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Antibiotic resistance and OXA-type carbapenemases-encoding genes in airborne *Acinetobacter baumannii* isolated from burn wards

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ABSTRACT

The study was conducted to investigate drug resistance, OXA-type carbapenemases-encoding genes and genetic diversity in airborne *Acinetobacter baumannii* (*A. baumannii*) in burn wards. Airborne *A. baumannii* were collected in burn wards and their corridors using Andersen 6-stage air sampler from January to June 2011. The isolates susceptibility to 13 commonly used antibiotics was examined according to the CLSI guidelines; OXA-type carbapenemases-encoding genes and molecular diversity of isolates were analyzed, respectively. A total of 16 non-repetitive *A. baumannii* were isolated, with 10 strains having a resistance rate of greater than 50% against the 13 antibiotics. The resistance rate against ceftriaxone, cyclophosvnamide, ciprofloxacin, and imipenem was 93.75% (15/16), but no isolate observed to be resistant to cefoperazone/sulbactam. Resistance gene analyses showed that all 16 isolates carried OXA-51, and 15 isolates carried OXA-23 except No.15; but OXA-24 and OXA-58 resistance genes not detected. The isolates were classified into 13 genotypes (A-M) according to repetitive extragenic palindromic sequence PCR (REP-PCR) results and only six isolates had a homology ≥90%. In conclusion, airborne *A. baumannii* in the burn wards had multidrug resistance and complex molecular diversity, and OXA-23 and OXA-51 were dominant mechanisms for resisting carbapenems.

1. Introduction

A. baumannii is an aerobic gram-negative coccobacillus and also one of the major pathogens responsible for nosocomial infections [1]. It has the third highest isolation rate among common gram-negative bacilli in clinical practice in China, after *Escherichia coli* and *Klebsiella pneumonia* [2]. Carbapenem antibiotics are the most common treatment for serious *A. baumannii* infections. However, due to the wide clinical application of these drugs, *A. baumannii* has developed an increasing resistance to them, which has become a serious global public health problem [3].

The mechanism of *A. baumannii* resistance to carbapenem antibiotics is very complex. Production of carbapenemases is

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Table 1 – Multi-PCR primers and amplicon sizes for genes encoding OXA carbapenemases.

Primer	Sequence (5′ → 3′)	Length (bp)	Reference
OXA-51	F: TAATGCTTTGATCGGCCTTG R: TGGATTGCACTTCATCTTGG	353	[17]
OXA-23	F: GATCGGATTGGAGAACCAGA R: ATTTCTGACCGCATTTCCA	501	[17]
OXA-24	F: GGTTAGTTGGCCCCCTTAAA R: AGTTGAGCGAAAAGGGGATT	246	[17]
OXA-58	F: AAGTATTGGGGCTTGTGCTG R: CCCCTCTGCGCTCTACATAC	599	[17]

considered the most important mechanism of resistance. The carbapenemases are divided into three classes, Class A, Class B, and Class D. The most common of these classes is Class D, which is also referred to as OXA-type carbapenemases. According to the characteristics of their nucleotide sequences, OXA-type carbapenemases can be subdivided into 4 types, OXA-23, OXA-24, OXA-51, and OXA-58 [4,5].

A. baumannii strains are opportunistic pathogens that can stay in the air and even be transmitted via respiratory droplets, causing infection particularly in susceptible individuals [6–12]. However, information about airborne *A. baumannii* in the hospital environments is so far rare. To fill the literature gap, we collected *A. baumannii* using an Andersen-6 stage air sampler, which is suitable for collecting viable bacteria, from a burn ward environment in a general hospital in Tai'an, China over six months [13]. In this study, we analyzed their resistance, OXA-type carbapenemases-encoding genes, and molecular diversity, aiming to support the effective prevention and control of nosocomial infections caused by *A. baumannii*.

2. Materials and methods

2.1. Sampling site and strategies

Burn wards in a general hospital in Tai'an City, China were selected as the sampling sites. Every burn ward has a floor area of 22.5 m^2 with four beds, and is ventilated by one air-conditioning vent on the ceiling. There is no direct ventilation with the external environment. Only four patients were present and the door was closed during the sampling.

From January 2011 to June 2011, airborne *A. baumannii* was collected at a calibrated flow rate of 28.3 L/min using Andersen 6-stage air sampler [13]. MacConkey agar was used as the sampling medium [14]. The sampler was placed in the center of burn wards and the corridor at a height of approximately 1.5 m above the ground. At each location, sampling time was 10 min and three duplicate air samples were collected once per week. Temperature and relative humidity were monitored throughout the sampling periods and were 23 ± 1 °C and 78 ± 3 °C respectively [12,13].

2.2. Isolation and identification of A. baumannii

After sampling, the MacConkey agar medium was cultured for 48 h in a 35 °C incubator [14], and non-repetitive suspected *A. baumannii* colonies were picked, identified using a VITEK system (BioMerieux, Marcy 1′Etoile, France), and further confirmed by PCR amplification of the inherent OXA-51 gene [15].

2.3. Susceptibility test

Susceptibility to 13 commonly used antibiotics, including amikacin (AK), ceftazidime (CAZ), ciprofloxacin (CIP), gentamicin (CN), ceftriaxone (CRO), cyclophosvnamide (CTX), cefepime (FEP), imipenem (IMP), Meropenem (MEM), ampicillin/sulbactam (SAM), cefoperazone/sulbactam (SCF), tri-methoprim/sulfamethoxazole (SXT), piperacillin/tazobactam (TZP), was determined. *Escherichia coli* (ATCC25922) and *Pseudomonas aeruginosa* (ATCC27853) were used as the susceptibility test quality control. Result evaluation and quality control were performed according to the 2010 CLSI standards [16].

2.4. Detection of OXA-type carbapenemases-encoding genes by multiplex PCR

Bacterial DNA was extracted according to the instructions of bacterial genome extraction kit (QIAGEN, Mississauga, Ontario, Canada). The extracted product was stored at −20 °C. OXA-23, OXA-24, OXA-51, OXA-58 were amplified by multiplex PCR [17], using primers (Table 1) from Sangon Biotechnology Co., Ltd. (Shanghai, China). The reaction system had a total volume of 50 μL and consisted of 5 μL of 10× PCR buffer, 4 μL of 2.5 mmoL/L dNTP, 1 μL each of 20 pmol/L primers, 3 μL of 25 mmoL/μL $MgCl_2$, 5 U Taq DNA polymerase, and 3 μL of DNA template. The amplification consisted of an initial denaturation at 94 °C for 5 min, 30 cycles of denaturation at 94 °C for 25 s, annealing at 52 °C for 40 s, and extension at 72 °C for 50 s. A final extension for 6 min at 72 °C was also applied. PCR products were analyzed by 2 °C agarose gel electrophoresis at 100 V for 1 h. Photos were taken with a gel imaging system (Tanon-2500, Shanghai, China).

Standard strain containing OXA-type carbapenemases-encoding genes was not available; therefore, the PCR procedure was repeated twice for all the isolates, and the positive strains were analyzed visually based on the amplicon sizes.

2.5. REP-PCR

The primers [18] REP1 (5′-ATGTAAGCTCCTGGGATTCAC-3′) and REP2 (5′-AAGTAAGTGACTGGGGTGAGCG -3′) were synthesized by TaKaRa (Dalian, China). The reaction system had a