Characteristics of airborne micro-organisms in a neurological intensive care unit: Results from China

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Abstract
Objective: To describe the characteristics of airborne micro-organisms in the environment in a Chinese neurological intensive care unit (NICU).
Methods: This prospective study monitored the air environment in two wards (large and small) of an NICU in a tertiary hospital in China for 12 months, using an LWC-1 centrifugal air sampler. Airborne micro-organisms were identified using standard microbiology techniques.
Results: The mean ± SD number of airborne bacteria was significantly higher in the large ward than in the small ward (200 ± 51 colony-forming units [CFU]/m³ versus 110 ± 40 CFU/m³, respectively). In the large ward only, the mean number of airborne bacteria in the autumn was significantly higher than in any of the other three seasons. A total of 279 airborne micro-organisms were identified (large ward: 195; small ward: 84). There was no significant difference in the type and distribution of airborne micro-organisms between the large and small wards. The majority of airborne micro-organisms were Gram-positive cocci in both wards.
Conclusion: These findings suggest that the number of airborne micro-organisms was related to the number of patients on the NICU ward.

Keywords
Nosocomial infection, air environment, neurological intensive care unit, bacteria

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Introduction

Nosocomial infection is one of the major complications experienced by patients when they are treated in a neurological intensive care unit (NICU). Patients being treated in an NICU are often in poor physical condition, which increases their risk of nosocomial infection. Nosocomial infection frequently results in a prolonged length of stay, and increases both the risk of mortality and the cost of medical treatment. The environment within a hospital acts as a source of infection for patients, with the air environment being one of the most important factors. There is a growing body of evidence that the aerial dispersal of some nosocomial pathogens can seed widespread environmental contamination, and that this may be contributing to the spread of infection in hospital wards. The air within hospital lobbies has been shown to be contaminated with micro-organisms, including airborne bacteria (mean concentration: $7.2 \times 10^2$ colony-forming units [CFU]/m$^3$), Gram-negative bacteria (mean concentration: $1.7 \times 10$ CFU/m$^3$), and fungi (mean concentration: $7.7 \times 10$ CFU/m$^3$). Environmental factors that may significantly influence the airborne concentrations of these biological agents should be managed, to minimize airborne levels. During a 3-month sampling period, a study undertaken in two hospitals in Turkey demonstrated that the mean numbers of live micro-organisms in air samples were 224.44 CFU/m$^3$ (hospital 1) and 536.66 CFU/m$^3$ (hospital 2). The authors concluded that microbial loads in the atmospheres of the two hospitals varied greatly depending on the number of people in the environment. A 1-year prospective study undertaken in a tertiary hospital in China demonstrated mean total Aspergillus counts of 7.73, 8.94, 13.19 and 17.32 CFU/m$^3$ in the bone marrow transplant department, intensive care unit, neurosurgery intensive care unit, and outdoors, respectively. The authors concluded that clinical infection may originate from the hospital environment.

Exposure to biological agents (including micro-organisms) is associated with a wide range of major public health issues such as infectious diseases, acute toxic effects and allergies. Hospitalized patients could be at significantly increased risk of bioaerosol exposure. The development of nosocomial infection is determined by the complex relationship between the pathogens, environment, and patients. Since air serves as a major medium for disease transmission, the air environment, which is an important part of the hospital ward, is important in the occurrence of nosocomial infection.

Of the over 40 legally-recognized infectious diseases, more than one-third can be spread through the air, with airborne transmission ranking as the number one method of transmission. Micro-organisms in the air take the form of a bioaerosol, mainly as a result of medical activities and activities of the patients themselves. There are many types of microbial aerosols in the air and most of them may cause nosocomial infection. Therefore, microbiological monitoring of the air in public places, especially in hospitals, plays a vital role in preventing nosocomial infection.

Airborne micro-organism levels have been reported for hospital clean rooms such as operating rooms, hospital rooms, intensive care units, surgical units, haematological wards, and maternity wards. As there is limited published information about the characteristics of microbial distribution in the air environment in NICUs, this present study aimed to monitor the air environment in an NICU in a tertiary hospital in China, and to identify the micro-organisms that were present.
Materials and methods

Study design

This prospective study monitored the air environment of the NICU, Kailuan General Hospital, Hebei United University, Tangshan, Hebei Province, China for 12 months between October 2010 and September 2011. The NICU consisted of a large ward with six beds and a small ward with two beds, both of which met the requirements of the National Health and Family Planning Commission of the People’s Republic of China. According to the regional characteristics of climate, March to May was defined as spring, June to August as summer, September to November as autumn, and December to February as winter. The study protocol was approved by the Medical Ethics Committee of Kailuan General Hospital (reference number: KLGH-NICU-NI-01).

Air sampling methods and colony counting

Air sampling was undertaken with an LWC-1 centrifugal air sampler (Kangjie Instrument Research Institute, Liaoyang, China), which worked in a similar manner to a Reuter centrifugal air sampler. Blood agar strips (Beiruite Bio-technology, Zhengzhou, China) were used to culture bacterial colonies and Sabouraud agar plates (Beiruite Bio-technology) were used to culture fungal colonies from the air environment in the NICU. The person who operated the LWC-1 centrifugal air sampler was kept 0.5 m away from the sampler, to avoid contamination of the experimental sampling with bacteria carried on or exhaled by the operator. The time for each air sampling was 1 min. Blood agar strips and Sabouraud agar plates were sealed after sampling and immediately transported to the microbiology laboratory for culture. The number of bacterial colonies on the blood culture strips and the number of fungal colonies on the Sabouraud agar plates were recorded using standard microbiology techniques.

Air samples were collected from the NICU between 09.00 h and 10.00 h on every second and fourth Tuesday of each month during the study period, giving a total of 24 sampling times. This air sampling time was selected because it covered a period when there were numerous clinical activities and frequent traffic of people in and out of the NICU. Routine disinfection measures (usually undertaken at 08.00 h for 2 h) were stopped on the sampling days and patients in the ward were either lying in bed or sitting in a chair at the time of air sampling.

In the large ward, one central point and four corner points were set up as the air sampling locations, with the corner points being 1 m away from the wall. In the small ward, three points on a diagonal line were selected, one central point and two corner points, with the corner points being 1 m away from the wall. The air sampling height was 1.5 m from the ground and the air sampling points were always >1 m away from the wall and any windows.

Results were calculated as the geometric mean of the samples, expressed as CFU per m³ of air (CFU/m³) as described previously. The mean number of bacteria for the 24 sampling times (i.e. 24 mean values calculated from 120 samples [five air samples per sampling time] in the large ward and 24 mean values calculated from 72 samples [three air samples per sampling time] in the small ward) and the mean number for each season were calculated (i.e. six sampling times per season). This study focused on the mean number of bacteria only, as the number of fungi was expected to be very low.
**Bacterial and fungal culture and identification**

The culture and identification of the bacteria and fungi collected in the air samples were conducted in accordance with the National Clinical Laboratory Procedures and other research.\(^\text{24}\) Once at the microbiology laboratory, blood agar strips and Sabouraud agar plates were cultured in an incubator (Heraeus, Hanau, Germany) at 35°C for 48 h for bacteria, and at 28°C for 7 days for fungi. The concentration of airborne bacteria (CFU/m\(^3\)) was determined using the number obtained from counting the colonies formed on blood agar strips after culture.\(^\text{24}\) Bacteria were identified using API\(^\text{®}\) identification strips and a fully automated bacterial identification system (VITEK\(^\text{®}\) 2 COMPACT; Biomérieux SA, Marcy l’Etoile, France) after Gram staining. Fungal cultures were identified using routine morphological tests, as described previously.\(^\text{23,24}\)

**Statistical analyses**

All statistical analyses were performed using the SPSS\(^\text{®}\) statistical package, version 13.0 (SPSS Inc., Chicago, IL, USA) for Windows\(^\text{®}\). Continuous variables were described by mean ± SD and compared using a two-sample Student’s \(t\)-test. Categorical variables were described by percentages and compared using \(\chi^2\)-tests. Wilcoxon rank-sum test and analysis of variance were used to analyse the type and distribution of the micro-organisms and the number of bacteria in the air environment of the NICU, according to the season. Fisher’s least significant difference (LSD) \(t\)-test was used to further test the two-sample comparison after the analysis of variance. All statistical tests were two-sided, and a \(P\)-value < 0.05 was considered statistically significant.

**Results**

Results of the air sampling undertaken for 12 months between October 2010 and September 2011 on the large and small wards of the NICU are presented in Table 1. The mean number of airborne bacteria in the large ward was significantly higher than that in the small ward \((P < 0.001)\).

In the large ward, the mean number of airborne bacteria varied significantly by season \((P < 0.001)\) (Table 2): in autumn, this number was significantly higher than that sampled during the other three seasons \((P < 0.05\) for each comparison). There was no significant difference in the mean number of airborne bacteria during the winter, spring, and summer in the large ward.

| Table 1. Number of bacteria in the air environment of two wards of the neurological intensive care unit (NICU) of a tertiary hospital in China that was monitored for 12 months in order to identify the number and type of airborne microorganisms that were present. |
|---|
| **Parameter** | **NICU ward** | **Statistical significance\(^a\)** |
| | Large ward, 6 beds | Small ward, 2 beds |
| Number of bacteria CFU/m\(^3\) | 200 ± 51 | 110 ± 40 |

Data presented as mean ± SD.

\(^a\)The wards were compared using two-sample Student’s \(t\)-test. CFU, colony-forming units.
In the small ward, there were no significant differences in the mean numbers of airborne bacteria across the four seasons.

A total of 279 micro-organisms were identified from the air environment: 195 from the large ward; 84 from the small ward (Table 3). In the large ward, the most common micro-organisms were Gram-positive cocci (72.3%), with *Micrococcus luteus* (28.7%) being the most frequent species identified. Also identified were 35 (17.9%) Gram-positive bacilli, one (0.5%) Gram-negative cocci, six (3.1%) Gram-negative bacilli and 12 (6.2%) fungi. Similarly, in the small ward, the most common micro-organisms were Gram-positive cocci (67.9%), with *Micrococcus luteus* (29.8%) being the most frequent species identified. Also identified were

### Table 2. Number of bacteria in the air environment of two wards of the neurological intensive care unit (NICU) of a tertiary hospital in China that was monitored for 12 months stratified according to the season in which the air samples were collected.

| NICU ward | Large ward, 6 beds | Small ward, 2 beds |
|-----------|--------------------|--------------------|
| Season    |                    |                    |
| Autumn, n = 6 | 234 ± 42 | 107 ± 39 |
| Winter, n = 6 | 182 ± 55* | 118 ± 45 |
| Spring, n = 6 | 198 ± 45* | 106 ± 38 |
| Summer, n = 6 | 186 ± 46* | 112 ± 41 |

Data presented as mean ± SD n bacteria per air sampling time (colony-forming units/m³).

*P < 0.001, overall for large ward by season (analysis of variance).

*P < 0.05 compared with autumn (Fisher’s least significant difference t-test).

### Table 3. Number and type of micro-organisms found in the air environment of two wards of the neurological intensive care unit (NICU) of a tertiary hospital in China, monitored for 12 months.

| NICU ward | Large ward, 6 beds | Small ward, 2 beds |
|-----------|--------------------|--------------------|
| Pathogen  | n                  | %                  | N                   | % |
| Gram-positive cocci | 141 | 72.3 | 57 | 67.9 |
| *Micrococcus luteus* | 56 | 28.7 | 25 | 29.8 |
| *Staphylococcus epidermidis* | 30 | 15.4 | 12 | 14.3 |
| *Staphylococcus haemolyticus* | 17 | 8.7 | 7 | 8.3 |
| Other coagulase-negative | | | | |
| *Staphylococci* | 29 | 14.9 | 11 | 13.1 |
| *Staphylococcus capitis* | 2 | 1.4 | – | – |
| *Staphylococcus warneri* | 2 | 1.0 | – | – |
| *Enterococcus faecium* | 3 | 1.5 | – | – |
| Other Gram-positive cocci | 2 | 1.0 | 2 | 2.4 |
| Gram-positive bacilli | 35 | 17.9 | 20 | 23.8 |
| *Bacillus subtilis* | 30 | 15.4 | 17 | 20.2 |
| *Corynebacterium* | 5 | 2.6 | 3 | 3.6 |
| Gram-negative cocci | 1 | 0.5 | – | – |
| *Neisseria gonorrhoeae* | 1 | 0.5 | – | – |
| Gram-negative bacilli | 6 | 3.1 | 1 | 1.2 |
| *Acinetobacter baumannii* | 3 | 1.5 | 1 | 1.2 |
| *Pseudomonas aeruginosa* | 1 | 0.5 | – | – |
| *Enterobacter cloacae* | 1 | 0.5 | – | – |
| *Acinetobacter lwolfii* | 1 | 0.5 | – | – |
| Fungi | 12 | 6.2 | 6 | 7.1 |
| Total | 195 | 100.0 | 84 | 100.0 |
20 (23.8%) Gram-positive bacilli, one (1.2%) Gram-negative bacilli and six (7.1%) fungi. There was no significant difference in the distribution of the types of micro-organisms sampled from the air environment between the large and the small wards (Wilcoxon rank-sum test).

Discussion

As viruses, bacteria and fungi can form solid bioaerosols in the air (which can then cause infection by invading the human body through mucous membranes, skin lesions, and the gastrointestinal and respiratory tracts), monitoring bioaerosols accurately and precisely is very important for understanding the relationship between these aerosols and the spread of infectious disease. Patients in the NICU are particularly vulnerable to developing nosocomial infections because they are often elderly and they might also have underlying diseases, swallowing dysfunctions, disturbances of consciousness, and various motor impairments.25–27 The rate of nosocomial infection ranges between 24 and 50% in NICUs.1,28–30 Nosocomial infection can result in a prolonged hospital stay and an increased risk of death.28 Therefore, monitoring and understanding the distribution and the characteristics of any airborne micro-organisms is important in the prevention and control of nosocomial infection in NICUs.

Most hospital-based airborne micro-organism studies have been performed in clean rooms or departments where the risk of infection is the greatest.12,20,21 Relatively little is known about microbial contamination in the NICU. This present study investigated the characteristics of micro-organisms sampled from the air environment of two wards in the NICU of a tertiary hospital in China, over a 12-month period. The mean ± SD numbers of airborne bacteria in the large and small wards were 200 ± 51 CFU/m³ and 110 ± 40 CFU/m³, respectively. The mean number of airborne bacteria in the large ward was significantly higher than that in the small ward, which was probably due to the greater number of beds, higher turnover of patients and larger number of visitors in the large ward. Although the mean number of airborne bacteria in this present study was much higher than that reported elsewhere,9 the level still met the hygienic standard for disinfection in hospitals in China.31 The mean number of airborne bacteria in the NICU in the present study was lower than that previously reported in hospital lobbies,8 probably because the location, the methods of disinfection and other risk factors were different between the two studies. In accordance with the requirements of the Chinese Ministry of Health, one wall-mounted air disinfection machine was installed for each 13 m² in the NICU ward investigated in this present study. The NICU was also disinfected manually by cleaning staff three times a day at 08.00 h, 14.00 h, and 20.00 h, with each cleaning session lasting 2 h.

The present study also showed that, in the large ward only, the mean number of airborne bacteria in the autumn was significantly higher than in any of the other three seasons. This seasonal variation in the large ward might be related to the fact that autumn has higher rates of cerebral vascular disease due to the change of temperature from summer to autumn, which results in a higher number of patients being accommodated in the large ward. These current findings suggest that the number of airborne micro-organisms was closely related to the number of the patients on the NICU ward and on a larger, busier ward, it was also related to the season.

In this present study, 279 airborne micro-organisms were identified; 195 of which came from the large ward, and 84 from the small ward. There was no significant difference in the type and distribution of the airborne micro-organisms between the large
and small wards, which suggests that the size of the ward and the number of the patients were not factors that influence the type and distribution of airborne micro-organisms.

This present study demonstrated that the most common airborne micro-organisms were Gram-positive cocci in both the large and small wards. The proportion of airborne Gram-negative bacilli was less than that previously reported, whereas the number of fungi was slightly higher.\textsuperscript{32} These findings might be related to improvements made within this institution, in terms of the antimicrobial drug management of Gram-negative bacilli in the NICU. Although the proportion of airborne Gram-negative bacilli was not very high in the present study, the species that were identified were mainly pathogens that cause nosocomial infection. Further bacterial homology analysis is required to determine whether the airborne Gram-negative bacilli were the same bacteria causing nosocomial infection in the patients in the NICU.

With regard to the higher number of fungi found in the present study, this was thought to be due to the humidity and temperature within the NICU being suitable for fungal growth, and the fact that the wall-mounted air disinfection machine was not designed to target airborne fungi. These present findings suggest that improvements to the antifungal disinfection regimen in the NICU, such as spraying with quaternary amine, are required.

This study only investigated the number and type of airborne micro-organisms in an NICU of one hospital over 12 months, which is a limitation of its findings. Future research should investigate NICUs in a larger number of hospitals over longer periods of time.

In conclusion, the present study demonstrated that the number of airborne bacteria in an NICU was affected by the number of patients on the ward, and in the large ward, it was also affected by the season. The majority of airborne micro-organisms were Gram-positive cocci in both wards. Overall, these findings suggest that, in order to prevent nosocomial infections in NICUs, it would be better to have smaller wards with two beds, improved air disinfection (particularly for Gram-negative bacilli and fungi), and improve awareness of the risk of airborne micro-organisms among healthcare professionals, so that hygiene in the NICU and the wider hospital can be improved further.

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Declaration of conflicting interest
The authors declare that there are no conflicts of interest.

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