Molecular Characteristics of a Series of Clinical Isolates of Drug-Resistant Acinetobacter baumannii ST219 Strain: The Implications of a Sequence Analysis of the blaOXA-51-like

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Abstract

The drug-resistant Acinetobacter baumannii ST219 strain was sporadically isolated in the emergency intensive care unit of Tokai University Hospital in 2014 after an outbreak in 2013. The isolates were identical in their antimicrobial susceptibility pattern, the fingerprint pattern of rep-PCR, and the molecular properties including mutations in drug-resistant genes and decreased expression of the efflux pump and the outer membrane porin genes, but they were found to possess different sequence types based on the findings of multilocus sequence typing: ST208 and 219. The analysis of the blaOXA-51-like sequences showed that all were blaOXA-66. Given these findings, all of the isolates were considered to be subclones derived from the same strain. A sequence analysis of the blaOXA-51-like of A. baumannii would therefore be useful for investigating the relationship of nosocomial infections.

Introduction

Acinetobacter baumannii is a strictly aerobic, non-fermenting, non-fastidious, non-motile, catalase-positive, oxidase-negative, gram-negative bacteria that is known to be a major pathogen of nosocomial infection in immunocompromised patients [1]. Outbreaks of antimicrobial-resistant A. baumannii have become a major clinical problem [1-3]. All strains of A. baumannii possessed a chromosomally encoded blaOXA-51-like, some of which provided resistance to carbapenems when the molecular milieu around the gene promoted its expression, such as ISAba1 [4]. The blaOXA-51-like has been reported to have sequence variations with over 40 variants [5,6].

We previously reported an outbreak of an amikacin- and ciprofloxacin-resistant A. baumannii sequence type (ST)219 strain that affected 15 patients in the emergency intensive care unit of Tokai University Hospital from September to October in 2013 [3]. Intensive control measures were implemented, including the replacement of the water supply system, which was considered to be a bacterial reservoir, and thus could successfully control the outbreak. However, sporadic cases of A. baumannii, with an identical pattern of antimicrobial susceptibility, were subsequently detected in 2014. The present study was undertaken to elucidate the molecular characteristics for antimicrobial resistance and typing for epidemiology in drug-resistant (DR) A. baumannii in 2014.

Materials and Methods

After an outbreak of DR-A. baumannii that affected 15 patients from September to October 2013 [3], DR-A. baumannii was sporadically detected in sputum specimens from six patients from January to October 2014 (TS-A. baumannii-2014-1 to -6). These patients were treated in the emergency intensive care unit (57 beds, including 3 beds in the severe burn care unit) of Tokai University Hospital (total 804 beds) for serious burns, traffic injuries, or cerebral hemorrhaging. Routine microbial examinations were performed on a weekly basis in clinical specimens from the patients’ sputum, urine (via catheter), venous blood and wounds, among other bodily fluids. One drug-susceptible A. baumannii (TS-A. baumannii-2014-7) was used as a control for quantitative RT-PCR (qRT-PCR).

Antimicrobial susceptibility testing, and screening of carbapenemase, MBL, ESBL and AmpC

Bacterial identification and antimicrobial sensitivity tests were performed using the MiroScan WalkAway 96 Plus kit (Beckman Coulter, Inc., CA, USA) in accordance with the CLSI 2010 guidelines [7] for Imipenem (IPM), Meropenem (MEMP), Piperacillin (PPIC), Ceftazidine (CAZ), Cefcapene (CFFP), Sulbactam/Carbapenem (S/C), Aztreonam (AZT), Cefozopran (CZOP), Gentamicin (GM), Tobramycin (TOB), Amikacin (AMK), Levofloxacin (LVFX), Ciprofloxacin (CFFP), Minocycline (MINO), Fosfomycin (FOM) and Sul-famethoxazole-Trimethoprim (ST). The criteria for multiple drug-resistant A. baumannii was resistance to IPM (MIC > 16 µg/mL), AMK

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(MIC > 32 μg/mL) and CPFX (MIC > 4 μg/mL), and DR-A. baumannii was defined as resistance to at least 2 of the drugs, according to the Japanese National Guideline concerning the prevention of infections and medical care for patients with infections.

A. baumannii isolates were screened for the production of metallo-β-lactamase (MBL) by Double-Disk Synergy Tests (DDST) (Eiken Chemical Co., Ltd. Tokyo, Japan) using extended-spectrum β-lactamase (ESBL), in accordance with the CLSI 2010. The isolates were screened for the production of AmpC using 3-aminophenylboronic acid monohydrate (Kanto Chemical Co., Inc., Tokyo, Japan).

Molecular typing

The first detected isolate of A. baumannii in each patient was used for the following molecular characterizations. DNA was extracted using the QIAamp DNA Mini Kit (QIAGEN GmbH, Hilden, Germany). A. baumannii isolates were screened for gene homology by the repetitive-sequence-based-polymerase chain reaction (rep-PCR), as described previously [8]. The BOX-PCR primer was used (5'-CTA CGG CAA GGC GAC GCT GAC G-3'). The temperature profiles were as follows: initial denaturation at 95°C for 3 min, 30 cycles of denaturation at 90°C for 30 s, annealing at 32°C for 1 min, extension at 65°C for 8 min, and a final extension at 65°C for 16 min.

Multilocus sequence typing (MLST) was performed as described previously [9]. The MLST sequences were uploaded into the A. baumannii MLST sequence type database (http://pubmlst.org/abumannii/) to determine the alleles and sequence types (ST). Clonal complexes (CCs) were assigned using the eBURST V3 software program (http://eburst.mlst.net/v3/) and defined as single locus variants (SLVs) and double locus variants (DLVs). The annealing temperature of the PCR amplification used in this study was 55°C for gltA, gyrB, recA, and cpm60, and 50°C for gdhB, gpi, and rpoD. The amplification products were purified with a QIAGEN DNA purification kit (QIAGEN GmbH). The DNA sequencing was performed using an ABI3500XL Genetic Analyzer (Applied Biosystems, Life Technologies Japan Ltd., Tokyo, Japan).

PCR assay for β-lactamase and armA

The resistance genes were examined via a multiplex PCR for blaOXA-32-positive, blaOXA-24-positive, blaoxa-11-like, blaoxa-19-like, and ISAbA1 genes, as described previously [2,10-12]. The amplification of the blaoxa-24-like genes was performed as described previously [6,11]. The armA gene, which encodes 16S rRNA methylases and confers high resistance to aminoglycosides (AGs), was screened by PCR using primers as described previously [13].

Sequencing of OXA-type β-lactamase, and gyrA, and parC

Sequencing of OXA-type β-lactamase was performed as described previously [14]. The quinolone-resistance-determining regions (QRDRs) of gyrA and parC were amplified and analyzed as described previously [14,15]. The DNA sequencing of the amplified DNA products was performed using an ABI3500XL Genetic Analyzer (Applied Biosystems).

Quantitative RT-PCR (qRT-PCR)

The RNA templates were extracted using the RNeasy™ Mini Kit (QIAGEN GmbH). The expression of three different resistance-modulation-division (RND) family pump-encoding genes (adeB, adeG, adeF) and two outer membrane porin-encoding genes (oprD and carO) were analyzed by quantitative RT-PCR using the StepOnePlus™ Real-Time PCR System (Applied Biosystems) [2,16-18]. The three previously characterized genes adeB, adeG, and adeF encoded the RND pumps in the adeABC, adeFG, and adeIFK operons, respectively. The housekeeping gene 16S rRNA was used as a control [19-21]. Reactions (20 μL) were set up using 400 nM primers and 2 μL of the CDNA template (diluted 1:10) with SYBR® Premix Ex Taq™ II (Tli RNase H Plus) and ROX plus (Takara Bio Inc., Shiga, Japan).

The data were analyzed using the StepOne™ software program.

Discussion

Clinical isolates of an outbreak in 2013 and subsequent sporadic detection in 2014 of DR-A. baumannii in the emergency intensive care unit of Tokai University Hospital showed resistance to a broad spectrum of antimicrobials except for carbapenems, and were found to have blaoxa-11-like carrying blaoxa-66 with ISAbA1 alterations

Table 1: The antimicrobial susceptibility pattern of the clinical isolates of Acinetobacter baumannii.

<table>
<thead>
<tr>
<th>Strain</th>
<th>β-Lactams</th>
<th>MIC (μg/mL)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>AGs</td>
</tr>
<tr>
<td></td>
<td>IPM</td>
<td>MEPM</td>
</tr>
<tr>
<td>TS-A. baumannii-2014-1</td>
<td>s1(S)</td>
<td>s1(S)</td>
</tr>
<tr>
<td>TS-A. baumannii-2014-7</td>
<td>s1(S)</td>
<td>s1(S)</td>
</tr>
</tbody>
</table>

Table 2: The characteristics of antimicrobial resistance in the clinical isolates of Acinetobacter baumannii.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Efflux pump expression</th>
<th>Outer membrane porin expression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>abeB</td>
<td>oprD</td>
</tr>
<tr>
<td>TS-A. baumannii-2014-1</td>
<td>0.34</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>abeG</td>
<td></td>
</tr>
<tr>
<td>TS-A. baumannii-2014-2</td>
<td>0.17</td>
<td></td>
</tr>
<tr>
<td></td>
<td>abeU</td>
<td></td>
</tr>
<tr>
<td>TS-A. baumannii-2014-7</td>
<td>0.69</td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Relative expression of efflux pumps and outer membrane porin in clinical isolates Acinetobacter baumannii by quantitative RT-PCR.

Figure 1: The results of the rep-PCR in clinical isolates of A. baumannii.

Lanes 1 was negative control of the PCR. Lanes 2 to 7 indicate drug resistant-A. baumannii (TS-A. baumannii-2014-1 to -6), and lane 8 is drug susceptible-A. baumannii (TS-A. baumannii-2014-7), respectively. Lane M is a marker. TS- A. baumannii-2014-1 to -6 showed an identical pattern with a homologous rate of identity > 97%. TS-A. baumannii-2014-7 had different fingerprint pattern (less than 70% similarity, respectively).

of genes responsible for AGs and FQs, and decreased expression of the efflux pump and outer membrane porin-encoding genes. These findings suggested that these isolates of A. baumannii shared a molecular basis for the same susceptibility pattern to antimicrobials. These findings are compatible with collateral susceptibility to carbapenem and consistent with a previous report indicating that the combination of OXA-type β lactamases with ISAb1 and deficiency of outer membrane porins deficiency alone does not confer carbapenem resistance, and that overexpression of the efflux pumps may be necessary [2,12,22].

Isolates of TS-A. baumannii-2014-1 to -6 showed identical molecular characteristics, such as the fingerprint pattern, OXA type, drug resistant genes and expression of efflux pumps. A. baumannii can survive for long-term periods of time in the hospital environment, causing sporadic and endemic infection [2,3,23]. We experienced an outbreak twice in the past in the emergency intensive care unit and burn unit of our University Hospital. DR-A. baumannii ST208 was involved in an outbreak in 2011, where the air fluidity bed was identified as a reservoir. An outbreak of DR-A. baumannii in 2013 was detected from the water systems including hands-free automatic tap and water...
mixture side of the joint tube. Effective measures to minimize the risk in the wet environmental reservoir included strict sanitary management of the water systems in order to prevent future outbreaks. The MLST sequences between ST208 and ST219 differed by only a single gpi base, and these isolates closely resembled one another in the molecular characteristics for resistance against each drug. This finding suggested that the ST208 and ST219 isolates were closely related in terms of genetics and might have been derived as subclones from the same origin.

In the detection of subsequent isolates, a reservoir was not identified despite environmental sampling for bacterial culture at several times. A more intensive environmental surveillance will be needed to identify the reservoir should DR-A. baumannii-2014 continue to be detected. However, the reinforcement of environmental disinfection, including clinical surfaces, and ensuring hand hygiene with alcohol containing antiseptic will be crucial for reducing the risk of cross-transmission in healthcare facilities [24].

In conclusion, a detailed molecular analysis of DR-A. baumannii would provide important knowledge for controlling nosocomial infections. Even when a different ST strain is detected, the sequencing of the blaOXA-51-like would be useful for determining the clonality, which would thus make it possible to identify the relationship of A. baumannii infection.

Acknowledgments
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References