REP-PCR tracking of the origin and spread of airborne *Staphylococcus aureus* in and around chicken house

Abstract *Staphylococcus aureus* was used as an indicator to study the origin and spread of microbial aerosol in and around chicken houses. Air samples indoor, upwind (10 and 50 m), and downwind (10, 50, 100, 200, and 400 m) of four chicken houses were collected using Andersen-6 stages sampler. The concentrations of *S. aureus* were determined for every sample site. Isolation of *S. aureus* from chicken feces was performed according to the standard method. The genetic relationship among the isolates was determined by profiles of PCR-amplified repetitive extragenic palindromic (REP-PCR) elements. The results showed that the concentrations of *S. aureus* indoor of four chicken houses were higher than those upwind and downwind sites ($P < 0.05$ or $P < 0.01$), but there were no significant concentration differences among downwind sites ($P > 0.05$). The fingerprints and the phylogenetic tree indicated that a part of the *S. aureus* (55.6%, 10/18) isolates from indoor air had the same REP-PCR fingerprints as feces isolates. Consequently, most isolates (57.1%, 20/35) from downwind 10, 50, 100, 200, even 400 m had the same REP-PCR fingerprints as those from indoor or feces. These data indicated that some isolates from downwind and indoor originated from the chicken feces. However, those isolates from upwind had low similarity (similarity index 0.6–0.87) to those from indoor or feces. Therefore, the isolates upwind were not from the chicken feces or indoor. These results suggest that microbes in chicken feces can be aerosolized and spread indoor and outdoor, especially to downwind of the chicken houses. It should have an important epidemiological and public health significance.

Z. Zhong¹, T. Chai¹, H. Duan¹,
Z. Miao¹, X. Li¹, M. Yao¹, W. Yuan¹,
W. Wang¹, Q. Li¹, B.-A. Zucker²,
G. Schlenker²

¹College of Animal Science and Veterinary Medicine, Research Center for Animal Disease Control Engineering, Shandong Agricultural University, Tai'an, Shandong Province, P.R. China, ²Institute of Animal Hygiene and Environmental Protection, The Free University of Berlin, Berlin, Germany

Key words: Chicken house; Airborne *Staphylococcus aureus*; PCR-amplified repetitive extragenic palindromic; Similarity identification; Epidemiological significance.

T. Chai
College of Animal Science and Veterinary Medicine
Shandong Agricultural University
Daizong Str. 61
Tai'an, Shandong 271018
P.R. China
Tel.: +86 538 824 1503
Fax: +86 538 824 1503
e-mail: chaitj117@163.com

Received for review 3 September 2008. Accepted for publication 27 July 2009.
© Indoor Air (2009)

Practical Implications

Thus, the use of *S. aureus* as an indicator to study the origin and spread of airborne pathogens from chicken houses is potentially useful for enhancing public health and understanding the airborne epidemiology of this pathogen. Meanwhile it can provide evidence for studying the spreading model of airborne pathogens.

Introduction

In modern poultry industry, high animal density in relatively constricted space has led to the increase of harmful gases and microbial aerosols in and around animal houses (Jarnych, 1976). The ventilation systems in poultry houses accelerate air exchange between inside and outside, which allows large amount of microbial and harmful materials to run off to the environment of the animal rooms, spreading common pathogens or drug-resistant pathogens (Donaldson,

1999; Shinn et al., 2000; Wathes et al., 1997) to humans and animals.

Many studies have demonstrated that some airborne pathogens can spread to distant area through open air, including severe acute respiratory syndrome-associated coronavirus (Yu et al., 2004) and anthrax (Meselson et al., 1994). In April and May 1979, an unusual anthrax epidemic occurred in Sverdlovsk, Union of Soviet Socialist Republics. The escape of an aerosol of anthrax pathogen at the military facility caused the outbreak (Meselson et al., 1994). In 1981,

foot-and-mouth virus transmitted from France to Southern England, leading to the outbreak of this disease (Donaldson et al., 1982).

In 2001 and 2002, aerosol of *Bacillus anthracis* infected postal workers and led to the death of many of them (CDC, 2003). The transmission of severe acute respiratory syndrome that swiped Southeast Asia was partly because of aerial spread (Eubank et al., 2004; Weiss and McMichael, 2004). The more typical aerial transmission is the measles, which had caused the infection in students who had not been vaccinated (Riley et al., 1978). Recent studies have shown that airborne bacteria and fungi can disperse locally or over long distance (Brown and Hovmoller, 2002; Lee et al., 2007; Mims and Mims, 2004; Stewart et al., 1995). It is very clear that microbial aerosol is a great threat to human and animal health.

In the past, to determine whether microbial aerosol spread from animal shed to environment was to monitor the airborne microbial concentration, which is usually higher around or within the animal shed (Chai et al., 1999, 2001; Franz, 1980; Kim and Kim, 2007). Alternatively, whether microbes come from animal house may be determined by their sensitivity to antibiotics (Chai et al., 2003; Yao et al., 2007). However, the efficacy of these methods is limited, usually unable to confirm the ultimate source. Only do the genes are identified in bacteria from animal feces, indoor and outdoor aerosol, shall evidence be sufficient. Therefore, we performed PCR-amplified repetitive extragenic palindromic (REP-PCR) analysis on *Staphylococcus aureus*, comparing the genetic relationship of different isolates in the current study, in addition to the determination of the concentration of *S. aureus* inside and different distance outside of four poultry houses. These studies are necessary to resolve the source of microbial aerosols and their spreading path, providing scientific bases for animal disease control and warning.

Methods and materials

The chicken houses

This study investigated four chicken farms in Tai'an, Shandong Province, China between March 2006 and May 2007. These farms are similar geographically and are isolated from nearby villages. There are no buildings and high crops around the farms. Farm A was located to the side of a mound, 3000 m away from the nearest village. Farms B, C and D were located to the east of villages with distances about 500–1000 m. Farms A and B used fans for ventilation and farms C and D were not ventilated.

A 'Three Cups Wind Anemometer DEM6' (Marine Meteorological Instrument Factory, Tianjin, China) was used to measure velocity (direction and speed).

Table 1 Description of chicken houses studied

CH	N	Layout	Indoor			Outdoor			
			T (°C)	RH (%)	WS (m/s)	T (°C)	RH (%)	WS (m/s)	WD
A	6000	Floor unit	26	40	0	21	50	1–3	South
B	4200	Cage unit	26	34	0	29	50	1–3.1	Southeast
C	3000	Cage unit	31	44	0	35	36	1.5–3	Southwest
D	3500	Cage unit	29	60	0	32	75	0–1.5	South

CH, chicken house; N, number of chicken; T, temperature; RH, relative humidity; WS, wind speed; WD, wind direction.

Conditions of these chicken houses are shown in Table 1.

Air and feces samples and *Staphylococcus aureus* identification

Two Andersen-6 stage samplers (Andersen, 1958) were used to collect indoor microbes. Samplers were set in the center of the chicken rooms, 1 m above ground. Samples were collected for 1–5 min at airflow rate 28.3 l/min. Outdoor microbes were collected, 1.5 m above ground, with airflow rate 40 l/min for 0.5–8 min using two Reuter-centrifugal sampler (RCS; Biotest Diagnostiken Corp., Fairfield, NJ, USA) samplers, which were set at upwind 10 and 50 m or downwind 10, 50, 100, 200, and 400 m from the houses. Each location was sampled 5 times. The sample media was Baird-Park agarose (lot number 060227; Tian He, Hangzhou, China).

The collected samples were cultured at 37°C for 24 h. All positive colonies were test for 'KOH reaction' to determine whether they were Gram-positive or Gram-negative. Gram-positive colonies were cultured in Baird-Park agarose media, followed by biochemistry identification with API 20 Steph (bioMerieux, Marcy-l'Etoile, France). The number of *S. aureus* colonies was recorded and aerosol concentration (CFU/m³ air) was calculated (CFU/m air) after correction according to Andersen correction table. *Staphylococcus aureus* cultures were frozen at –20°C in LB broth containing 20% glycerol.

Twenty feces samples were collected at random from each poultry house and diluted to 10- and 10³-fold, and then inoculated in Baird-Park agarose media. Culture, identification, and storage were performed as described above.

Template preparation

Staphylococcus aureus was inoculated in heart brain infusion broth with shaking for 18 h. The culture was then centrifuged in 1.5-ml Eppendorf tube (KD1876, Shanghai, China) for 2 min. Upon removal of the supernatant, the pellet was suspended in 100-μl TE buffer (10-mM Tris-HCl, 1-mM EDTA, pH 8.0) and

incubated with 0.2-mg/ml lysostaphin at 37°C for 20 min followed by digestion with proteinase K (0.5 mg/ml) for 1 min at 55°C and extraction with phenol, phenol-chloroform and chloroform. The DNA was precipitated with absolute ethanol and finally resuspended in TE buffer.

REP-PCR

Differences of *S. aureus* can be distinguished by comprehensive analysis of PCR-amplified repetitive DNA. REP-PCR primers (REP1: 5'-ATGTAAGCTC-CTGGGATTAC-3' and REP2: 5'-AAGTAAGT-GACTGGGGTGAGCG-3') were synthesized by Shanghai Biotech Inc. (Songjiang District, Shanghai, China). The PCR reaction mixture (25 µl) contained 10 × buffer 2.5µl, 200-µM dNTP, 1.5-U Taq DNA polymerase, 1.5-mM MgCl₂ (TaKaRa, Dalian Liaoning, China), primers 200 pM each and template DNA 1 µl. The amplification was carried out by incubation of the mixture for 2 min at 94°C for pre-denaturation, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 42°C for 1 min and extension at 72°C for 1 min. A final extension was performed at 72°C for 5 min.

PCR product was resolved by 1.0% agarose gel electrophoresis (1 × TAE, EB staining). Molecular size marker was DL2000Maker (TaKaRa). Gel image was photographed with Tanon-2500 (Shanghai, China). To reduce the variation of experiment, all isolates from the same poultry farm were analyzed by a single experiment.

REP-PCR data collection and clustering analysis

Variance analysis was performed using SPSS 13.0 (Statistical Package for the Social Science, Chicago, IL, USA). The concentration of the *S. aureus* was represented by the median, and the range was represented by the minimum and maximum. PCR product profile was recorded by computer software. A value '1' was assigned when a band exist and a value '0' was assigned when a band was absent in a sample. The matrix was automatically generated from the gel image by software (Gel Image System V4.00; Tianneng Technology Co., Ltd, Shanghai, China). Bacterial isolates were clustered with unweighted pair group method using average algorithm and dendrogram was constructed with NTSYS-pc 2.10 software (Applied Biostatistics Inc., Stony Brook, NY, USA) (Rohlf, 2006). During the analysis, each isolate was regarded as an operational taxonomic unit. When two isolates had identical REP-PCR profile or when genetic similarity was greater than 90%, they were considered to be the progeny of the same cell population. Conversely, if the similarity was less than 90%, their genetic relationship was considered distinct (Borges et al., 2003; Van der Zee et al., 2005).

Results

Airborne *Staphylococcus aureus* density

The densities of airborne *S. aureus* indoor were 45, 23, 27, and 51 CFU/m³ air. At 10 and 50 m upwind, *S. aureus* was isolated from farms A and D. The densities were: 4 and 1 CFU/m at 10 and 50 m upwind of farm A; 5 and 2 CFU/m³ at 10 and 50 m upwind of farm D. The *S. aureus* densities for the four farms were 10, 8, 4, and 9 CFU/m at 10 m downwind; 7, 3, 3, and 4 CFU/m at 50 m downwind. The densities gradually decline as the distance increased to 100 and 200 m downwind from the four farms (Table 2). At 400 m downwind, *S. aureus* can only be isolated from farms A and B (1 CFU/m for both).

The densities of *S. aureus* indoor in farms B and C were significantly greater than that of outdoor, regardless of the distance from the poultry house ($P < 0.05$). In farms A and D, the densities indoor were greater than those at 10 and 50 m upwind ($P < 0.05$). There were no significant differences among different distances downwind for the four farms ($P > 0.05$). However, the densities at 10 and 50 m downwind were significantly higher than those at the same distance upwind ($P < 0.05$) (Table 2).

REP-PCR clustering analysis

The similarity index is 100% when REP-PCR profiles are identical. The figure has clearly shown the similarities among the genes of isolates. In farm A (Figure 1a), isolates Feces-3, Feces-4, Indoor-3, and Indoor-5 were identical (similarity index 100%). In addition, they were 91% similar to Feces-1. Feces-8 was identical to Indoor-6, Downwind10m-5, Downwind50m-3. Indoor-8 was identical to Downwind50m-4, Downwind100m-2, 3, and 4. These data suggest that feces microbes can become aerosol, which may spread outdoor. However, Feces-3 was 80% and 77% similar

Table 2 The concentration of airborne *Staphylococcus aureus* in four chicken houses (CFU/m; $n = 5$)

Chicken house		UW50m	UW10m	Indoor	DW10m	DW50m	DW100m	DW200m	DW400m
A	Max.	5	13	121	49	28	10	2	2
	Min.	0	0	11	2	0	0	0	0
	Median	1	4	45	10	7	3	1	1
B	Max.	0	0	78	17	9	5	3	2
	Min.	0	0	19	1	0	0	0	0
	Median	0	0	23	8	3	2	2	1
C	Max.	0	0	67	13	7	4	2	0
	Min.	0	0	3	0	0	0	0	0
	Median	0	0	27	4	3	1	1	0
D	Max.	7	16	137	31	13	6	3	0
	Min.	0	0	9	1	0	0	0	0
	Median	2	5	51	9	4	2	1	0

CFU, colony forming unit; UW, upwind; DW, downwind.

Fig. 1 Dendrogram of *S. aureus* strains in four chicken houses based on REP-PCR profiles. (a) Dendrogram of *S. aureus* strains in Farm A based on REP-PCR profiles (b) Dendrogram of *S. aureus* strains in Farm B based on REP-PCR profiles (c) Dendrogram of *S. aureus* strains in Farm C based on REP-PCR profiles (d) Dendrogram of *S. aureus* strains in Farm D based on REP-PCR profiles

to Upwind10m-1 and Upwind50m-2, respectively, suggesting their sources of origin were different.

Similarly, in farm B (Figure 1b), isolates Feces-2 and Feces-3 were identical to Indoor-6. Indoor-1 was identical to Downwind10m-3 and Downwind10m-4 and Downwind100m-2. Indoor-5 was identical to Indoor-7 and Downwind50m-2. Downwind10m-2 was identical to Downwind50m-1. Downwind10m-5 was identical to Downwind50m-3. However, Downwind10m-1 was 78% similar to Feces-4, Feces-7, and Indoor-2. Downwind200m-1 was 63.6% similar to Feces-9 and Indoor-10.

In farm C (Figure 1c), isolate Feces-2 was identical to Indoor-4. Feces-4 was identical to Feces-7 and

Indoor-1. Feces-1 was identical to Downwind10m-2 and Downwind10m-4. These data suggested that indoor airborne *S. aureus*, as well as some outdoor isolates, was from feces (60%, 3/5). Downwind10m-1 was identical to Downwind50m-3, suggesting that downwind microbes were from upwind. In contrast, Downwind50m-2 was 62% similar to Feces-3 and Indoor-4.

In farm D (Figure 1d), Feces-2 was identical to Indoor-3 and Indoor-7. Feces-3 was identical to Downwind10m-1. Indoor-4 was identical to Downwind10m-2 and Downwind50m-2. However, Feces-9 was 75% similar to Upwind50m-1. No *S. aureus* was isolated from 400 m or further downwind farm D.

Discussion

REP-PCR was established by Versalovic et al. (1994). An REP element is a conserved genomic DNA sequence with a consensus sequence of 38 bp containing a 5-bp variable loop between the palindrome stem and six degenerated positions at both ends. The palindromic sequence and the ability to form a frame are the key for it to have high level of conservation. Using one or more pairs of primer, the REP element can be amplified. Upon clustering analysis of the REP-PCR product using computer program, similarity indices can be obtained. In turn, REP-PCR profile can represent the relatedness of bacterial strains. Theoretically, isolates of the same species, same type and identical genetic composition should have specific DNA fingerprints (Ichiyama et al., 1991). Thus, REP-PCR can be applied to the identification of bacterial species, types or isolates. It has been shown that this method is reproducible and accurate. Vecchio et al. (1995) showed that their PCR products were highly reproducible and stable when trying to distinguish 170 stains of *Mycoplasma pneumoniae* using RW3A of RepMP3 as primer. Van der Zee et al. (1999) showed that REP-PCR results were similar to pulse field gel electrophoresis (PFGE) analysis. PFGE of large restriction fragment can distinguish many bacteria and yeast pathogens. The identification ability by PFGE can be as high as 87–100%. Echeverrigaray et al. (1999) analyzed 16 strains of commercial yeast strain and classified these strains into three groups. It is evident that REP-PCR is accurate and reliable. Many researchers have used it for bacterial pathogen studies and molecular epidemiology investigation (Francois et al., 2005; Ibenyassine et al., 2006; Jurković et al., 2007).

Comparing the densities of airborne *S. aureus* indoor with outdoor (Table 2), it is clear that there were much more airborne microbes indoor than outdoor at any distance ($P < 0.05$), which suggests that microbial aerosol forms continuously from birds and feces, accumulates and spread to the environment. In the present experiment, no *S. aureus* was isolated from downwind 400 m from farms C and D, which might have resulted from the death of microbes under the effect of UV light, temperature and relative humidity (Che, 1986). It is also possible that long-time sampling might have stressed the microbes resulting in death (Marthi et al., 1990; Stewart et al., 1995).

Although *S. aureus* was isolated from upwind of farms A and D, the concentration was very low, being 1 and 2 CFU/m³ at 50 m upwind, 4 and 5 CFU/m at 10 m upwind, respectively. The microbial densities of downwind 10 and 50 m were higher than that of upwind, suggesting microorganism from upwind was not the major source of downwind of the chicken houses and those microbes downwind were originated

from the poultry house. The insignificant differences between microbial concentration downwind 10 and 400 m ($P > 0.05$) indicate that *S. aureus* can spread long distance.

As shown in Figure 1, *S. aureus* isolated from feces were identical to 55.6% of the airborne isolates (four farms being respectively 4/5, 80%; 1/4, 25%; 2/5, 40%; and 3/4, 75%), suggesting that airborne isolates were the same genetic origin as that of feces isolates. Similarly, isolates downwind were identical to 57.1% (10/18, 55.6%; 4/8, 50%; 3/5, 60%; and 3/4, 75%, respectively for the four farms) those from indoor and feces. In contrast, upwind isolates were more distinct genetically from indoor and feces ones, with similarity indices being 60–87%. In farm A, Upwind10m-1 and Upwind10m-3 were 85.6% similar to Feces-2. Upwind50m-1 and Upwind50m-2 were 78.4% and 77.2% similar to Feces-6, respectively. In farm D, Upwind50m-1 was 74% similar to Feces-9. Upwind50m-2 was 60% similar to Feces-2. Upwind10m-1 and Upwind10m-2 were 87% and 72% similar to Feces-7, respectively. Upwind10m-3 was 82% similar to Feces-9. It can be seen from the figure that the greater the similarity index, the closer is the genetic relationship. Studies have confirmed that isolates with similarity indices greater than 90% are the same species or the same type, being the progeny of one cell. In contrast, isolates with similarity indices between 60 and 87% were genetically distant.

Hence from this study, it can be seen that by using the accurate REP-PCR technology *S. aureus* in feces was found to have been aerosolized and had spread to the outdoor air, especially downwind of the chicken houses. This suggests that the ambient air outside of these chicken houses can be polluted by airborne pathogens, posing a threat to the health of neighboring human inhabitants and the chickens themselves. In their study on the transmission dynamics of antibiotics-resistant bacteria, Austin and Anderson (1999) pointed out that such transmission may prove difficult to eradicate, ultimately requiring careful surveillance and effective infection control measures. Thus, the use of *S. aureus* as an indicator to study the origin and spread of airborne pathogens from chicken houses is potentially useful for enhancing public health and understanding the airborne epidemiology of this pathogen.

Acknowledgements

This project was financially supported by the National Natural Science Foundation of China 'Study on spread model of microorganism aerosol from animal stable' (No.30571381) and the International scientific and technological cooperation project 'Monitoring of Animal-borne Zoonoses' (No.2009DFA32890).

References

- Andersen, A. (1958) New sampler for the collection, sizing, and enumeration of viable airborne particles, *J. Bacteriol.*, **76**, 471–484.
- Austin, D.J. and Anderson, R.M. (1999) Transmission dynamics of epidemic methicillin-resistant *Staphylococcus aureus* and vancomycin resistant enterococci in England and Wales, *J. Infect. Dis.*, **179**, 883–891.
- Borges, L.G., Vechia, V.D. and Corça, G. (2003) Characterization and genetic diversity via REP-PCR of *Staphylococcus aureus* isolates from polluted waters in southern Brazil, *FEMS Microbiol. Ecol.*, **43**, 173–180.
- Brown, J.K.M. and Hovmoller, M.S. (2002) Aerial dispersal of pathogens on the global and continental scales and its impact on plant disease, *Science*, **297**, 537–541.
- Centers for Disease Control and Prevention (CDC) (2003) Follow-up of deaths among U.S. Postal Service workers potentially exposed to *Bacillus anthracis* – District of Columbia, 2001–2002, *MMWR Morb. Mortal. Wkly Rep.*, **52**, 937–938.
- *Chai, T.J., Chai, J.Q. and Mueller, W. (1999) Airborne microorganisms of a calf stable and spreading to its environment, *Chin. J. Prev. Vet. Med.*, **21**, 311–313. (in Chinese).
- Chai, T.J., Zhang, J.X., Hou, Y.P. and Zhao, Y.L. (2001) Spread of microorganism aerosol in animal house to its environments, *Anim. Husbandry Vet. Med.*, **33**, 10–12.
- Chai, T.J., Zhao, Y.L. and Liu, W.B. (2003) The resistance against antibiotics of bacteria from a poultry house and their spreading to surroundings of the house, *Chin. J. Prev. Vet. Med.*, **25**, 209–214 (in Chinese).
- Che, F.X. (1986) The air microbiology and it's research with evolvement, *Environ. Sci.*, **7**, 85–90 (in Chinese).
- Donaldson, A.I., Gloster, J., Harvey, L.D. and Deans, D.H. (1982) Use of prediction models to forecast and analyse airborne spread during the foot-and-mouth disease outbreaks in Brittany, Jersey and the Isle of Wight in 1981, *Vet. Rec.*, **110**, 53–57.
- Donaldson, A. (1999) Airborne spread of foot-and-mouth disease, *Microbiol. Today*, **26**, 118–119.
- Echeverrigaray, S., Fernanda, S. and Toroczka, Z. (1999) Use of RAPD analysis for differentiation among six enological *Saccharomyces* strains, *World J. Microbiol. Biotechnol.*, **16**, 143–146.
- Eubank, S., Guclu, H., Anil Kumar, V.S., Marathe, M.V., Srinivasan, A., Toroczka, Z. and Wang, N. (2004) Modeling disease outbreaks in realistic urban social networks, *Nature*, **429**, 180–184.
- Francois, P., Huyghe, A. and Charbonnier, Y. (2005) Use of an automated multiple-locus, variable – number tandem repeat-based method for rapid and high-throughput genotyping of *Staphylococcus aureus* isolates, *J. Clin. Microbiol.*, **43**, 3346–3355.
- Franz, H. (1980) *Experimentelle Untersuchungen ueber die atmosphaerische Ausbreitung von Luftkeimen aus Stallanlagen und aus kuenstlichen Keimquellen*, Dissertation, Stuttgart, Universitaet Hohenheim.
- Ibenyassine, K., AitMhand, R., Karamoko, Y., Cohen, N. and Ennaji, M.M. (2006) Use of repetitive DNA sequences to determine the persistence of enteropathogenic *Escherichia coli* in vegetables and in soil grown in fields treated with contaminated irrigation water, *Lett. Appl. Microbiol.*, **43**, 528–533.
- Ichiyama, S., Ohta, M., Shimokatu, K., Kato, N. and Takeuchi, J. (1991) Genomic DNA fingerprinting by pulsed field gel electrophoresis as an epidemiological marker for study of nosocomial infections caused by methicillin-resistant *Staphylococcus aureus*, *J. Clin. Microbiol.*, **29**, 2690–2695.
- Jarnych, V.S. (1976) *Aerosole in der Veterinaermedizin*, Berlin, VEB Deutscher Landwirtschafts-verlag (in German).
- Jurković, D., Križková, L., Sojka, M., Takacova, M., Dusinsky, R., Krajcovic, J., Vandamme, P. and Vancanneyt, M. (2007) Genetic diversity of *Enterococcus faecium* isolated from Bryndza cheese, *Int. J. Food Microbiol.*, **116**, 82–87.
- Kim, K.Y. and Kim, C.N. (2007) Airborne microbiological characteristics in public buildings of Korea, *Build. Environ.*, **42**, 2188–2196.
- Lee, A.K.Y., Lau, A.P.S., Cheng, J.Y.W., Fang, M. and Chan, C.K. (2007) Source identification analysis for the airborne bacteria and fungi using a biomarker approach, *Atmos. Environ.*, **41**, 2831–2843.
- Marthi, B., Fieland, V.P., Walter, M. and Seidler, R.J. (1990) Survival of bacteria during aerosolization, *Appl. Environ. Microbiol.*, **56**, 3463–3467.
- Meselson, M., Guillemin, J. and Hugh-Jones, M. (1994) The Sverdlovsk anthrax outbreak of 1979, *Science*, **266**, 1202–1208.
- Mims, S.A. and Mims, F.M. III (2004) Fungal spores are transported long distances in smoke from biomass fires, *Atmos. Environ.*, **38**, 651–655.
- Riley, E.C., Murphy, G.R. and Riley, L. (1978) Airborne spread of measles in a suburban elementary school, *Am. J. Epidemiol.*, **107**, 421–432.
- Rohlf, F.J. (2006) *NTSYS-pc: Numerical Taxonomy and Multi-variate Analysis System [M], Version 2.10*, Exeter Software, 2000, New York, Setauket.
- Shinn, E.A., Smith, G.W., Prospero, J.M., Betzer, P., Hayes, M.L., Garrison, V. and Barber, R.T. (2000) African dust and the demise of Caribbean coral reefs, *Geophys. Res. Lett.*, **27**, 3029–3032.
- Stewart, S.L., Grinshpun, S.A., Willeke, K., Terzieva, S., Ulevicius, V. and Donnelly, J. (1995) Effect of impact stress on microbial recovery on an agar surface, *Appl. Environ. Microbiol.*, **61**, 1232–1239.
- Van der Zee, A., Verbakel, H., van Zon, J.C., Frenay, I., van Belkum, A., Peeters, M., Buiting, A. and Bergmans, A. (1999) Molecular genotyping of *Staphylococcus aureus* stains: comparison of repetitive element sequence-based PCR with various typing methods and isolation of a novelepidecity marker, *J. Clin. Microbiol.*, **37**, 342–349.
- Van der Zee, A., Heck, M., Sterks, M., Harpal, A., Spalburg, E., Kazobagora, L. and Wannet, W. (2005) Recognition of SCCmec types according to typing pattern determined by multienzyme multiplex PCR-amplified fragment length polymorphism analysis of methicillin-resistant *Staphylococcus aureus*, *J. Clin. Microbiol.*, **43**, 6042–6047.
- Vecchio, D.V.G., Petroziello, J.M., Gress, M.J., McCleskey, F.K., Melcher, G.P., Crouch, H.K. and Lupski, J.R. (1995) Molecular genotyping of methicillin resistant *Staphylococcus aureus* via fluorophore-enhanced repetitive-sequence PCR, *J. Clin. Microbiol.*, **33**, 2141–2144.
- Wathes, C.M., Holden, M.R. and McMichael, A.J. (1997) Concentrations and emission rates of aerial ammonia, nitrous oxide, methane, carbon dioxide, dust and endotoxin in UK broiler and layer houses, *Br. Poult. Sci.*, **38**, 14–28.
- Weiss, R.A. and McMichael, A.J. (2004) Social and environmental risk factors in the emergence of infectious diseases, *Nat. Med.*, **10**, 70–76.
- Yao, M.L., Zhang, B. and Chai, T.J. (2007) Antibiotic resistance of airborne *Escherichia coli* from hen house and rabbitry and their spreading to surroundings, *J. Northwest A & F University (Nat. Sci. Ed.)*, **8**, 60–64 (in Chinese).
- Yu, I.T.S., Li, Y., Wong, T.W., Chan, A.T., Lee, J.H., Leung, W., Dennis, Y.C. and Tommy, H. (2004) Evidence of airborne transmission of the severe acute respiratory syndrome virus, *N. Engl. J. Med.*, **350**, 1731–1739.