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Detection of *Acinetobacter baumannii* in the Oropharynx of Long-Term Hospitalized Patients and the Expression of *IntI* Gene Induced by Different Antibiotics

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Abstract

Acinetobacter baumannii is the main drug resistant bacteria in clinic at present, and its drug resistance is still rising rapidly. Integrons play an important role in bacterial acquisition of exogenous drug resistance genes. This study investigated *A. baumannii* colonization in oropharynx and the integron gene carrying, and the expressions of integrase gene were determined when exposed to the different concentrations of antibiotics. 64 hospitalized patients were collected during January 2019 to June in respiratory department of our hospital (the hospitalized time more than 14 days) in our experiment. All throat swab collections were used for DNA extraction, and *A. baumannii* identification and integron gene detection were done by PCR assay. *A. baumannii* strains isolated from the oropharynx were identified by MALD-TOF-MS technology and the drug resistance was also analyzed. When expose to a series of ceftazidime and imipenem (10 μg/mL, 5 μg/mL, 1 μg/mL, 0.1 μg/mL, 0.01 μg/mL), the expressions of integron gene in the strains originated from oropharynx were determined by qRT-PCR assay. The results of the PCR showed that 60 patients had detected the *A. baumannii Ab-ITS* gene and rA gene in throat swabs, wherein 43 patients with symptoms of infection and 17 without symptoms of infection. There was no significant difference in *Ab-ITS* gene and rA gene detection rates in the symptoms of infection and no symptoms of infection ($P > 0.05$). In total 60 cases of samples, 29 cases had detected the *IntI* genes. 6 strains of *A. baumannii* isolated from the throat swabs of hospitalized patients were multi-drug resistant bacteria with *IntI* gene and variable region genes. Gene sequencing analysis revealed that the variable region gene cassettes were aacA4-catB8-aadA1-qacEdelta1. When exposed to the concentration of 1 μg/mL and 5 μg/mL ceftazidime, the mRNA expres-

sion of *IntI* had significantly increase compared to the negative control ($P < 0.05$). When exposed to the concentration of 0.1 μg/mL, 1 μg/mL and 5 μg/mL imipenem, the mRNA expression of *IntI* had significantly increase compared to the negative control ($P < 0.05$). The results indicated that oropharyngeal microflora includes multidrug-resistant *A. baumannii* and carries ingegron genes in long-term hospitalized patients. The improper use of ceftazidime and imipenem might also contribute to the up-regulation of the integrase genes expression and the enhancement of bacterial resistance, which requires more attention in clinical work.

Keywords

Acinetobacter baumannii, Colonization, Integron, Antibiotic

1. Introduction

Currently, the outbreak of *Acinetobacter baumannii* in hospital is a serious public health problem. Especially in the past 20 years, *A. baumannii* resistance to imipenem and meropenem had enhanced significantly increased, and that lead the clinical treatment for *A. baumannii* infection to become more and more difficult [1]. It is known that oral pharyngeal colonization of patients with low immunity *A. baumannii* was the important reason to induce severe pneumonia and ventilator-associated pneumonia [2].

Integrants are an important mobile genetic element in gram-negative bacteria with effective capture and exogenous gene expression function. The core structure of integrants is the integrase, which belonged to the tyrosine family with the responsible for catalyzing the gene cassette capture and rearrangement, as part of the integration of sub-variable region [3]. When increasing bacterial integrase expression, which acquires the ability of exogenous resistance gene also increases [4]. However, there was still lacking of research on the effects of antibiotics on *A. baumannii* integrase gene expression.

In this study, we used the PCR assay to detect the oropharynx colonized *A. baumannii* in long-term hospitalized patients in our hospital, and the integron gene was also detected. The integrase gene expression of *A. baumannii* when exposed to a series of concentration of antibiotic were determined by qRT-PCR assay.

2. Material and Methods

2.1. Respiratory Department Patient Throat Swab Collection

64 hospitalized patients were collected from January 2019 to June in respiratory department of our hospital (the hospitalized time more than 14 days) in our experiment. The average age of patients in the experiment was (62 ± 32.4) years old. Patients with tuberculosis, fungal infection, tumor and other primary pulmonary diseases, as well as allergic constitution and multiple drug allergy were

excluded. At the same time, excluded did not follow the prescribed medication, unable to judge the curative effect, or incomplete data affect the curative effect.

The 46 patients have significantly increased the number of neutrophile and C-reactive protein (CRP), and respiratory infection symptoms. The other 18 patients had normal level of neutrophile and CRP. When collecting the throat swabs, the tongue spatulas were gently pressed the patients' tongue, the sterile saline moistened cotton swab wiped patient's secretions on each side of palatal and tonsil [5]. The patients' throat swabs were used for bacterial culture and the genomic DNA extraction.

2.2. Bacterial Identification and Drug Resistance Analysis

The throat swabs were inoculated in Columbia CNA blood agar plate immediately (Hopebio, Qingdao, China) and cultured in 35˚C with 5% CO_2 for 24 h. Then the visible colonies were collected and identified by matrix-assisted laser desorption ionization-time of flight mass spectrometer (MALD-TOF-MS) (MALDI-TOF VITEK-MS system, BioMrueux, France). The VITEK 2-compact system (BioMrueux, France) was used to analysis the drug resistance of *A. baumannii* separated from patient throat swabs. The Kirby-Bauer (KB) method was used for determining the sensitivity of tigecycline and cefoperazone/sulbactam sodium.

2.3. Detection *A. baumannii* Gene and Integrated Gene in Throat Swab

Bacterial genomic DNA were extracted by bacterial genomic DNA purification kit (Tiangen Biotech, Beijing, China), the purified DNA was used for the *Ab-ITS*, *rA* and *IntI* genes detection. The *Ab-ITS* primer was specifically targeted to *A. baumannii*, and *rA* primer was specifically targeted to all bacterial of the *Acinetobacter* family. When both *Ab-ITS* gene and *rA* gene detected, we determined the *A. baumannii.* The primers sequences and annealing temperatures were listed in **Table 1**.

The PCR reaction volumes were 25 μL, including 2 × PCR master mix 12.5 μL, each of the primers (10 μM) 0.5 μL, the bacterial genomic DNA 2 μL, sterile distilled water was added to 25 μL. The PCR reaction was started at 94˚C for 1 min, then the following reactions were 94˚C denaturation for 30 s, 55˚C annealing for 30 s, 72˚C extension for 1 min. The following reactions were 35 cycles, and the final reaction was 72˚C extension for 1 min. The PCR products were analysed by agarose electrophoresis.

2.4. The Integrate Gene in *A. baumannii* Isolated from Throat Swab

The *A. baumannii* originated from the culture of throat swab. The bacterial genomic DNA were purified by the bacterial genomic DNA purification kit, and *IntI* gene was detected by PCR assay. The PCR reactions were followed by the method mentioned above.

Table 1. The primers sequence in the experiment.

Genes	Primer sequence (5'-3')	Produce length (bp)
Ab-ITS	F: CATTATCACGGTAATTAGTG R: AGAGCACTGTGCACTTAAG	208
rA	F: CCTGAATCTTCTGGTAAAAC R: GTTTCTGGGCTGCCAAACATTAC	425
IntI	F: ACGAGCGCAAGGTTTCGGT R: GAAAGGTCTGGTCATACATG	564
16s rRNA	F: ACGAGCGCAAGGTTTCGGT R: GAAAGGTCTGGTCATACATG	-
IntI variable region	F: GGCATCCAAGCAGCAAG R: AAGCAGACTTGACCTGA	variable

2.5. The *A. baumannii* Integrase Gene Expression When Exposed to Different Concentrations of Ceftazidime and Imipenem

A serial of concentrations (10 μg/mL, 5 μg/mL, 1 μg/mL, 0.1 μg/mL, 0.01 μg/mL) of ceftazidime and imipenem were prepared and two drugs were added into LB broth respectively. In the control group, the LB broth was without any antibiotic. The *A. baumannii* strains isolated from throat swab were inoculated in the LB broth mentioned above. All the broth were cultured in 37˚C with shaking at 200 rpm for 8 h.

At the end of experiment, the bacteria were collected for total RNA extraction using bacteria total RNA extraction kit (Sangon Biotech, Shanghai, China), than the cDNA was synthesized by reverse transcription kit (Vazyme, Nanjing, China). The *A. baumannii IntI* mRNA expression was determined by qRT-PCR assay using AceQ Universal SYBR qPCR Master Mix (Vazyme, Nanjing, China). All the experiments were replaced for thrice and expressed as mean ± standard deviation.

3. Results

3.1. The Detection of *A. baumannii* and Integration Gene in the Throat Swabs

In this study, 60 patients had detected the *A. baumannii Ab-ITS* gene and rA gene in throat swabs (Figure 1), wherein 43 patients with symptoms of infection and 17 without symptoms of infection. There was no significant difference in *Ab-ITS* gene and rA gene detection rates in these two groups ($P > 0.05$). In total 60 cases of samples, 29 cases had detected the *IntI* genes (Figure 2).

3.2. Isolation and Drug Resistance of *A. baumannii* in Throat Swabs of Hospitalized Patients

Only 6 strains of *A. baumannii* were isolated from the throat swabs of hospitalized patients. They were named as strains 1 to 6, all of which carried the *IntI*

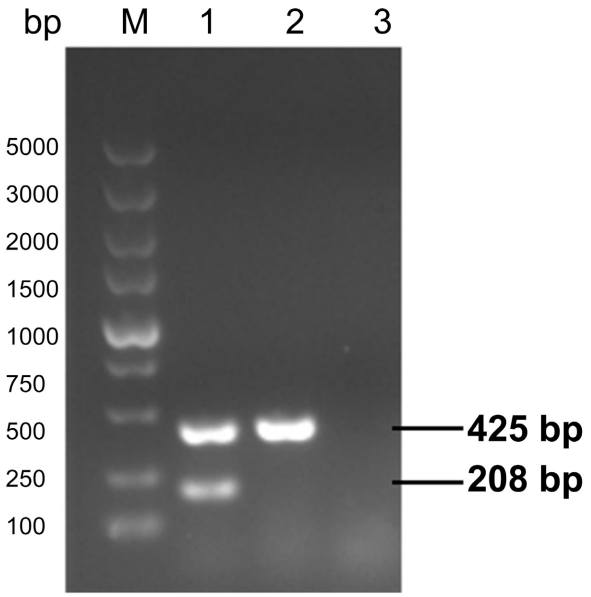

Figure 1. The result of *A. baumannii* identification of by PCR assay. M: DNA Marker, 1: *A. baumannii*, 2: *Acinetobacter*, 3: Negative.

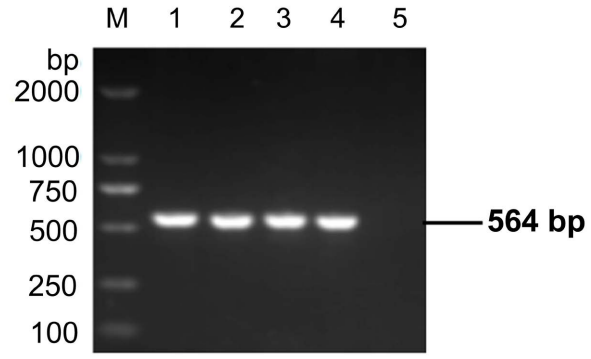

Figure 2. The result of *IntI* genes detection by PCR assay. M: DNA Marker, 1: *A. baumannii*, 2: *Acinetobacter*, 3: Negative.

gene and were multi-drug resistant bacteria. The *A. baumannii* drug resistance were shown in **Table 2**.

Six strains of *A. baumannii* detected two molecular weight *IntI* variable region genes, and 6 strains of *A. baumannii* detected a band with a size of approximately 2300 bp (**Figure 3**). Gene sequencing analysis revealed that the 2300 bp gene cassette was aacA4-catB8-aadA1-qacEdelta1.

3.3. The *IntI* Gene Expression Exposed to Ceftazidime and Imipenem

For the 6 strains of *A. baumannii*, both ceftazidime and imipenem can increase the expression of *IntI* gene. When exposed to the concentration of 0.01 μg/mL and 0.1 μg/mL ceftazidime, the mRNA expression of *IntI* had no significant increase compared to the negative control ($P > 0.05$); when exposed to concentration to 1 μg/mL and 5 μg/mL, the mRNA expression of *IntI* had significantly

Table 2. The drug resistance of *A. baumannii* isolated from the throat swabs.

Antibiotics	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6
Ceftriaxone	+	+	+	+	+	+
Ceftazidime	+	+	+	+	+	+
Cefepime	+	+	+	+	+	+
Ampicillin/Sulbactam	+	+	+	+	+	+
Ciprofloxacin	+	+	+	+	+	+
Levofloxacin	+	+	+	−	+	−
Imipenem	+	+	+	+	+	+
Piperacillin/Tazobactam	+	+	+	+	+	+
Gentamicin	+	+	+	+	+	+
Tobramycin	+	+	+	+	+	+
Sulphamethoxazole	+	+	+	+	+	+
Amikacin	+	+	+	+	+	+
Cefoperazone/Sulbactam	+	+	+	+	+	+
Tigecycline	+	−	+	−	−	−

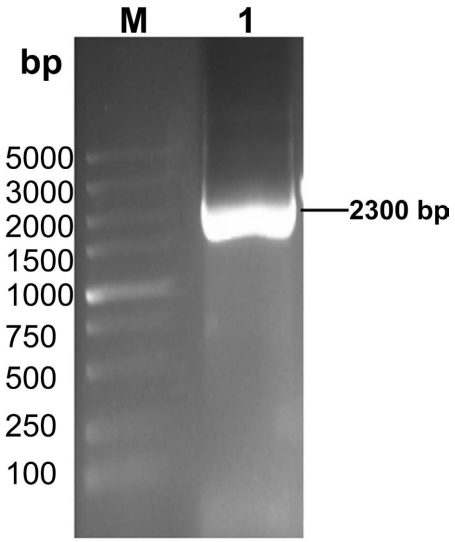

Figure 3. The amplification of *IntI* variable region gene by PCR assay. M: DNA Marker, 1: *IntI* variable region gene.

increase compared to the negative control ($P < 0.05$); when exposed to concentration to 10 μg/mL, the mRNA expression of *IntI* had significantly increase compared to the negative control ($P < 0.05$) (**Figure 4**).

When exposed to the concentration of 0.01 μg/mL imipenem, the mRNA expression of *IntI* had no significant increase compared to the negative control ($P > 0.05$); when exposed to the concentration of 0.1 μg/mL, 1 μg/mL and 5 μg/mL imipenem, the mRNA expression of *IntI* had significantly increase compared to the negative control ($P < 0.05$); when exposed to the concentration of

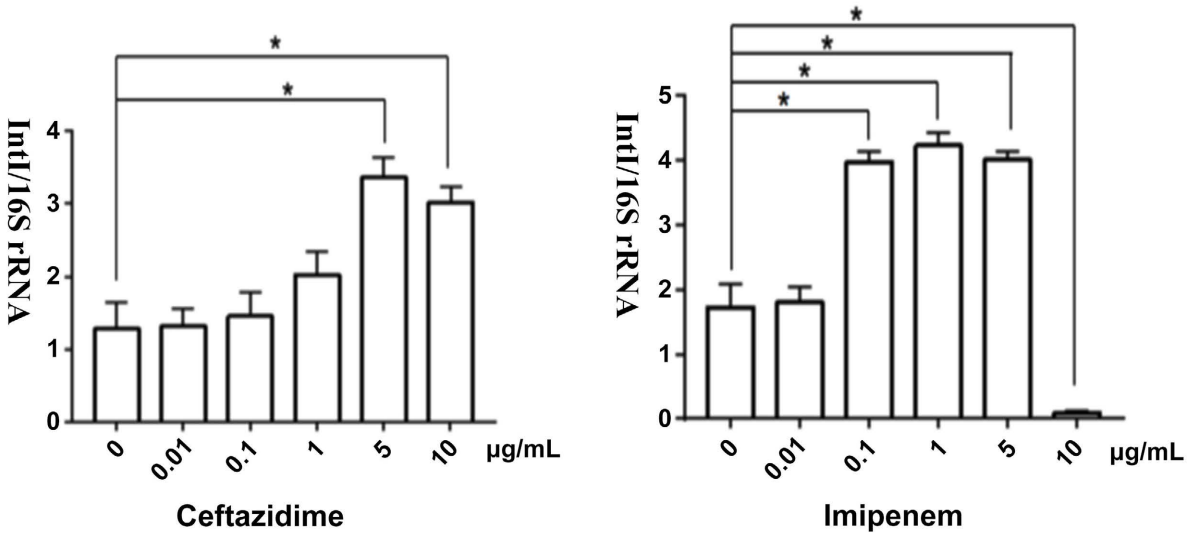

Figure 4. The *IntI* gene expression exposed to ceftazidime and imipenem. *: compared with normal control group (the concentration was 0), $P < 0.05$.

10 μg/mL imipenem, the mRNA expression of *IntI* had significantly decrease compared to the negative control ($P < 0.05$) (**Figure 4**).

4. Discussion

A. baumannii is the main pathogen causing nosocomial infections. This bacterium is a gram-negative bacterium. It is a strictly aerobic, non-lactose-fermented pathogenic bacterium. It has no flagella and no power, but it has strong vitality and exists widely in nature. *A. baumannii* mainly causes infections in ICU, respiratory medicine, brain surgery and other patients, and induces bacteremia, pneumonia, meningitis, peritonitis, endocarditis, etc. [6]. Our research found that the detection rate of *A. baumannii* gene in the pharynx in the long-term hospitalized patients (>14 days) in the department of respiratory medicine in our hospital is extremely high, reaching 93.75% (60/64). In addition, the detection rates of *A. baumannii* gene in the pharynx of patients between with and without infection symptoms were similar. We speculated that long-term hospitalized patients were likely to be cross-infected by other patients and caused *A. baumannii* genes to be detected in the pharynx, and the no infected symptoms patients with the colonized *A. baumannii* in the pharynx needs the attention of clinicians [7]. When the patient's immunity is reduced, the colonized *A. baumannii* may also cause infection [8].

In recent years, the drug resistance of *A. baumannii* has increased rapidly, making its treatment extremely difficult [9]. There are many factors for *A. baumannii* to acquire multi-drug resistance, but the mobile element plays an important role in the process of bacteria acquiring exogenous drug resistance genes [10]. Integrons are important movable elements of gram-negative bacteria [11]. Clinical pathogenic bacteria mainly carried type I integrons [12]. The type I integrons had strong ability to capture the antibiotic resistance genes and partici-

pated in recruiting and expressing a large number of drug resistance genes to make the bacterial obtaining drug resistance [13]. At the same time, integrons could also help drug-resistant genes to spread horizontally between different species of bacteria, which is extremely unfavorable for controlling the outbreak of multi-drug resistant bacteria in the hospitals [14].

As early as 1989, Strokes and his colleagues proposed the concept of integron. Integron is a mobile genetic element that can effectively capture and express foreign genes. The traditional structure consists of three parts: 5'-conserved segment (5'-CS), 3'-conserved segment (3'-CS) and the variable region. All integrons have three core structures [15]. The first core structure is integrase (Int I), which belongs to the tyrosine recombinase family and is responsible for catalyzing the capture and rearrangement of foreign genes. The second core structure is the recombination site attI of the integron which could bind to the attC site of the exogenous gene cassette [16]. The third core structure is the Pc promoter. Once the exogenous gene after being integrated, the Pc promoter begins to mediate its expression. According to different integrase gene sequences, it can be divided into 6 categories. Among them, 4 of I, II, and III are the most studied, and integrons of type I are the earliest discovered and most widely distributed integrons, and they are also among *A. baumannii* [17]. The most reported type of integron, and some areas have reported the detection of type II integrons in *A. baumannii*, but type II integrons could not exist alone, and must exist in a way that cooperates with type I integrons [18]. In this study, we found that the 6 strains of *A. baumannii* all carried the gene cassette in the form of aacA4-catB8-aadA1-qacEdelta1. Studies by other researchers had found that the multi-drug-resistant *A. baumannii* isolated in recent years generally carrying the drug resistance gene cassette of aacA4-catB8-aadA1 [19]. Our results indicated that the structure of gene cassette in *A. baumannii* isolated from the respiratory department of our hospital were consistent within other regions of our country.

Integrase is a key structure for integrons to capture and integrate exogenous drug resistance genes. Up-regulation of bacterial integrase expression will enhance its ability to capture exogenous drug resistance genes and directly lead to the rapid enhancement of bacterial drug resistance [20]. Research by Hocquet and his colleagues found that the bacterial SOS response occurred and the expression of its integrase gene was also up-regulated [21]. SOS response is a stress response made when bacterial chromosomal DNA is severely damaged. SOS response was activated when bacterial DNA molecules were severely damaged and normal replication and repair systems could not complete DNA replication [22]. In this case, a variety of bacterial genes/proteins were induced to express, such as lexA and RecA. The bacterial lexA and RecA have two-way regulation functions [23]. Under normal circumstances, lexA binds to the lexA site of the integrase promoter (Pint) region, thereby inhibiting the SOS response; when the bacterial chromosome is severely damaged, a large amount of single-stranded DNA (ssDNA) is produced, and the ssDNA-RecA complex is formed [24]. The ssDNA-

RecA complex will cause the degradation of lexA protein, which will initiate the bacterial SOS response, and during this process, the expression of bacterial integrase genes will also be upregulated. It was reported that a variety of antibiotics, such as β-lactams, aminoglycosides and quinolones, might cause serious damage to bacterial chromosomes during the treatment process and then directly or indirectly initiate the SOS response. Guerin E *et al.* reported that ampicillin, ciprofloxacin, mitomycin C, and trimethoprim could up-regulate the expression of integrase genes in *Escherichia coli* and *Vibrio cholerae* [25]. Chen and his colleagues reported that azithromycin could up-regulate the expression of integrase genes of *Pseudomonas aeruginosa* [26].

5. Conclusion

The above results indicated that the use of antibiotics might also contribute to the up-regulation of the integrase genes expression and the enhancement of bacterial resistance, which requires more attention in clinical work. In clinical treatment, choosing effective antibiotics and using them in sufficient amounts to avoid the inability to completely kill bacteria and cause the up-regulation of integrase expression to induce bacterial resistance. However, the specific implementation plan in the course of clinical antibiotic treatment still needs further study.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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