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RESEARCH ARTICLE

$PM_{2.5}$-associated bacteria in ambient air: Is $PM_{2.5}$ exposure associated with the acquisition of community-acquired staphylococcal infections?

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Abstract

Particulate matter (PM), a major component of air pollution, is an important carrier medium of various chemical and microbial compounds. Air pollution due to PM could increase the level of bacteria and associated adverse health effects. Staphylococci as important opportunistic pathogens that cause hospital- and community-acquired infections may transmit through air. This study aimed to obtain knowledge about the concentration of airborne bacteria as well as staphylococci associated with particulate matter with a diameter of less than 2.5 micrometers ($PM_{2.5}$) in ambient air. The impact of meteorological factors including ultraviolet (UV) index, wind speed, temperature, and moisture on microbial concentrations was also investigated. Quartz filters were used to collect $PM_{2.5}$ and associated bacteria in ambient air of a semiarid area. Airborne bacteria were quantified by culture method and *Staphylococcus* species identified by molecular methods. The mean (SD) concentration of $PM_{2.5}$ and airborne bacteria was 64.83 (24.87) μg/m^3 and 38 (36) colony forming unit (CFU)/m^3, respectively. The results showed no significant correlation between the levels of $PM_{2.5}$ and concentrations of bacteria ($p < 0.05$). Staphylococcus species were detected in 8 of 37 (22%) samples in a concentration from 3 to 213 CFU/m^3. *S. epidermidis* was detected with the highest frequency followed by *S. gallinarum* and *S. hominis*, but *S. aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) were not detected. No significant correlation between the concentrations of bacteria with meteorological parameters was observed ($p < 0.05$). Our finding showed that, although the study area is sometimes subject to air pollution from $PM_{2.5}$, the concentration of $PM_{2.5}$-associated bacteria is relatively low. According to the results, $PM_{2.5}$ may not be a source of community-associated staphylococcal infections.

Keywords Airborne bacteria · Air pollution · $PM_{2.5}$ · *Staphylococci*

Introduction

Staphylococci are spherical non-spore forming, gram-positive bacteria found on the skin and in the nose of humans as well as in the environment such as water and soil. *Staphylococcus aureus* is an opportunistic human pathogen that causes a wide variety of nosocomial infections such as pneumonia. Among *Staphylococcus aureus* strains, methicillin-resistant *Staphylococcus aureus* (MRSA) is of the highest importance from the public health point of view. MRSA infections lead to an increase in morbidity and mortality in hospital settings worldwide [1, 2].

Although *Staphylococcus aureus* and MRSA are among the most important causative agents of hospital-acquired infections, they are increasingly emerged as important causes of

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community-acquired infections [3]. Susceptible individuals such as children, elderly, and people with the weakened immune system are at particular risk from community-acquired staphylococcal infections [4].

Furthermore, other species of *Staphylococcus* known as coagulase-negative staphylococci (CoNS) including *S. epidermidis, S. haemolyticus* and *S. hominis* can also cause hospital and community–acquired staphylococcal infections with varying degrees of severity [5]. Direct person-to-person contact is the most common route of staphylococcal infection transmission. However, airborne transmission is also considered as a route for the dissemination of staphylococcal infections [2]. Study of Madsen et al. (2018) showed that airborne MRSA and *S. aureus* are present on particulate matter (PM) with aerodynamic diameters which can penetrate lung alveoli [6]. In other words, the biological composition of PM, bacterial and fungal aerosols, may cause health problems such as systemic inflammatory. Among blood markers of inflammation, interleukin-6 positively associated with exposure to *Staphylococcus* spp. [7].

PM is an important carrier medium of various chemical and microbial compounds, such as pathogenic bacteria [8–10]. PM has been recognized as a major component of air pollution. Air pollution associated with PM has recently become a primary concern in some countries such as Iran, which affects human health, resulting in more morbidity and mortality [11]. Urbanization, the increase of mobile sources and stationary sources reside in the vicinity of urban areas are the main factors affecting air pollution. In the last years, due to the increase of desertification, the air quality in arid and semi-arid areas especially in the Middle East countries such as Iran has also been affected by region and local dust events [12, 13]. Among PM, $PM_{2.5}$ are particularly important because they can pose a higher risk to human health than PM_{10} (particles with an aerodynamic diameter less than 10 μm) [8]. It was reported that exposure to high levels of $PM_{2.5}$ associated with adverse health effects such as respiratory problems and pneumonia [8, 14].

Considering the importance of $PM_{2.5}$ as carrier of airborne microorganisms [15] and increase in the prevalence of community-acquired staphylococcal infections, it is needed to investigate the role of air pollution associated with $PM_{2.5}$ on the microbial infections. Since the change in concentration of PM could affect the load of airborne microorganisms in the atmosphere, this study was designed to (1) obtain information about the concentration of airborne bacteria as well as staphylococci associated with $PM_{2.5}$ in ambient air of a semi-arid area, (2) investigate the meteorological factors that may affect the concentration of airborne microorganisms.

Methods

Sampling site

Isfahan, as the third largest city in Iran, with a population of about 1.6 million is located in the center of country in a position of 32. 38′ 41 N, 51. 40′ 03 W. Isfahan with a semi-arid climate is affected by relatively high levels of $PM_{2.5}$ and PM_{10} [11]. Air sampler was placed on the rooftop of a building in the city center of Isfahan at the height of 8 m from the ground level. To reduce biasing effects on the $PM_{2.5}$ measurement, the sampling site was selected in a location relatively far away from any potential PM sources such as construction sites. The location of sampling site is presented in Fig. 1.

Sample collection

An air sampler (PQ200 Ambient Air Particulate Sampler) with a flow rate of 16.7 L/min was used to collect $PM_{2.5}$ in a period of 24 h from 9AM-9AM. A total of 37 air samples were collected during a one-year period from November 2018 to October 2019 on approximately ten days intervals (3–4 samples per month). Precombusted (500°C, 1 h) 47 mm quartz fiber filters were used to collect $PM_{2.5}$. Filters were weighed using an analytical balance in sterile conditions before and after sampling and the mass concentration of $PM_{2.5}$ was calculated and expressed as microgram per cubic meter (μg/m^3). Half of the filters were then subjected to extraction and analysis of airborne bacteria associated with $PM_{2.5}$.

Meteorological parameters including temperature, relative humidity (RH), wind speed, and ultraviolet (UV) index were also recorded during the sample collection based on the data of Isfahan metrological organization.

Detection of airborne bacteria and staphylococci

Each filter was suspended in 7 ml sterile phosphate buffer solution (PBS), shaken for 15 min and then sonicated for 5 min. The washing procedure was repeated with an additional 3 ml PBS. Aliquots, 250 μl, of the suspension spread-plated onto tryptic soy agar (TSA), in duplicate. The TSA plates were incubated at 30 °C for 2–3 days. The growing colonies were enumerated and calculated as colony-forming units per cubic meter (CFU/m^3).

Bacterial colonies were Gram-stained and characterized based on the colony and cell morphology. Isolation of staphylococci was performed based on the cell morphology and catalase testing. Isolated colonies were further identified by a polymerase chain reaction (PCR) assay using the *Staphylococcus* genus-specific primer set Staph 756F and Staph 750R (Table 1). *S. aureus* identification was performed using a species-specific primer set that code for the

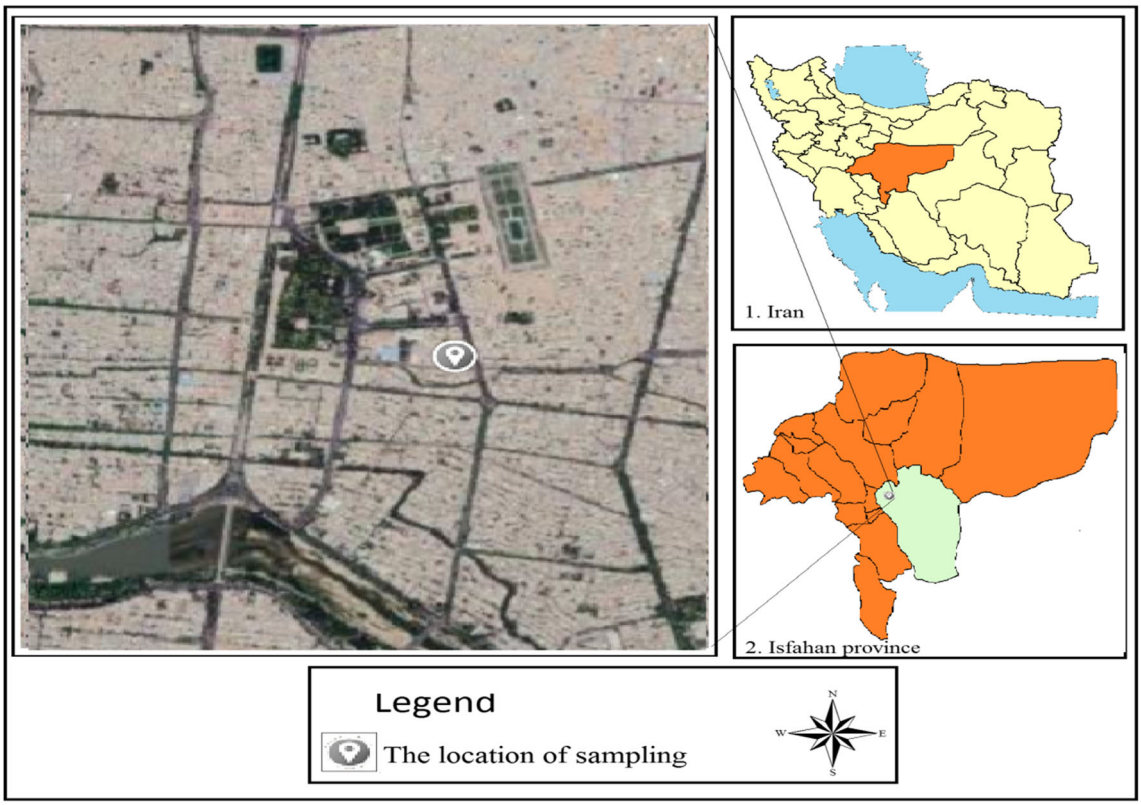

Fig. 1 Sampling site location

thermonuclease (*nuc*) gene (Table 1). *S. aureus* (ATCC 29,213) was used as a positive control. Confirmed *Staphyloccous spp*. and *S. aureus* isolates were analyzed for the presence of *mecA* gene (Table 1). Extraction of DNA and PCR reaction was performed as described by Shamsizadeh et al. (2017) [16].

Molecular identification of Staphylococcus species

PCR amplification of certain *Staphylococcus* species was performed by universal primers Eubac 27F and 1492R which amplify an approximately 1,420-bp fragment of the 16 s ribosomal RNA (rRNA) gene [20]. DNA sequencing of the amplified gene was performed, and DNA sequences were analyzed using BLAST algorithms and databases from the National Center for Biotechnology Information (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Statistical analysis

Statistical analyses were performed with SPSS 22. Kolmogorov-Smirnov's normality test was performed for identifying the use of parametric or non-parametric tests. For comparison of concentration differences of $PM_{2.5}$ and $PM_{2.5}$-associated bacteria in different seasons, the Kruskal-Wallis test was used. The Spearman's rank was used to evaluate the correlation between the analyzed parameters. P-values < 0.05 were considered statistically significant.

Table 1 Primers used in the study

Primer	Sequence (5' → 3')	Amplified fragment (bp)	Reference
Staph756F	AACTCTGTTATTAGGGAAGAACA	756	[17]
Staph750R	CCACCTTCCTCCGGTTTGTCACC		
*S. aureus*F	GCGATTGATGGTGATACGGTT	280	[18]
*S. aureus*R	AGCCAAGCCTTGACGAACTAAAGC		
*Mec*A147F	GTGAAGATATACCAAGTGATT	112	[19]
*Mec*A112R	ATCAGTATTTCACCTTGTCCG		

Results and discussion

PM can serve as carrier of various kinds of microorganisms such as pathogenic bacteria, and therefore changes in concentration of PM may lead to increase of the numbers of microorganisms associated with PM. The $PM_{2.5}$ concentrations ranged from 13 to 125 μg/m^3, with an average of 64.83 μg/m^3 (Table 2). The results of $PM_{2.5}$ concentrations showed that 89% of samples exceeded the limit value of 25 μg/m^3 as recommended by WHO (2006) [21]. In recent years, soil-derived dust particles have been the major component of PM and consequently air pollution in Isfahan. Increased aridity in the Middle East region such as Iran, due to climate change, has been led to enhanced dust activity and events which contribute to air pollution. In consistent with our results, the mean concentration of $PM_{2.5}$ in Isfahan during 2013–2016 was reported in a range of 37.29–56.15 μg/m^3 [11]. Alghamdi et al. (2014) reported a mean concentration of 60 μg/m^3 for $PM_{2.5}$ in Jeddah, Saudi Arabia [22]. However, in a study in Ahvaz, Iran, the city which was affected by severe Middle East dust (MED) storms, average concentration of $PM_{2.5}$ during the sampling period was 85.5 μg/m^3 in normal days which increased to 475 μg/m^3 in dust days [12]. As presented in Fig. 2, a high level of $PM_{2.5}$ was obtained in March which was related to the dust events in the region. Exposure to excessive PM is a risk factor for the acquisition of respiratory diseases [14, 23]. Study of Qin et al. (2019) showed the change in pharyngeal microbiota profiles after exposure to high concentrations of PM. They concluded that this change may lead to an increase in respiratory diseases [24].

The mean concentration of total bacteria was 37.82 CFU/m^3 and ranged from not detected to 290 CFU/m^3 (Table 2). In study of fang et al. (2007) in Beijing, China, the culturable airborne bacteria was found in a concentration from 71 to 22,100 CFU/m^3 with a mean of 2,217 CFU/m^3 [25]. In Hangzhou, China, concentration of airborne bacteria ranged from < 12 CFU/m^3 to 3259 CFU/m^3 with a mean of 292 CFU/m^3 [26]. Jeon et al. (2011) found a concentration of 330 CFU/m^3 for total bacteria in non-Asian dust days in Seoul, whereas the concentration was increased to about seven times higher (2212 CFU/m^3) for dust days [27]. Consistent with our finding, Alghamdi et al. (2014) found lower concentrations of bacteria associated $PM_{2.5}$ in Jeddah, Saudi Arabia, in a range of 45–591 CFU/m^3 [22]. Concentration of bioaerosols and its composition in outdoor environments can be affected by some factors such as environmental conditions prevailing at the site, wind direction and wind speed [28]. Therefore, detection of low concentration of airborne bacteria in the study is probably due to the semi-arid climate of the region. Furthermore, it seems that airborne bacteria are relatively low distributed in

Table 2 The values of analyzed parameters

Variable	Mean ± SD	Minimum	Maximum
Airborne bacteria (CFU/m^3)	38 ± 36	ND	290
Staphylococci (CFU/m^3)	12 ± 48	ND	213
$PM_{2.5}$ (μg/m^3)	64.83 ± 24.87	13	125
UV index	5 ± 3	1	9
Wind speed (m/h)	3.05 ± 1.16	1.58	6
Temperature (°C)	20.5 ± 9.9	1.3	33.5
Moisture(%)	27.7 ± 15.8	6.5	80

ND: not detected

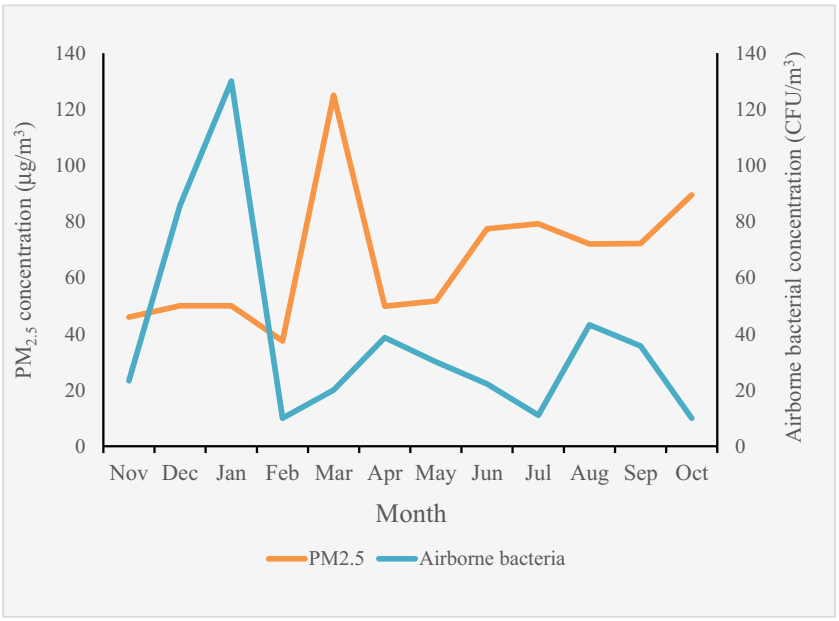

Fig. 2 Changes in concentration of $PM_{2.5}$ and associated airborne bacteria during the sampling period

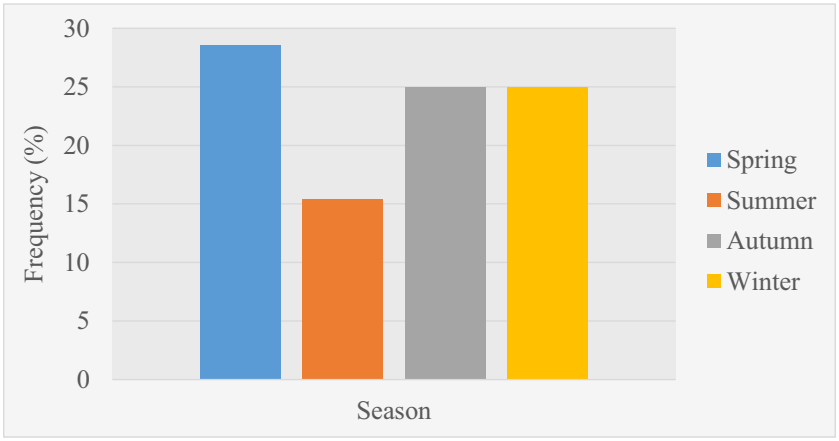

Fig. 3 Frequency of staphylococci detection in different seasons

fine particles ($PM_{2.5}$). In other words, particles with an aerodynamic diameter of more than 5 μm show significantly higher concentrations of microorganisms than smaller ones [26].

The highest bacterial concentration was obtained in autumn with an average of 69 CFU/m^3, whereas the lowest mean concentration (28 CFU/m^3) was seen in summer (Fig. 2). However, no significant differences were observed between bacterial counts in different seasons ($p < 0.05$). There are different reports about the seasonal variation of bacterial concentrations. Some studies reported more airborne bacteria in summer and autumn and lower in spring and winter [26, 29].

Staphylococcus spp. was found in 8 of 37 (22%) air samples in a concentration of 3-213 CFU/m^3 with a mean of 12 CFU/m^3 (Table 2). As other PM2.5-associated bacteria, the lowest frequency of detection of *Staphylococcus* species was observed in the summer (Fig. 3). Since relative humidity and temperature play a major role in survival of bioaerosols in ambient air [28], lower presence of viable bacteria in the summer season is related to low humid and hot climatic conditions of the region. In the study of Fang et al. (2014) in Hangzhou, 11.28% of isolated bacteria from 789 isolates identified as the *Staphylococcus* species. The most common detected species was *Staphylococcus cohnii* (2.53%), whereas *Staphylococcus aureus* was found only in 0.63% of isolates [26]. Various species of *Staphylococcus* were also detected in the urban air of Beijing, as the second dominant genus in a frequency from 8.94–16.28% at three sampling sites, and *S. aureus* was detected in a frequency of 1.6% in one sampling point [25]. Lu et al. (2018) found staphylococci in non-haze and haze days in Xi'an, China with a relative abundance of 0.059% and 0.042%, respectively [30]. However, in our study, *S. aureus* was not detected in any of air samples. The results of sequencing analysis showed the presence of four *Staphylococcus spp.* in our ambient air samples. *S. epidermidis* was detected with the highest frequency (82.4% of isolates), followed by *S. equorum* (13.5%), *S. gallinarum* (3.4%), and *S. hominis* (0.7%). The *Staphylococcus* spp. found in the present study correspond with those detected by Mirhossein et al. (2016a and 2016b) who found *S. epidermidis* and *S. hominis* but not *S. aureus* in air samples of indoor environments and hospitals, respectively in Isfahan [31, 32]. In agreement with our results, MRSA was also not found in samples collected from outdoor environments on the European side of Istanbul, Turkey; but

Table 3 Correlation matrix of the analyzed parameters in ambient air

Variable	Bacteria	Staphylococci	$PM_{2.5}$	UV index	Wind speed	Temperature	Relative humidity
Bacteria	1						
Staphylococci	0.952**	1					
$PM_{2.5}$	-0.315	-0.285	1				
UV index	-0.234	-0.307	0.206	1			
Wind speed	-0.256	-0.185	0.251	0.006	1		
Temperature	-0.24	-0.327	0.394	0.934**	0.119	1	
Relative humidity	0.25	0.334*	-0.189	-0.78**	-0.051	-0.725**	1

*Correlation significant at the 0.05 level (2-tailed)

**Correlation significant at the 0.001 level (2-tailed)

CoNS were observed. Among fifteen methicillin-resistant CoNS isolates which were identified by the disk diffusion method, *Staphylococcus hominis* was found at the highest frequency (11 from 15) [33]. In study of Polymenakou et al. (2007) during an intense African dust event in the Eastern Mediterranean, bacterial pathogens such as *Acinetobacter lwoffi, Haemophilus parainfluenzae*, *Streptococcus pneumoniae*, and *Streptococcus mitis* were found at small particle sizes. However, they not detected any species of *Staphylococcus*, such as *S. aureus* [13].

Our results also showed no detection of the *mecA* gene, a genetic element found in the methicillin-resistant *Staphylococcus* (MRS) spp., in the staphylococcal isolates. In contrast, Mirhosseini et al. (2016) detected *mecA* with a frequency of 17% in airborne staphylococci found in different hospital wards in Isfahan [32]. Perez et al. (2012) found MRSA in 36% of the sampled homes in city of Philadelphia, USA [34]. *S. aureus* and MRSA were also detected in 100% and 66% of households' indoor air of residential apartments within the metropolitan area in South Korea, respectively [35]. It seems that residential air may be a source of *S. aureus* and antibiotic resistance of *Staphylococcus* species and community-associated staphylococcal infections [34]. It has been reported that most MRSA and *S. aureus* are associated with larger particles with a size between 7 and 12 μm [6], which indicates lower health problems. Our results showed no significant correlation between the concentration of airborne bacteria as well as staphylococci with $PM_{2.5}$ concentration (Table 3) ($p < 0.05$). Consistent with our results, in Alghamdi et al. (2014) study a correlation between $PM_{2.5}$ and airborne bacterial concentration was not found [22]. In contrast, Jeon et al. (2011) reported a significant positive correlation between culturable bacterial population levels with total suspended particles (TSP) and PM_{10} in dust days, whereas no significant correlation was found during non-Asian dust (NAD) days [27]. Although, there was no correlation between bacterial and $PM_{2.5}$ concentrations, a reverse relationship between the $PM_{2.5}$ levels and bacterial concentrations was observed (Fig. 2). The reverse relationship which has also been observed in other studies may be related to the composition of $PM_{2.5}$ and the presence of compounds such as soot and metals which affects the microbial viability [22, 36].

Table 3 shows the Spearman's correlation coefficients between the concentration of $PM_{2.5}$ and $PM_{2.5}$-associated bacteria with meteorological parameters. Our results showed no significant correlation between the concentrations of $PM_{2.5}$-associated bacteria with meteorological parameters ($p < 0.05$). It has been reported that the effect of meteorological factors on microorganisms associated with PM is complex and well not understood [29]. Alghamdi et al. (2014) reported that wind speed positively correlated with the load of microorganisms associated with PM [22]. However, our results showed a negative but no significant relationship between $PM_{2.5}$-associated bacteria and wind speed, which may be related in part to the dilution effect of atmospheric diffusion [30].

Conclusions

Our results indicated that although the studied region is projected to experience air pollution due to the relatively high levels of $PM_{2.5}$, concentration of $PM_{2.5}$-associated bacteria is relatively low. Based on the detection of low numbers of CoNS and no detection of *S. aureus* and MRSA in ambient air, our results suggest that $PM_{2.5}$ may not be a source of community-associated staphylococcal infections. However, more research is needed about the role of larger particles in the transmission of staphylococci in ambient air.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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