Brief Original Article

Bacteremia caused by an *Acinetobacter junii* strain harboring class 1 integron and diverse DNA mobile elements

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Abstract

Introduction: Infections caused by *Acinetobacter junii* are rarely reported. However, some outbreaks of septicemia in neonates and pediatric oncology patients, as well as meningitis, peritonitis, and ocular infection have been described. Since it is highly infrequent to find the molecular characterization of *A. junii* strains in literature, in this study we described the molecular characterization of *A. junii* isolates recovered from blood samples of a renal transplant patient.

Methodology: The case was defined as a catheter-related bacteremia caused by *A. junii*. The patient responded favorably after catheter removal and treatment with ciprofloxacin.

Results and Conclusion: The complete molecular characterization of the isolate showed that it harbored a class 1 integron and diverse DNA mobile elements. This explains its genomic plasticity for acquiring antimicrobial resistance determinants and for adapting to a nosocomial niche.

Key words: *Acinetobacter junii*; integron; antibiotic resistance; insertion sequences

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Introduction

Few reports describe *Acinetobacter junii* infections that affect patients who have had prior antibiotic therapy, invasive procedures or malignancy [1]. However, some outbreaks of septicemia in neonates and pediatric oncology patients, as well as meningitis [2], peritonitis [3], and ocular infection [4] have been described [5-7].

In contrast to *A. baumannii* infections, the infections caused by *A. junii* are easy to treat because this bacterium is commonly susceptible to antimicrobial agents [5]. Still, carbapenem-resistant *A. junii* producing OXA-type carbapenemases and IMP-4 have been described [8,9]. The presence of plasmids containing antibiotic resistance genes, commonly found in *A. baumannii*, shows that horizontal genetic transfer may be possible between both species of *Acinetobacter*. By this genetic exchange the therapeutic options to treat *A. junii* infections might be limited.

Mobile elements and resistance determinants associated to antibiotic resistance have been well studied for *A. baumannii* [10-12], in contrast there are few reports of these elements in *A. junii* [13,14].

Genetically characterized *A. junii* strains is rarely described in literature. This study was undertaken to investigate whether *A. junii* can be a reservoir of mobile elements. In addition, we studied the occurrence of resistant determinants in *A. junii* which had previously been described in *A. baumannii* isolates from our lab, to investigate their possible intra-species transfer.

Methodology

Bacterial Strain

Positive growth were obtained in two blood culture sets (taken at two different moments) and in the blood culture drawn through the catheter hub (BacT/Alert; bioMérieux, Marcy l'Etoile, France) from a 41 years old female patient. She was admitted to the hospital for pathological fracture of the left tibia secondary to

brown tumor. She had a history of papillary thyroid carcinoma and renal transplant in 2006 treated with tacrolimus and corticosteroids since that date.

The isolates were analyzed using VITEK 2 Compact (bioMérieux, Marcy l'Etiole, France) system. Polymrase chain reaction (PCR) amplifications and sequence analysis of the 16S rRNA and *rpoB* genes were carried out to confirm the identification [15]. Sequencing was performed on both DNA strands using ABIPrism 3100 BioAnalyzer equipment for sequencing (Macrogen Inc., Seoul, Republic of South Korea).

Antibiotic susceptibility

The antibiotic susceptibility test was performed using the VITEK 2 System (bioMerieux, Marcy, L'Etoile, France) employing the panel AST-082 (GNS susceptibility card). The minimum inhibitory concentration (MIC) results were interpreted using the Clinical and Laboratory Standards Institute (CLSI) categories.

Molecular Techniques

Total DNA of the *A. junii* isolates was prepared and used as template for PCR reactions. PCR reactions were carried out using the GoTaq enzyme according to manufacturer's instructions (Promega, Madison, USA), and the products were detected by agarose gel electrophoresis. To reveal the presence of resistance determinants against different antibiotics families (*tet*(A), *tet*(B), *bla*$_{ADC}$, *bla*$_{OXA-23}$, *bla*$_{OXA-51}$, *bla*$_{OXA-58}$, *bla*$_{CTXM-2}$, *bla*$_{SHV}$, *bla*$_{VEB}$, *qnrB*, *qnrS*, *aadB*, *aacC2*, *aac(6')-Ib*, *aadA1*, *aphA1*, *sul1*, *sul2*, *sul3*, *strA*, *strB*, *dfr18*, *dfr9*, *dfr20*, *floR*), mobile elements (IncP, IncW, IncA/C, IncN, IncFII, pRAY, Tn*1331*, Tn*3*, IS*26*, IS*440*, IS*Aba1*, IS*Aba125*, IS*1008*, IS*CR2*, AbaR-type resistance island), and integrons, specific primers were used [17,18,19,20,21] .

All positive PCR amplification products were sequenced on both DNA strands, using an ABI Prism3100 BioAnalyzer equipment (Macrogen Inc., Seoul, Republic of South Korea) and nucleotide sequences were analyzed using the Blast v2.0 software (http://www.ncbi.nlm.nih.gov/BLAST/).

Results and Discussion

In the recovered isolates the bionumber obtained using the Vitek system was 0040000100500100, which gives an identification of *Acinetobacter junii* with 98 % probability. To confirm the identification, 16S RNA and *rpoB* gene amplifications were conducted. Sequence analysis revealed a 99% identity with the sequences corresponding to the 16S ribosomal RNA gene of *A. junii* (Accession number JX840368) and 100 % of identity with RNA polymerase subunit B (*rpoB*) gene of *A. junii* (Accession number JQ924568). Both isolates were susceptible to ampicillin/sulbactam (≤ 2 μg/ml); piperacillin-tazobactam (≤ 4 μg/ml), ceftazidime (4 μg/ml), cefepime (2 μg/ml); carbapenems (imipenem ≤ 1 μg/ml; meropenem ≤ 0.25 μg/ml), ciprofloxacin (≤ 0.25 μg/ml) rifampicin (0,19 μg/ml) and colistin (≤ 0.5 μg/ml) and resistant to gentamicin (≥ 16 μg/ml); amikacin (≥ 64 μg/ml); and TMP-SMX (≥ 4 μg/ml).

Due to the fact there is little information published on the molecular mechanisms and mobile elements associated to antimicrobial resistance in *A. junii*, we decided to study the resistance determinants and mobile elements based on their wide distribution in our country [22,19].

Among the PCR reactions carried out to detect resistance determinants, positive results were only obtained for *sul1*, *sul2*, *strA*, *strB*, *aphA1* and *aac(6')-Ib* (Table 1), which explained the resistance found in the strains to gentamicin, amikacin and TMP-SMX.

The mobile elements found in the *A. junii* isolates were the insertion sequences IS*26*, IS*Aba1*, IS*Aba125*, IS*1008* and IS*CR2*, which is in agreement with the high occurrence of insertion sequences in *Acinetobacter* spp. [12,13]. Negative results were obtained to determine the presence not only of plasmids belonging to the incompatibility plasmids tested in this study, but also of transposons mostly present in our clinical isolates.

We observed the presence of one class 1 integron, whereas we obtained negative results for class 2 integron amplification, which is the most wide-spread class of integron in our *A. baumannii* strains in our region. The amplification of the variable region (vr-1) of the integron revealed the presence of the gene cassettes *arr3-aac(6')-Ib*, genes that codify for a rifampin ADP-ribosylating transferase and an aminoglycoside-(6')-N-acetyltransferase, respectively.

The association between the IS*26* and the *aphA1* gene, encoding for APH (3')-I aminoglycoside phosphotransferase enzyme and conferring kanamycin and neomycin resistance, is frequent in *A. baumannii* strains [23,24]. Thus we decided to test the association of these two genes in our strain obtaining positive results for the amplification reactions carried out. This result showed that in *A. junii,* the *aphA1* gene can also be linked with IS*26* as it was described for *A. baumannii*.

Concerning the phenotype of these strains, the presence of the *aac(6')-Ib* gene, can explain the observed resistance to amikacin. However, no resistance to rifampicin was observed suggesting that the *arr3* gene cassettes maybe weakly expressed or do not confer resistance to our strains.

Table 1. Antibiotic resistance determinants, mobile elements and integrons studied with the corresponding results obtained in the *Acinetobacter junii* strains.

Antibiotic resistance determinant, mobile elements and integrons	Results
Resistance determinants	
bla_{OXA-23}	-
bla_{OXA-51}	-
bla_{OXA-58}	-
bla_{ADC}	-
bla_{SHV}	-
$bla_{CTX-M-2}$	-
bla_{VEB-1}	-
aac(6')-Ib	+
aphA1	+
aadB	-
aacC2	-
tet(A)	-
tet(B)	-
qnrS	-
qnrB	-
strA	+
strB	+
sul1	+
sul2	+
sul3	-
dfr9	-
dfr18	-
dfr20	-
floR	-
Plasmids	
IncP	-
IncW	-
IncFII	-
IncA/C	-
IncN	-
pRAY	-
Insertion sequence and transposons	
Tn*3*	-
Tn*1331*	-
IS*825*	-
IS*Aba1*	+
IS*Aba125*	+
IS*26*	+
IS*1008*	+
IS*440*	-
IS*CR2*	+
Integrons	
intI1	+
vr-1	*arr-3 - aac(6')-Ib*
intI2	-

The molecular characterization of the *A. junii* isolates showed the presence of different mobile genetic elements and determinants associated to horizontal gene transfer; these elements might also play an important role in the acquisition and development of antibiotic resistance in this species.

Infections caused by species of *Acinetobacter* other than *A. baumannii* have been reported in literature [25,15]. The existence of molecular techniques that allow correct species identification give an important contribution to the epidemiology of non- *baumannii Acinetobacter* and to the knowledge on the real prevalence of these species and the type of infections associated with them. In addition, information regarding the antimicrobial resistance mechanisms and mobile genetics elements contained in these species can help to establish a more accurate treatment and stop their spreading.

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