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ORIGINAL ARTICLE

Polymerase chain reaction assay for the detection of *Acinetobacter baumannii* in endotracheal aspirates from patients in the intensive care unit

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Background: We aim to evaluate the efficacy of polymerase chain reaction (PCR) to detect *Acinetobacter baumannii* in endotracheal aspirates.
Methods: Endotracheal aspirates and clinical data were collected from patients who were admitted to the intensive care unit of Taipei Veterans General Hospital between April 1 and August 31 in 2006. Bacterial isolates from endotracheal aspirate cultures were phenotypically identified as *Acinetobacter calcoaceticus—A baumannii* complex using the API ID 32GN system. The presence of *A baumannii* in the aspirate was also directly detected by multiplex PCR.
Results: Ten of the 114 endotracheal aspirate cultures were positive for *A calcoaceticus—A baumannii* complex, and only nine of the isolates were confirmed as *A baumannii* by the multiplex PCR. Direct PCR detection showed that 40 (35.1%) of the endotracheal aspirates were positive for *A baumannii*. Using positive culture of *A baumannii* as the gold standard, the sensitivity of direct PCR detection was 100% (6 of 6), the specificity was 70.4% (38 of 54), the positive predictive value was 27.3% (6 of 22), and the negative predictive value (NPV) was 100% (38 of 38) among patients with *A baumannii* pneumonia. Among patients with *A baumannii* colonization, the sensitivity of direct PCR detection was 100% (3 of 3), the

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specificity was 70.6% (36 of 51), the positive predictive value was 16.7% (3 of 18), and the NPV was 100% (36 of 36).
Conclusion: Direct PCR detection of *A baumannii* in endotracheal aspirates has a high sensitivity and NPV as compared with culture-based methods. Further studies are needed to determine the clinical applicability of this rapid detection test.

Introduction

In the last decade, *Acinetobacter baumannii* has become an increasingly important cause of nosocomial infection, particularly ventilator-associated pneumonia, in intensive care units (ICUs).[1] The risk factors for ventilator-associated pneumonia because of *A baumannii* include a longer hospital stay, longer time on ventilation, prior episodes of sepsis, reintubation, and prior antibiotic use.[1] The diagnosis of *A baumannii* pneumonia is usually based on the symptoms of respiratory tract infection in addition to the analysis of radiographic and microbiologic data.[2]

The culture of specimens from the lower respiratory tract has been the most important diagnostic method for respiratory tract infections.[3] However, it takes time for the culture results to become available. The bacterial growth may also be inhibited because of the prior administration of empirical antibiotics, thus yielding false-negative culture results. Early detection of the causative pathogen can help the physician to prescribe appropriate antimicrobial agents, which may lead to a better prognosis of the patients. The polymerase chain reaction (PCR) has been adopted to detect the pathogens of respiratory tract infections over the last few years.[4] PCR on a single throat swab specimen may greatly simplify the diagnosis of lower respiratory tract infections caused by *Legionella* spp., *Mycoplasma pneumoniae*, or *Chlamydia pneumoniae*,[5] and it also plays an important role in the diagnosis of *Mycobacterium tuberculosis*.[6] To our knowledge, there is no current report detailing the efficacy of PCR in the direct detection of *A baumannii* from respiratory tract samples. The aim of this study was to evaluate the efficacy of using PCR to detect *A baumannii* from endotracheal aspirates.

Materials and methods

Specimen collection and study design

Endotracheal aspirates were prospectively and consecutively collected from patients admitted to the medical ICU of the Taipei Veterans General Hospital, who received endotracheal intubation and mechanical ventilation from April 1 to August 31, 2006. Each specimen was collected from the endotracheal tube using a sterile suction tube. The clinical data were prospectively collected and insured by the institutional review board. This was an observational study, and the management of the patients (including antimicrobial therapy) was not influenced by the results of the analysis.

Phenotypic and genotypic identification of *Acinetobacter* isolates

All the aspirated specimens were sent for bacterial culture. Isolates recovered from culture were subjected to phenotypic identification using the API ID 32GN system (bioMerieux, Marcy l'Etoile, France). Those specimens identified as *Acinetobacter calcoaceticus—A baumannii* complex were further identified to the genomic species level as *A baumannii* using a multiplex PCR method.[7] This PCR method included two pairs of primers. The first pair of primers, P-Ab-ITSF (5′-CATTATCACGGTAATTAGTG-3′) and P-Ab-ITSB (5′-AGAGCACTGTGCACTTAAG-3′), specifically amplified an internal 208-bp fragment of the 16S-23S ribosomal DNA intergenic spacer region of *A baumannii*. The second pair of primers, P-rA1 (5′-CCTGAATCTTCTGGTAA AAC-3′) and P-rA2 (5′-GTTTCTGGGCTGCCAAACATTAC-3′), which targeted a highly conserved region of the *recA* gene (about 425 bp) of *Acinetobacter* spp., was included as a reaction control. The reaction produced two bands if the bacteria were *A baumannii*, whereas only one band corresponding to the *recA* gene was seen if the bacteria were other *Acinetobacter* spp. Isolates identified as *Acinetobacter* spp. other than *A baumannii* were further identified to the genomic species level using 16S-23S ribosomal DNA intergenic spacer sequence analysis as described previously.[7]

For direct identification of *A baumannii* from the endotracheal aspirates, the samples were first submitted to a process of digestion and decontamination with 4% NaOH.[8] The specimens were then subjected to gene extraction using a PureGene DNA Purification kit (Gentra Systems, Minneapolis, MN, USA). The purified DNA was then used as a template in the multiplex PCR for identification of *A baumannii* using the same aforementioned primers and protocol. The results were available within 4 hours.

Definition of pneumonia

Pneumonia was defined as follows: (1) patients had one or more serial chest X-rays with one of the following: new infiltrate, progressive infiltrate, or consolidation; (2) patients had at least one of the following: fever ($>38^{\circ}C$) without other causes, leukopenia ($<4{,}000$ WBC/μL) or leukocytosis ($\geq 12{,}000$ WBC/μL), or altered mental status without other causes (in patients ≥ 70 years old); and (3) patients had at least two of the following: new onset of purulent sputum (or change in character of sputum, increasing respiratory secretions, or increasing suctioning requirements); new onset of worsening cough (or dyspnea,

tachypnea, rales, or bronchial breath sounds); or worsening gas exchange.[9] *Acinetobacter baumannii* pneumonia was defined as evidence of pneumonia in addition to culture from the endotracheal aspirate yielding only *A baumannii*. The colonization of *A baumannii* was defined as having a positive *A baumannii* culture from endotracheal aspirate without evidence of pneumonia. The Acute Physiology and Chronic Health Evaluation II score was used as previously defined.[10]

Statistical analysis

Analyses were performed using the SPSS statistical software (version 15.0; SPSS, Chicago, IL, USA). Qualitative variables were compared using the Pearson Chi-square test or Fisher's exact test. A p value less than 0.05 was considered statistically significant.

Results

Patient characteristics

A total of 114 patients were enrolled in this study, and 81 of them were men. Their age ranged from 17 to 97 years, with an average age of 70.7 years. The underlying diseases of the patients are listed in Table 1. Most of the patients had at least one underlying disease and were critically ill, with a mean Acute Physiology and Chronic Health Evaluation II score of 20.86 ± 7.02. The API ID 32GN system revealed that 10 of the 114 cultures were positive for *A calcoaceticus—A baumannii* complex. However, one of the isolates was identified by the genotypic method as *Acinetobacter* genomic species 13TU. Therefore, only nine patients had positive culture results for *A baumannii*. Direct PCR detection of *A baumannii* showed that 40 (35.1%) of the 114 patients were positive, whereas 74 (64.9%) demonstrated negative PCR results in their endotracheal aspirates. The comparison of the underlying diseases between the patients with positive and negative PCR results showed that only hematological malignancy was significantly more prevalent among the patients positive for *A baumannii* (Table 2).

Table 1 The underlying diseases of the patients ($n = 114$)

Underlying diseases	*n* (%)
Hypertension	49 (43)
Operation within 1 mo	41 (36.0)
Solid tumor	37 (32.5)
Type 2 diabetes mellitus	35 (30.7)
Shock	20 (17.5)
Cerebral vascular disease	18 (15.8)
Coronary artery disease	13 (11.4)
Chronic renal failure	13 (11.4)
Chronic obstructive pulmonary disease	12 (10.5)
Liver cirrhosis	8 (7.0)
Hematological malignancy	6 (5.3)
Collagen vascular disease	4 (3.5)

The efficacy of direct PCR detection in patients with *Acinetobacter baumannii* pneumonia

Among the patients included in our study, 60 had pneumonia. Among them, six (10%) had a diagnosis of *A baumannii* (initially identified by API ID 32GN, then confirmed by PCR) pneumonia, whereas 22 (36.7%) had positive results by means of direct PCR detection. Using those samples with a positive culture for *A baumannii* as a standard, the sensitivity of direct PCR detection was 100% (6 of 6), the specificity was 70.4% (38 of 54), the positive predictive value was 27.3% (6 of 22), and the negative predictive value was 100% (38 of 38), as shown in Table 3. There was no significant difference regarding the underlying diseases between patients with positive and negative PCR results. There was also no significant difference regarding the in-hospital mortality rate, 14-day mortality (since ICU admission), or the length of hospital stay (Table 4).

The efficacy of direct PCR detection in patients with *Acinetobacter baumannii* colonization

Among the 114 patients, 54 patients revealed no evidence of pneumonia. Among these 54 patients, 18 had positive PCR results and 36 had negative results for *A baumannii*. Using positive culture of *A baumannii* as a standard, the sensitivity of direct PCR detection was 100% (3 of 3), the specificity was 70.6% (36 of 51), the positive predictive value was 16.7% (3 of 18), and the negative predictive value was 100% (36 of 36). There was also no significant difference regarding the underlying diseases and outcomes between the patients with positive and negative PCR results in this group (Table 4).

Discussion

The intravascular space and respiratory tract are the two most common sources of *A baumannii* bacteremia, and both are associated with a high mortality rate.[11,12] The early diagnosis of *A baumannii* pneumonia with prompt application of appropriate therapy may prevent systemic involvement. The time required for the identification of *A baumannii* based on culture methods is at least 3 days. PCR is a method that combines rapid nucleic acid extraction, amplification, and detection.[13] The time for microbiological identification using multiplex PCR can be as short as 3—4 hours, which greatly improves the turnaround time.[14] Although PCR has been applied for the diagnosis of a diverse range of respiratory pathogens,[15] our study is the first prospective observational study to evaluate the clinical utility of PCR on specimens from the respiratory tract for the detection of *A baumannii*.

Among patients with pneumonia, our study demonstrated the high sensitivity and negative predictive value of direct PCR detection for determination of *A baumannii*. However, the application of PCR was limited by the low positive predictive value, which might be because of the following possibilities. First, negative culture results could have been obscured by prior antimicrobial therapy, which was applied when pneumonia was first suspected. As such, the dead bacterial remnants would fail to grow on culture

Table 2 Comparison of underlying diseases between patients with positive and negative results by means of direct PCR detection of *Acinetobacter baumannii* in endotracheal aspirates

Underlying diseases	PCR (−) ($n = 74$)	PCR (+) ($n = 40$)	p
Hypertension	32 (43.2)	17 (42.5)	1.000
Type 2 diabetes mellitus	22 (29.7)	13 (32.5)	0.926
Liver cirrhosis	7 (9.5)	1 (2.5)	0.257
Operation within 1 mo	28 (37.8)	13 (32.5)	0.717
Shock	13 (17.6)	7 (17.5)	1.000
Solid tumor	27 (36.5)	10 (26.0)	0.298
Hematological malignancy	1 (1.4)	5 (12.5)	0.02
Cerebral vascular disease	12 (16.2)	6 (15.0)	1.000
Coronary artery disease	6 (8.1)	7 (17.5)	0.215
Chronic obstructive pulmonary disease	6 (8.1)	6 (15.0)	0.339
Chronic renal failure	10 (13.5)	3 (7.5)	0.538
Collagen vascular disease	2 (2.7)	2 (5.0)	0.611

Data are presented as *n*(%).
PCR = polymerase chain reaction.

plates but could be detected by PCR. Second, the more sensitive nature of PCR detection might lead to the detection of a small number of *A baumannii*, which might not be detected in culture or be outgrown by the other bacteria present in the aspirate sample. Third, the PCR results might be attributed to a false positive. However, the inclusion of a negative PCR control minimized this possibility. Fourth, the more sensitive PCR method might detect a small number of *A baumannii*, that is, however, not the responsible agent for the pneumonia.

Despite the rapid detection of *A baumannii* by PCR, there was no difference regarding the outcome between the patients with positive and negative PCR results. A possible reason for this observation was that patients from both groups had comparable underlying diseases and were both critically ill. Moreover, this was an observational study, and no change in intervention occurred after a positive result. Because most of the *A baumannii* isolates were multidrug resistant, it is essential to determine whether a prompt change in antibiotic treatment after a positive PCR result would improve a patient's outcome. The high negative predictive value of PCR in the diagnosis of *A baumannii* pneumonia might help to limit the prescription of unnecessary broad-spectrum antibiotics for those patients with negative PCR results. On the other hand, before a more comprehensive prospective study is conducted to elucidate the clinical significance of positive PCR results in pneumonic patients who have negative culture results, there should be an awareness of the overestimation of the significance of the PCR detection to avoid overuse of broad-spectrum antibiotics.

The PCR detection of *A baumannii* in patients without evidence of pneumonia was most likely because of *A baumannii* colonization. *Acinetobacter baumannii* can be part of the bacterial flora of the human body,[16] and the respiratory tract is an important site of colonization.[17] *Acinetobacter baumannii* colonization has been reported in the nasopharynx and other sites of the respiratory tract.[18] In the evaluation of *A baumannii* colonization, direct PCR detection also demonstrated a high sensitivity and high negative predictive value. In this study, because of the small number of patients, we did not determine whether PCR detection of *A baumannii* colonization could predict

Table 3 The comparison of direct PCR detection and phenotypic identification in addition to PCR verification of *A baumannii* among patients with *A baumannii* pneumonia or colonization

	Direct PCR detection, *n*	
	(−)	(+)
A baumannii pneumonia		
Culture (+)[a]	0	6
Culture (−)[a]	38	16
Total	38	22
A baumannii colonization		
Culture (+)[a]	0	3
Culture (−)[a]	36	15
Total	36	18

[a] The genomic species *A baumannii* identified by the culture method was initially identified using the API ID 32GN system and then further verified by PCR.
A baumannii = *Acinetobacter baumannii*; PCR = polymerase chain reaction.

Table 4 The comparison of outcomes between patients with positive and negative PCR results

	Outcome		p
	PCR (−)	PCR (+)	
Pneumonia group			
14-d mortality	8/38	1/22	0.135
In-hospital mortality	17/38	9/22	0.986
Length of hospital stay (d)	46.42 ± 40	51.64 ± 31	0.582
Colonization group			
14-d mortality	5/36	5/18	0.273
In-hospital mortality	14/36	11/18	0.210
Length of hospital stay (d)	41.53 ± 42	49.44 ± 56	0.817

PCR = polymerase chain reaction.

future *A baumannii* infection. Nevertheless, the high negative predictive value of PCR detection of *A baumannii* colonization might implicate a role for this method in outbreak control in ICUs or nursing institutions. The rapid nature and high negative predictive value of PCR detection might be used to identify individuals who need to be isolated during an *A baumannii* outbreak.

Despite the promising preliminary results, there are limitations to our study. Gram stain was not used to check the quality of the aspirates, and it might be difficult to distinguish real infections from colonization, even when positive culture results and a pulmonary infiltrate were obtained. Gram stain is a dependent diagnostic test for the early etiological diagnosis of bacterial pneumonia.[19] To yield a reliable microbial etiology during the early diagnosis of *A baumannii* pneumonia that will prevent overuse of antibiotics, a prospective study that uses PCR combined with Gram stain verification or even invasive diagnostic methods[20] might be warranted.

In conclusion, our study demonstrated that direct PCR detection for the early identification of *A baumannii* from endotracheal aspirates was fast and had a high sensitivity and negative predictive value. Further study is required to explore the potential role of this approach in the diagnosis of *A baumannii* pneumonia and outbreak control.

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