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Presence of the Tet M Determinant in a Clinical Isolate of *Acinetobacter baumannii*

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The *tet*(M) gene encodes a protein which is related to tetracycline ribosomal protection, one of the mechanisms of tetracycline resistance. A *tet*(M) gene that is 100% homologous to the *tet*(M) gene of *Staphylococcus aureus* has been found in a clinical isolate of *Acinetobacter baumannii*, which also carries the *tet*(A) gene encoding a tetracycline efflux pump.

Acinetobacter baumannii is an opportunistic nosocomial pathogen whose importance has steadily risen due to its facility to develop resistance to a great variety of antimicrobial agents, including tetracyclines (13). Tetracyclines are bacteriostatic antibiotics which inhibit protein synthesis by preventing the attachment of aminoacyl-tRNA to the ribosomal acceptor side (4). To date, the main mechanisms responsible for tetracycline resistance have been identified as (i) expression of efflux pumps and (ii) ribosomal protection. *tet*(M) is one of the genes related to the ribosomal protection function and, together with the *tet*(O) gene, is the most extensively characterized of the whole group. Moreover, this gene has generally been associated with conjugative chromosomal elements which code for their own transfer, such as Tn*916* and Tn*1545* (4).

To our knowledge, the only data published on the mechanisms of resistance to tetracycline in *A. baumannii* were those of Guardabassi et al. (5), who found the Tet A and Tet B determinants in clinical and aquatic strains of this microorganism. In their article, the authors also reported on the search for the presence of other determinants, including Tet M. Unfortunately, they did not succeed in this effort. In spite of the description of different tetracycline determinants in *A. baumannii,* little is known about the mechanisms in *Acinetobacter* that are responsible for tetracycline resistance. Thus, the aim of the present study was to analyze the molecular mechanisms of resistance to tetracycline in 15 clinical isolates of *A. baumannii* and to characterize the Tet M determinant found in one of the studied strains.

To achieve this aim, a total of 15 epidemiologically unrelated isolates of *A. baumannii* were recovered from different biological samples, mainly respiratory secretions, and submitted to the Clinical Laboratory of Microbiology of the Hospital Clinic of Barcelona, Spain (14). These isolates were identified as *A. baumannii* by using standard biochemical procedures according to the criteria of Bouvet and Grimont (3) and by amplified ribosomal DNA restriction analysis (ARDRA) (12). The MICs of tetracycline and minocycline were determined by using Mueller-Hinton medium (2) with the Etest (AB Biodisk, Sölna, Sweden) in accordance with the manufacturer's instructions. The MICs of tetracycline were also determined by a microdilution method, in accordance with the guidelines established by the National Committee for Clinical Laboratory Standards (8). The amplification of 2,039 bp corresponding to the whole gene of *tet*(M) in *A. baumannii* was established several times by PCR with the primers specific for this gene, namely, *tet*M1 (5′-TGGGCTTTTGAATGGAGGAA-3′) and *tet*M2 (5′-ATCTCCTCCTTTACACTTTA-3′), under the conditions previously described (9), except that the annealing temperature was 50 instead of 55°C. The prevalence of this gene was determined by PCR by analyzing the 15 epidemiologically unrelated clinical isolates of *A. baumannii* (14). Moreover, the presence of the *tet*(A) gene was established in these strains by using the conditions described by Guardabassi et al. (5). The PCR products of both the *tet*(A) and *tet*(M) genes were recovered from the agarose gel and purified with the Concert rapid purification system according to the manufacturer's instructions (Gibco BRL, Life Technologies Inc., Gaithersburg, Md.). The sample was then directly processed for DNA sequencing by using the dRhodamine terminator cycle sequencing kit and was analyzed with an automatic DNA sequencer (ABI PRISM 377; Perkin-Elmer, Emeryville, Calif.). The DNA sequencing for the *tet*(M) gene was repeated twice in order to avoid the effects of contamination.

Cloning and transformation procedures were performed with a TA cloning kit dual promoter (Invitrogen, Groningen, The Netherlands). The dot blot analysis was performed as described previously (10).

After performing dot blotting, amplification, and sequencing of the *tet*(M) gene, we found that the *tet*(M) gene was present in one clinical isolate of *A. baumannii*, A5-22 (Fig. 1 and 2), and that this gene was 100% homologous to the *tet*(M) gene identified in *Staphylococcus aureus* strain Mu50 (EMBL accession number AP003359) (6). Since our strain also carried a Tet A determinant (A. Ribera, I. Roca, J. Ruiz, I. Gibert, and J. Vila, submitted for publication), the *tet*(M) gene from the *A. baumannii* strain was cloned in a vector and transformed in an *Escherichia coli* DH5α strain to demonstrate the activity of Tet M by itself. The MICs of tetracycline and minocycline (Table 1) were 512 and 8 μg/ml, respectively, for the *A. baumannii* strain and 128 and 8 μg/ml, respectively, for the transformed *E.*

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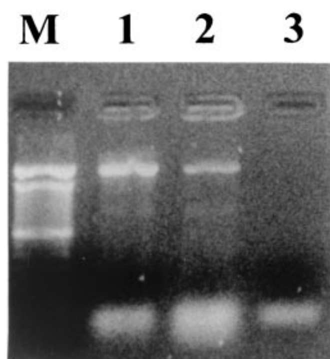

FIG. 1. PCR amplification of the *tet*(M) gene. Lane M, 100-bp DNA ladder(GIBCO-BRL, Life Technologies); lane 1, *A. baumannii* strain A5-22; lane 2, *E. coli* DH5α transformed strain; lane 3, *E. coli* DH5α wild-type strain.

coli strain, while the MICs of these antibiotics for the *E. coli* wild-type strain were 0.75 and 1.5 μg/ml, respectively. The differences between the MICs of tetracycline for the *A. baumannii* strain and the transformed strain could be explained by additional mechanisms of tetracycline resistance in the *A. baumannii* strain, such as the efflux determinant Tet A among others, which is able to pump tetracycline but not minocycline out of the cell (4).

Previous experiments suggest that tetracycline resistance is inducible, although it is unclear whether this is due to the *tet*(A) gene, the *tet*(M) gene, or to other tetracycline resistance mechanisms.

Therefore, our finding is another example where a *tet* gene, such as *tet*(M), that has been identified as having a gram-positive origin has been identified in a gram-negative bacterium, in this case, *A. baumannii*. It is now known that the number of gram-negative bacteria carrying *tet* genes as well as other types of genes with gram-positive origins is increasing (1, 4, 7).

The results of the prevalence study (Table 2) showed a low frequency of the *tet*(M) gene in the clinical isolates (1 out of 15 isolates, or 6.6%). In contrast, the results suggested a higher prevalence of the *tet*(A) gene among these clinical isolates (6 out of 15 isolates, or 40%); these results are consistent with those of Guardabassi et al. (5), who found that *tet*(A) and *tet*(B) are the genes responsible for tetracycline resistance that are most frequently encountered in clinical isolates of *A. baumannii*. It is known that Tet A confers resistance to tetracycline but not to minocycline and that Tet B confers resistance to both antibiotics (4). Therefore, our results support these data, as the *tet*(A) gene was identified in the strains resistant to tetracycline but not to minocycline, while the gene was not found in the strains resistant to both antibiotics. These strains may possess the Tet B determinant, which would confer resistance to both antibiotics. Another possibility may be the presence of mutations in the 16S rRNA gene (11) in these strains, since they have recently been identified as conferring resistance to tetracyclines in *Helicobacter pylori*. However, further studies are necessary to confirm this hypothesis.

In the present study, the identification of the *tet*(M) gene in a clinical isolate of *A. baumannii* that also carries the *tet*(A) gene is a novel finding. The fact that the gene is 100% homologous to the *tet*(M) gene of *S. aureus* suggests a horizontal transference of genetic material between gram-positive and gram-negative bacteria.

TABLE 1. MICs of tetracycline and minocycline for the *A. baumannii* strain and for both transformed and wild-type *E. coli* strains

Strain	MIC (μg/ml)	
	Tetracycline	Minocycline
A5–22	512	8
E. coli (wild type)	1	1
E. coli (transformed)[a]	128	8

[a] Transformed with the plasmid pCR-II in which the *tet*(M) gene from the *A. baumannii* strain (A5–22) was cloned.

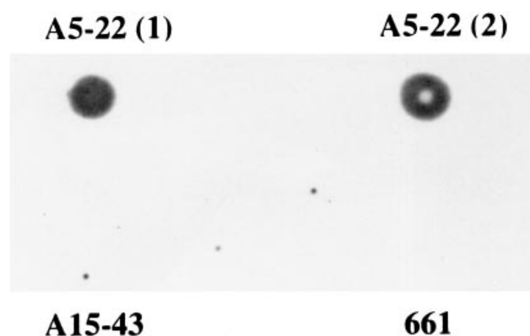

FIG. 2. Dot blot analysis using an amplified *tet*(M) product as the probe. The experiment was done with two different genomic DNA extracts from strain A5-22 and with the genomic DNA extract from strains 661 and A15-43, which contained the *tet*(A) gene but did not possess the *tet*(M) gene. The dot blot was positive for the two genomic DNA extracts of strain A5-22 and was negative for the other strains.

TABLE 2. MICs of tetracycline and minocycline for 15 strains of *A. baumannii* and results of the PCR amplification of the *tet*(A) and *tet*(M) genes

Strain	MIC (μg/ml)		PCR result	
	Tetracycline	Minocycline	*tet*(A)	*tet*(M)
A5–22	512	8	+	+
623	512	1	+	−
F13	512	2	+	−
661	256	1	+	−
A15–43	512	1	+	−
AC236	256	1	+	−
6948V	512	32	−	−
5985V	>512	32	−	−
215I	512	32	−	−
709-R	>512	32	−	−
6F	>512	32	−	−
O14–47	>512	32	−	−
L30	3	0.25	−	−
46I	16	0.50	−	−
93	3	0.125	−	−

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