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Introduction

Outbreaks caused by multidrug resistant Acinetobacter baumannii strains have been intensively documented worldwide (Zarrilli et al. 2013). With the increasing number of carbapenem-resistant A. baumannii, outbreaks of A. baumannii were expanding. One data showed that resistance to carbapenem increased from 1.0% in 2003 to 58.0% in 2008 in Detroit Medical Center health system (Reddy et al. 2010). In China, A. baumannii has recently become an important pathogen causing the nosocomial infection in hospitals. The infection rate of A. baumannii is always in the top three in total clinical isolates of Gram-negative bacilli, second only to Escherichia coli and Klebsiella pneumoniae. In 2014, A. baumannii isolated from non-fermenting Gram-negative bacilli was always in the first place in clinical tests (Wang et al. 2013; Hu et al. 2014; Hu et al. 2015).

The analysis results of multilocus sequence typing (MLST) demonstrated that multiple STs were prevalent in the outbreak regions. Ying reported that ST208 was the most prevalent, followed by ST191 and ST729 in the 398 isolates collected in seven regions of South China in 2012 (Ying et al. 2015). Through the whole genome sequencing, it was found that blaOXA-23-like-producing A. baumannii ST208 strains emerged and rapidly spread in Hangzhou First People's Hospital (Chen et al. 2018). Just recently, Jeon et al. (2018) reported that sequence type (ST) 191 was the predominant clone, followed by the ST208 and ST369 in the hospital in Korea.

At present, A. baumannii strains were mainly isolated from the clinical specimens, as blood, urine, tissues, and sputum (Bogaerts et al. 2006; Zong et al. 2008; Mendes et al. 2009; Yang et al. 2010; Chen et al. 2013). In our laboratory, we have been focused on the epidemiology of A. baumannii isolates from sputum (Jiang et al. 2013; Jiang et al. 2014). However, some

Abstract

To understand the molecular epidemiology and antibiotic resistance of air and clinical isolates of Acinetobacter baumannii, the intensive care unit settings of a hospital in Northern China were surveyed in 2014. Twenty non-duplicate A. baumannii isolates were obtained from patients and five isolates of airborne A. baumannii were obtained from the wards' corridors. Pulsed field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) were used to analyze the homology relationships of isolates. Resistance and resistance genes were detected by drug susceptibility test and PCR. The results demonstrated that all isolates can be classified into eight PFGE types and four sequence types (ST208, ST195, ST369 and ST530). A pair of isolates from patients (TAaba004) and from the air (TAaab012) that share 100% similarity in PFGE was identified, indicating that air might be a potential and important transmission route for A. baumannii. More than 80% of the isolates were resistant to carbapenems and aminoglycoside antibiotics. Twenty-four isolates, which were resistant to carbapenems, carried the blaoxa-23-like gene. The data indicated that air might be an alternative way for the transmission of A. baumannii. Hospitals should pay more attention to this route, and design new measures accordingly.

Key words: Acinetobacter baumannii, molecular epidemiology, antibiotic resistance, airborne, nosocomial infection
reports have shown that \textit{A. baumannii} can survive and transmit in the air (Allen et al. 1987; Obbard et al. 2003). To date, the homology relationship between the airborne \textit{A. baumannii} isolates from hospital air and those isolated from patients has not been extensively studied. In this study, twenty-five isolates of non-duplicate \textit{A. baumannii} were obtained from patients and the air of wards’ corridors during May – Jun. and Oct. – Nov. of 2014. The molecular epidemiological characterization of the isolated \textit{A. baumannii}, such as drug resistance, and types of resistance genes, was compared to understand the relationship between the airborne \textit{A. baumannii} and those isolated from patients.

**Experimental**

**Materials and Methods**

**Ethical approval.** Samples of patients were acquired during a routine checkup by medical staffs. The study was approved by the Ethics Committee of Tai’an City Central Hospital and carried out in accordance with the approved guidelines.

**Source of the isolates.** From May 12 to Jun. 5 and Oct. 11 to Nov. 15, 2014, \textit{A. baumannii} isolates were obtained from patients hospitalized in four different wards, including the intensive care unit (ICU), emergency intensive care unit (EICU), respiratory intensive care unit (RICU) and geriatrics ward (GW). Meanwhile, airborne \textit{A. baumannii} isolates were obtained from the air of the wards and adjacent corridors using the Andersen 6-stage air sampler. Each stage of the sampler has a plate with 400 holes of uniform diameter, according to the method described in previous studies (Andersen et al. 1958; Obbard et al. 2003). In brief, the sampler was placed in the center of wards and corridors (closed) at a height of 1.5 m above the ground. Airborne \textit{A. baumannii} was collected at a calibrated flow rate of 28.3 l/min for 20 min, and MacConkey agar (Oxoid, Basingstoke, England) was used as the sampling medium. At each sampling site, four duplicate air samples were collected once per week. The number of colonies was about 30–300 in each stage. Temperature and relative humidity were recorded throughout the sampling periods (temperature 20–25°C; relative humidity 73–83%).

**Bacterial identification and drug susceptibility test.** Bacterial identification was performed by using an VITEK-2 bacterial instrument (BioMerieux, Lyons, France) following the operation instruction. Drug susceptibility test was performed by two different methods: the sensitivity of tigecycline and polymyxin B was detected by the Etest method (AB Biodisk, Solna, Sweden); the sensitivity of the remaining fourteen antibiotics was detected using the minimal inhibitory concentration method. The susceptibility testing result of tigecycline was explained according to Food and Drug Administration (FDA) guideline (http://www.fda.org.uk/sitemap.aspx). The criteria of the susceptibility of other agents were adapted from the Clinical and Laboratory Standards Institute (CLSI) (http://clsi.org/standards/). The criteria of multidrug resistant (MDR) \textit{A. baumannii}, extensively drug-resistant (XDR) \textit{A. baumannii} and pandrug-resistant (PDR) \textit{A. baumannii} were referred to the previously described (Magiorakos et al. 2012).

**Pulsed field gel electrophoresis (PFGE).** Clonal relatedness of 25 \textit{A. baumannii} strains isolated in this study was determined by PFGE (Ribot et al. 2006). Briefly, the bacterial chromosomal DNA was digested with 60 U of Apal (Takara, Dalian, China) in a 37°C. \textit{Salmonella} serotype Braenderup strain (H9812) was digested with XbaI (Takara, Dalian, China) and used as the molecular weight standard, the DNA fragments were separated on agarose gel using a clamped homogeneous electric field electrophoresis-Mapper XA system (Bio-Rad, California, USA). The PFGE images were analyzed using the Gel Doc software (Bio-Rad, California, USA) and the genetic relatedness of the \textit{A. baumannii} isolates were evaluated using the BioNumerics software (Applied Maths, Sint-Martens-Latem, Belgium). The cutoff level of 85% was set to define clonal clustering of the PFGE types.

**Multilocus sequence typing (MLST).** The sequence types (STs) of \textit{A. baumannii} strains were determined by MLST using the previously described method (Bartual et al. 2003). To date, the homology relationship between the previously described (Magiorakos et al. 2012).

**Table I**

| Antibiotics                  | Susceptible | Resistant |
|-----------------------------|-------------|-----------|
|                             | Number      | Rate (%)  | Number      | Rate (%)  |
| Amikacin                    | 3           | 15.0%     | 17          | 85.0%     |
| Ceftazidime                 | 0           | 0.0%      | 20          | 100%      |
| Ciprofloxacin               | 0           | 0.0%      | 20          | 100%      |
| Levofloxacin                | 0           | 0.0%      | 20          | 100%      |
| Gentamicin                  | 0           | 0.0%      | 20          | 100%      |
| Tobramycin                  | 3           | 15.0%     | 17          | 85.0%     |
| Ceftriaxone                 | 0           | 0.0%      | 20          | 100%      |
| Cefotaxime                  | 0           | 0.0%      | 20          | 100%      |
| Cefepime                    | 0           | 0.0%      | 20          | 100%      |
| Imipenem                    | 0           | 0.0%      | 20          | 100%      |
| Meropenem                   | 0           | 0.0%      | 20          | 100%      |
| Trimethoprim/sulfamethoxazole| 6           | 30.0%     | 14          | 100%      |
| Piperacillin/tazobactam     | 0           | 0.0%      | 20          | 100%      |
| Tigecycline                 | 20          | 100%      | 0           | 0.0%      |
| Polymyxin B                 | 20          | 100%      | 0           | 0.0%      |
Note: “R” indicates resistant, “S” indicates susceptible.

from the adjacent corridors of the wards. Fifteen com-

A. baumannii strains. Twenty A. baumannii clinical strains were isolated from 356 patients and five strains were isolated from 4 out of 64 air samples in duplicate during this experimental period (8 weeks). Wherein two A. baumannii strains (TAaba015 and TAaba014) were isolated from the same air sample from ICU corridor in 08.11.2014.

Drug susceptibility patterns. A total of twenty-five non-duplicate A. baumannii isolates were obtained, including twenty from the clinical samples and five from the adjacent corridors of the wards. Fifteen com-

enly used antibiotics (amikacin, ceftazidime, cipro-

floxacin, levofloxacin, gentamicin, tobramycin, ceftri-

axone, cefotaxime, cefepime, imipenem, meropenem, trimethoprim/sulfamethoxazole, piperacillin/tazobac-

tam, tigecycline, and polymyxin B) were selected for drug susceptibility test. The result showed that only one out of the five airborne isolates (TAaba013) was non-MDR A. baumannii, which was only resistant to ceftazidime, ceftriaxone, cefotaxime, and cefepime. All twenty clinical isolates were MDR A. baumannii, and susceptible to tigecycline and polymyxin B.

Drug resistance genes. All isolates had the blaOXA-23-like resistant gene, and twenty-four had the blaOXA-51-like gene except one airborne isolate (TAaba014). However, other seven resistance genes of carbapen-

emes were not detected in this study. These results indicated that blaOXA-23-like gene was responsible for the carbapenemase resistance. In addition, twenty isolates carried the 16S rRNA methylase gene armA. No rmtB gene was detected in all isolates.

PFGE and MLST. As shown in Fig. 1, the isolates can be clustered into eight PFGE types (A-H) and four STs (ST208, ST369, ST195 and ST530). One airborne isolate (TAaba012) had the same PFGE and ST types as one clinical isolates (TAaba004). They were isolated from the same ward (EICU) during the same period (May – Jun 2014). Similarly, in another ward (intensive care unit, ICU), one airborne isolate (TAaba008) was found to be closely related to three clinical isolates (TAaba002, TAaba009, and TAaba011) in terms of PFGE and ST types (type D and ST208, respectively).

In the second sampling period (Oct. 11 to Nov. 15, 2014), we collected three non-reduplicate airborne isolates (TAaba013, TAaba014 and TAaba015). We did not find any clinical isolates with the same PFGE and ST types. In particular, the PFGE and ST types of TAaba013 were different from any isolates obtained in this study.

Discussion

A. baumannii is a predominant cause of nosocomial infections, and the organism has been detected in water, soil, and on the surface of human body. A recent study has shown that some A. baumannii environmental isolates are able to survive in tap, normal saline, and distilled water with a wide range of pH and temperature for a long period of time (Obiedat et al. 2014). It makes it harder for the prevention of A. baumannii spread in a nosocomial environment. A. baumannii is often trans-

mitted through physical objects, like stethoscopes, com-

puters, milk pumps, and headboards of beds. In this study, we investigated the genetic relationship of the A. baumannii isolates from the air and patients, and the possible transmission pathway through the air.
Fig. 1. Dendrogram showing the PFGE and MLST profiles of the *A. baumannii* isolates. The dendrogram was generated by the BioNumerics software. The sampling date, isolate number, ward and source, PFGE and ST types and resistance genes are shown for each isolate.
Our results show that one airborne *Acinetobacter baumannii* (TAaba012) isolated in the EICU and one clinical isolate (TAaba004) isolated in the same ward shares 100% similarity in PFGE and MLST, indicating *A. baumannii* might be transmitted through air. Another airborne isolate (TAaba008) obtained in ICU was also found to be the same strain as three clinical isolates (TAaba002, TAaba009, and TAaba011). Based on the genetic relationship of the airborne and clinical isolates, we speculate that there could exist an air-mediated spread of this *A. baumannii* strain. Moreover, many studies demonstrated that airborne *A. baumannii* could be detected in ICU and airborne transmission of *A. baumannii* was possible (Munoz-Price et al. 2013; Spellberg et al. 2013; Jakupogullari et al. 2016).

A total of twenty clinical isolates were obtained during the sampling period of this study, but only one isolate was found in the air, indicating airborne transmission might be affected by many factors, such as length of hospital stay, humidity, temperature, etc. Since our dataset are relatively small, we cannot draw any exclusive conclusion. Moreover, because horizontal gene transfer that can jeopardize clonal strains, PFGE is no longer the most effective technique for *A. baumannii* typing (Salipante et al. 2015), and our data can only confirm that the same strain was isolated from air and the specimens from patients, which could suggest an alternative route of transmission through air. More systematic studies with a longer period of sampling should be constructed to better understand the airborne pathway.

Treatment of *A. baumannii* infections is particularly difficult because of the resistance to a broad range of antibiotics, especially with the increasing of resistant to carbapenems (Lee et al. 2012). Although mechanisms of resistance to carbapenems were very complex, resistance genes play an important role in this process. It has been reported that bla\textsubscript{OXA-23}-producing *A. baumannii* were found worldwide (Le et al. 2008; Runnegar et al. 2010; Mosqueda et al. 2013). In this study, the analysis showed that all strains had bla\textsubscript{OXA-51-like} gene, one intrinsic gene in *A. baumannii*. Moreover, 96% strains (24/25) carried the bla\textsubscript{OXA-23-like} gene, except TAaba014. These results indicated that both genes were mainly responsible for the carbapenems resistance in this area, especially the bla\textsubscript{OXA-23-like} gene and it was generally believed that bla\textsubscript{OXA-23-like} gene was responsible for carbapenem resistance in China (Ruan et al. 2010; Liu et al. 2015). Additionally, it has been reported that 16S rRNA methylase gene armA was closely related to the aminoglycosides resistance (Yokoyama et al. 2003), and 80% (20/25) strains had the 16S rRNA methylase gene armA. All of the strains showed the resistance to amikacin, tobramycin and gentamicin. Antibiotic resistance is a serious global public problem.

Taken together, in this study, we isolated twenty clinical *A. baumannii* from patients and five airborne *A. baumannii* from the ward environment. Out of the five airborne *A. baumannii*, one was found to have the same PFGE and ST type with one strain isolated from patients, indicating that the air may be an important and potential transmission way for *A. baumannii*. Considering the seriousness of nosocomial infections of *A. baumannii*, prophylactic strategies in controlling the air transmission of *A. baumannii* should be considered when new measures are designed for controlling nosocomial infections.

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Author contributions

M. Jiang and Z. Zhang performed the main experiment and wrote the manuscript. Y. Mu and N. Li analyzed data. Z. Zhang and S. Han reviewed the manuscript and approved it.

Conflict of interest statement

The authors declared there was no any conflict of interests.

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