Partial characterization of a transposon containing the \( \text{tet}(A) \) determinant in a clinical isolate of \textit{Acinetobacter baumannii}

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A genomic library from one clinical isolate of \textit{Acinetobacter baumannii} was obtained to find genes responsible for tetracycline resistance. \textit{Escherichia coli} DH5\(\alpha\)-MDR transformants, selected on Mueller–Hinton agar supplemented with tetracycline 18 mg/L, were thoroughly characterized. In one clone, with an insert of 7070 bp, it was found that the resistance to tetracycline was mediated by the \( \text{tet}(A) \) gene (1200 bp) which encodes a tetracycline efflux pump. This gene was recovered together with \( \text{tetR}(A) \) (651 bp), the \( \text{tet}(A) \) repressor. Moreover, the partial sequence (2008 bp) of a transposase gene, tcpA, and 1316 bp corresponding to an IS, similar to that described in one strain of \textit{Salmonella typhi} (IS4321), were found. In this \textit{A. baumannii} clinical isolate, the \( \text{tet}(A) \) gene is located in a transposon. The structure of this transposon is similar to that of Tn1721, with the \( \text{tet}(A) \), \( \text{tetR}(A) \) and the regions between these genes, being closely related to those of Tn1721. The data indicate horizontal transfer of tetracycline resistance genes between \textit{A. baumannii} and other genera sharing the same ecological niches.

Keywords: tetracycline resistance, \textit{Acinetobacter baumannii}

Introduction

\textit{Acinetobacter baumannii} is a Gram-negative bacillus that is usually commensal, but in the past few decades has emerged as an important opportunistic pathogen, especially in the hospital setting.\(^1\) This microorganism has unique characteristics among nosocomial Gram-negative bacteria, such as resistance to desiccation, that favours its persistence in the hospital environment. This fact, together with a facility for developing resistance to most of the antibiotics currently available,\(^1\) explains in part at least the propensity of \textit{A. baumannii} to cause extended outbreaks, mainly located in intensive care units.\(^1\)

Tetracycline is one of the antibiotics to which \textit{A. baumannii} has developed resistance. This antibiotic acts by binding to the 30S ribosomal subunit, resulting in the inhibition of protein synthesis.\(^2\) Tetracycline-resistant bacteria generally express one of two different mechanisms: an efflux pump or a ribosomal protection system. Tetracycline resistance determinants \( A \) to \( E \), \( G \) and \( H \) among Enterobacteriaceae and other Gram-negative bacilli and determinants \( K \) and \( L \) among Gram-positive bacteria specify efflux pumps for tetracyclines that enable the bacteria to grow in the presence of therapeutic levels of tetracycline. Unlike Gram-positive bacteria, efflux determinants from Gram-negative bacteria have a common genetic organization, with all containing a structural gene and a repressor gene in opposing orientations and expressed from overlapping operator regions.\(^2\) The Gram-negative \( \text{tet} \) efflux genes are found on transposons inserted into a diverse group of plasmids from a variety of incompatibility groups, most of which are conjugative.\(^2\)

Guardabassi \textit{et al.}\(^3\) have reported the mechanisms of resistance to tetracycline in \textit{A. baumannii}, by finding the \( \text{Tet}(A) \) and \( \text{Tet}(B) \) determinants in clinical and aquatic strains. However, little is known about the genetic context of these determinants.

The aim of this study was to analyse the molecular mechanisms of resistance to tetracycline in a clinical isolate of \textit{A. baumannii} and estimate the prevalence of the genetic construct containing the \( \text{tet}(A) \) gene in other isolates of this microorganism.

Materials and methods

\textit{Strains, plasmids and growth conditions}

Clinical isolates of \textit{A. baumannii}, recovered from respiratory secretions, were submitted to the Clinical Laboratory of Microbiology of the Hospital Clinic of Barcelona. Isolates were identified as \textit{A. baumannii} by standard biochemical testing and by amplified ribosomal DNA restriction analysis (ARDRA).\(^1\) \textit{Escherichia coli} DH5\(\alpha\)-MDR (Gibco-BRL, Life Technologies Inc., Gaithersburg, MD, USA) was used as host strain in trans-
formation experiments. All cloning procedures were carried out with the phagemid vector pBluescript(SK(+/-)) (Stratagene Cloning Systems, La Jolla, CA, USA). When plasmids were to be maintained in E. coli strains, agar was supplemented with 18 mg/L of tetracycline and 100 mg/L of ampicillin.

DNA methodology

Basic DNA procedures, including restriction endonuclease digestions, ligations, transformations and agarose gel electrophoresis were carried out as described previously. To isolate plasmids, an alkaline lysis method was used. Genomic DNA was extracted using the Wizard Genomic DNA purification kit (Promega Corporation, Madison, WI, USA). DNA fragments obtained from the cloning procedures were purified from agarose gels using the Concert Rapid Purification System according to the manufacturer’s instructions (Gibco-BRL, Life Technologies Inc., Gaithersburg, MD, USA).

Construction of the genomic library and DNA sequencing

The genomic DNA of the clinical isolate A. baumannii (A5-22) was partially digested with Sau3AI. Fragment patterns were examined in 0.7% agarose gels and fragments of 4 to 9 kb were recovered and purified from the gel as noted. The recovered fragments were then directly cloned into the pBSK phagemid vector previously linearized with BamHI and treated with calf intestinal alkaline phosphatase. Thereafter, the pool of recombinant plasmids with different inserts was introduced into E. coli DH5α-MDR by heat-shock transformation and plasmid-containing DH5α clones were recovered on LB agar containing tetracycline 18 mg/L. Random screening of plasmids isolated from the transformants revealed the presence of inserts of ~7 kb. The whole insert from one representative recombinant plasmid was sequenced using the dRhodamine Terminator Cycle Sequencing kit and the manufacturer’s instructions (Gibco-BRL, Life Technologies Inc., Gaithersburg, MD, USA).

Computer analysis of sequence data

Nucleotide and amino acid sequences were analysed at the website of the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov/). The GenBank and protein databases were screened for sequence similarities. The EMBL accession number of our sequence (7077 bp) is AY196695.

Antimicrobial susceptibility assay

MICs of tetracycline and minocycline were determined on Mueller-Hinton agar by Etest (AB Biodisk, Solna, Sweden), according to the manufacturer’s instructions.

PCR amplification

The presence of the tet(A) gene in 22 epidemiologically unrelated A. baumannii strains and in E. coli DH5α strains (wild-type and transformed) was established by PCR amplification of a 954 bp fragment using primers specific for the gene and using the cycling conditions previously described by Vila et al. PCR products were resolved in agarose gel (2% w/v) and stained with ethidium bromide. In all cases, the products obtained were recovered and sequenced to establish the accuracy of the PCR. In the same manner, the amplification of a 212 bp starting in tnpA and terminating in the IS element was achieved using the primers: (1) 5'-CCGTCGGACGACACATTG-3' and (2) 5'-TCCGAATGAAA-GCCTGTCC-3' and the same cycling conditions.

Results

From a genomic library of the A. baumannii isolate, one clone of E. coli DH5α resistant to tetracycline was obtained. Analysis of the DNA sequence revealed that tetracycline resistance was due to the presence of the tet(A) gene. Together with tet(A), tetR(A), the tet(A) repressor gene, was also cloned. The partial sequence of a Tn3-like transposase gene, tnpA, and an IS element similar to that described in Salmonella typhi (IS4321) were also found at the same locus. The insert was 7077 bp in length (Figure 1), consisting of 2008 bp contributed by tnpA (partial sequence), 1316 bp by the IS element, 1200 bp by the tet(A) gene, 651 bp by tetR(A) and 75 bp by another transposase (partial sequence of a truncated and non-functional transposase, tnpA'). The tet(A) and the tetR(A) genes are adjacent, oriented divergently and share a central regulatory region (105 bp) with overlapping promoters and operators. The tnpA and the IS element are separated by 18 bp and the IS element and tetrR(A) by 324 bp. Follow-

![Figure 1](image-url)  
**Figure 1.** Gene arrangement of the partial transposon found in a clinical isolate of A. baumannii (A5-22) in comparison with the gene arrangement of Tn1721. The direction of the arrows shows the direction of transcription. The relative orientations of the three 38 bp inverted repeats, IRL, IRRl and IRRII, are symbolized by black arrowheads. tnpA' is a 5' truncated tnpA.
Tetracycline resistance in *A. baumannii*

<table>
<thead>
<tr>
<th>Strain</th>
<th>MIC (mg/L)</th>
<th>tetracycline</th>
<th>minocycline</th>
<th>tet(A) tnpA-IS</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em> DH5α</td>
<td>0.75</td>
<td>0.50</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>E. coli</em> DH5α (pBK)</td>
<td>256</td>
<td>0.50</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>A5-22 (<em>A. baumannii</em>)</td>
<td>&gt;256</td>
<td>8</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>623</td>
<td>&gt;256</td>
<td>1</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>661</td>
<td>256</td>
<td>1</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>A15-43</td>
<td>&gt;256</td>
<td>1</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>AC236</td>
<td>256</td>
<td>0.50</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3001</td>
<td>128</td>
<td>0.50</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3021</td>
<td>128</td>
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<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3175</td>
<td>64</td>
<td>0.25</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3183</td>
<td>64</td>
<td>0.25</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3198</td>
<td>64</td>
<td>0.25</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3207</td>
<td>128</td>
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<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3212</td>
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<td>0.25</td>
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<td>+</td>
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<tr>
<td>3233</td>
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<td>2</td>
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<td>–</td>
</tr>
<tr>
<td>3237</td>
<td>128</td>
<td>4</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Moreover, in 11 of these 12 strains (91.67%), and the *E. coli* transformant, a second PCR detected the *tnpA* followed by the IS element, suggesting that the genetic context in these isolates is the same as in *A. baumannii* A5-22.

**Discussion**

The presence of the Tet(A) determinant is herein described in one clinical isolate of *A. baumannii*. The gene responsible for tetracycline resistance appears to be part of a transposon, but whether the transposon remains functional has yet to be determined. However, its gene organization, transcription polarity and the nucleotide sequences of the genes responsible for tetracycline resistance, show striking similarity to those of the well-known Tn1721, strongly suggesting the presence of a Tn1721-like transposon in our clinical isolate of *A. baumannii*. However, despite significant similarity to Tn21, the nucleotide sequence of the *tnpA* gene differs markedly from its counterpart in Tn1721, being 100% identical with the *tnpA* described in *S. typhi* strain CT18 and in *P. aeruginosa*. Moreover, the nucleotide sequence of the IS has 99% homology with that of strain CT18 (IS4321). It is interesting to note that despite having the same transposase gene as that found in *A. baumannii*, and an almost identical IS element, the relative position of these genes in the CT18 strain differs from that in *A. baumannii* A5-22.

Tn1721-like elements have also been found in *Aeromonas* spp. and *Salmonella* spp. The presence of a Tn1721-like transposon in our isolate, not previously described in *A. baumannii*, together with the high similarity of the nucleotide sequences of the *tnpA* and the IS element with those present in other Gram-negative bacteria, suggest horizontal transfer between microorganisms sharing the same ecological niches.

Several events that could give rise to the situation with the IS element may have occurred. It could be a result of a recombination with the IRRI found in the Tn1721. Some of these genetic elements are thought to be responsible for the incorporation of genes of antibiotic resistance, including tetracycline determinants, in bacterial chromosomes. It is known that the dissemination of the IS elements is mediated by plasmids, and that posterior integration of the IS within the chromosome can occur.

The results of the PCR amplification of the 212 bp sequence containing a fragment of *tnpA* and the IS, suggest that this arrangement (*tnpA*-IS) is common in the tetracycline-resistant isolates of *A. baumannii* investigated and probably indicate a common structure to that in *A. baumannii* A5-22 (Table 1).

Little is known about the genetic basis of tetracycline resistance in *A. baumannii*. This article provides novel information about the resistance of this microorganism to tetracycline. We found a Tn1721-like transposon carrying the Tet(A) determinant in a clinical isolate of *A. baumannii*, suggesting horizontal transfer among different genera of Gram-negative bacteria sharing the same ecological niche.

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References


