di Giorgio et al. 1996; Kim and Kim 2007).

Once airborne microorganisms are exposed to a variety of environmental stresses, the most important ones are being the effects of temperature, relative humidity (RH), and wind speed (Li and Hou 2003; Kim and Kim 2007). To minimize the impact of meteorological variations, sampling was generally conducted under certain temperature, relative humidity (RH), and wind speed conditions in our study.

The limitation of this field study is that it was conducted only in the 3-month period in the spring, and it would not completely reflect the weather conditions of our country that has four distinct seasons. Moreover, the number of public buildings investigated in the present study was a relatively small size. Therefore, further larger studies should be performed intensively. Emphasis should be placed on routine air sampling in public buildings, which would provide a clearer picture of the relationship between airborne microbes and infection rates.

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