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Tetracycline Resistance in *Ureaplasma* spp. and *Mycoplasma hominis*: Prevalence in Bordeaux, France, from 1999 to 2002 and Description of Two *tet*(M)-Positive Isolates of *M. hominis* Susceptible to Tetracyclines▽

S. Dégrange, H. Renaudin, A. Charron, C. Bébéar, and C. M. Bébéar*

Laboratoire de Bactériologie EA 3671, Université Victor Segalen Bordeaux 2 and CHU de Bordeaux, 146 rue Léo Saignat, 33076 Bordeaux Cedex, France

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Twenty-four of 128 clinical isolates of *Mycoplasma hominis* and 6 of 276 clinical isolates of *Ureaplasma* spp. from Bordeaux, France (1999 to 2002), were resistant to tetracycline and harbored the *tet*(M) gene. For *M. hominis*, we also found an increase in tetracycline resistance and two *tet*(M)-positive isolates that were susceptible to tetracyclines.

Tetracyclines belong to the first-line treatment of human urogenital infections caused by *Mycoplasma hominis* and *Ureaplasma* spp. (2). High-level resistance to tetracyclines in *M. hominis* and *Ureaplasma* spp. has been associated with the presence of the *tet*(M) determinant (14, 15), the sole tetracycline resistance mechanism acquired by clinical isolates of human mycoplasmas (2).

The goal of this study was to determine the prevalences and mechanisms of resistance to tetracyclines among *M. hominis* and *Ureaplasma* species isolates obtained from patients in Bordeaux, France, from November 1999 to October 2002.

The growth conditions and antibiotic susceptibility testing of *Ureaplasma* and *M. hominis* strains have previously been described (18). One hundred twenty-eight isolates of *M. hominis* and 276 isolates of *Ureaplasma* spp., obtained from the lower urogenital tract and from semen, were studied for antibiotic susceptibility at the Pellegrin Hospital in Bordeaux. Antibiotic susceptibility testing was performed using the commercial SIR Mycoplasma kit (Bio-Rad), which includes tetracyclines, fluoroquinolones, macrolides, and related antibiotics.

Amplifications of the *tet*(M) and *M. hominis* 16S rRNA genes and of the IS*Mhom1* element were performed with primers previously described (8) or listed in Table 1. For reverse transcription-PCR (RT-PCR), RNAs were isolated from cultures of *M. hominis* PG21 (ATCC 23114) and *M. hominis* isolates MHa (3), 3425, and 3430 (this study) with the High Pure RNA isolation kit (Roche Diagnostics GmbH). mRNAs were reverse transcribed into cDNAs with the enhanced avian RT-PCR kit (Sigma) using primer tet2 (8).

The sequence directly upstream from the *tet*(M) gene of *M. hominis* isolates was determined by PCR using the Universal GenomeWalker kit (BD Biosciences Clontech) and by direct sequencing with an ABI Prism dRhodamine Terminator cycle sequencing ready reaction kit (Applied Biosystems).

Twenty-four of the 128 clinical isolates of *M. hominis* (18.75%) and 6 of the 276 clinical isolates of *Ureaplasma* spp. (2.2%) were classified as resistant to tetracycline by the SIR Mycoplasma kit according to the guidelines of the Comité de l'Antibiogramme de la Société Française de Microbiologie (6). MICs of tetracycline, doxycycline, and minocycline ranged from 32 to 64 μg/ml for the 24 *M. hominis* isolates and were >32 μg/ml for the 6 *Ureaplasma* isolates. All the tetracycline-

TABLE 1. Oligonucleotides used in this study

Primer target or designation	Primer sequence (5′→3′)	GenBank accession no. (reference)
M. hominis 16S rRNA		
Mh16S-1	TTTGATCCTGGCTCAG GATG	AJ002265 (11)
Mh16S-2	ACTCCTACGGGAGGCA GCAG	AJ002265 (11)
Mh16S-3	AAGAACTTCATCGTGC ACGC	AJ002265 (11)
Mh16S-4	TGAAGCGGTGAAATGC GTAG	AJ002265 (11)
Mh16S-5	GGGTATCTAATCCTGT TTGC	AJ002265 (11)
Mh16S-7	ATGATGATTTGACGTC ATCC	AJ002265 (11)
Intergenic space 16S-23S		
Mh16S-6	GGTGCATGGTTGTCGT CAGC	AJ002265 (11)
ITS-2	TCGCAGGTAATCACGTC CTTCATCG	AF443616 (12)
IS*Mhom1*		
IS*30F*	GCTCCACAAACGTAC CAAG	DQ973625 (this study)
IS*30R*	GCTATTGTAGTGTCTA AATC	DQ973625 (this study)

* Corresponding author. Mailing address: Laboratoire de Bactériologie EA 3671, Mycoplasma and chlamydiae infections in humans, Université Victor Segalen Bordeaux 2, 146 rue Léo Saignat, 33076 Bordeaux Cedex, France. Phone: (33) 5 57 57 16 25. Fax: (33) 5 56 93 29 40. E-mail: cecile.bebear@u-bordeaux2.fr.

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FIG. 1. Nucleotide sequence of the IS*30*-like element of *M. hominis* isolate 3425 and flanking regions. Amino acid positions are shown at right. The deduced amino acid sequence encoded by the single ORF is shown in single-letter code below the DNA sequence. Each of the three acidic residues that comprise the highly conserved active-site motif DDE is enclosed within a box. Methionine start codons are underlined. The limits of the IS element are indicated with brackets, and the IR sequences at the left (IR-L) and right (IR-R) termini are indicated by labeled arrows. Other IR sequences are indicated by opposing arrows. The direct repeat sequences that flank the IS are marked by asterisks below the corresponding sequence.

resistant isolates were *tet*(M) positive. None of the 16S rRNA mutations described for tetracycline-resistant mutants obtained in vitro (S. Dégrange et al., unpublished data) was found in the resistant *M. hominis* isolates.

In a previous multicentric study in France, including Bordeaux, the prevalence of tetracycline resistance was estimated to be around 3% of *M. hominis* and *Ureaplasma* species isolates in the early 1990s (1). Using the same susceptibility testing kit as described in that previous report (1), this study has shown that in Bordeaux, the percentage of *M. hominis* isolates resistant to tetracyclines increased significantly to 18.75% (24 of 128 isolates) between 1999 and 2002 from 2.8% (2 of 72) in 1992 (1). In contrast, this study did not show any increase in the tetracycline resistance rates of *Ureaplasma* spp., with 2.2% (6 of 276) of clinical isolates being resistant to tetracyclines from 1999 to 2002 compared to 3% (10 of 324) in 1992 (1). Studies performed before 1990 reported for *M. hominis* percentages of resistance to tetracyclines from 17% to more than 30% (7, 17). In ureaplasmas, tetracycline resistance has been reported to occur in 10% of isolates (17, 19). In contrast with our results, a recent study in the United States reported that 45 of 100 *Ureaplasma* species isolates were *tet*(M) positive between 2000 and 2004 (19).

As susceptibility to tetracycline has been observed in *tet*(M)-positive bacteria like *Streptococcus pneumoniae* (9), 10 tetracycline-susceptible isolates of both *M. hominis* and *Ureaplasma* species were screened by PCR for the presence of *tet*(M). Two *M. hominis* isolates, named 3425 and 3430, harbored the *tet*(M) determinant but were categorized as susceptible to tetracyclines, with MICs identical to those of the reference strain, PG21. Furthermore, no mutation was found in their 16S rRNA sequences.

In these two isolates, no mRNA transcripts of the *tet*(M) gene could be identified by RT-PCR. Studying the *tet*(M) sequence present in both isolates, we found no mutation in the gene or in the 400-bp region upstream of the gene in a comparison with the sequences of the *M. hominis* tetracycline-resistant isolate MHa (3) and those reported by Su et al. (16) and Burdett (4). However, in isolate 3425, a new insertion sequence (IS) was found inserted in the leader peptide sequence upstream from *tet*(M) (Fig. 1). Because it disrupted the leader peptide sequence, we speculated that this IS was probably involved in the lack of transcription of *tet*(M) in this isolate. The full insertion element, designated IS*Mhom1*, was 1,260 bp long, was bounded by 27-bp inverted repeats (IRs), and included a single open reading frame (ORF). A BLAST comparison with database sequences showed that the 336-amino-acid protein encoded by the single ORF was homologous to transposases of the IS*30* family isolated from other mycoplasma species. Alignment with the corresponding proteins from those mycoplasmas showed identities of from 40% with the transposase of *Mycoplasma fermentans* IS*1630* (5) to 57% with the transposase of the *Mycoplasma bovis* IS*30*-like element (10). The presence of this IS element was detected by PCR in the other isolate, 3430, but not in the region upstream from *tet*(M).

Lastly, the exposure of cells from isolates 3425 and 3430 to increasingly subinhibitory concentrations of doxycycline by serial passages, as previously reported (13), gave different results. After 20 passages, the doxycycline MIC of isolate 3425 stayed at its initial value of 0.12 μg/ml. Thus, the antibiotic pressure did not succeed in increasing the doxycycline MIC of isolate 3425 without losing the IS element in the *tet*(M) leader peptide sequence, as confirmed by PCR. In contrast, an increased resistance to doxycycline was observed after 3 passages of isolate 3430, with the doxycycline MIC increasing from 0.12 to 8 μg/ml and reaching 32 μg/ml after 10 passages. MICs of tetracycline and minocycline also increased to 128 and 16 μg/ml, respectively, corresponding to the resistance level observed in an *M. hominis* strain expressing the *tet*(M) gene. Furthermore, this determinant was transcribed again in isolate 3430 from the third passage, as revealed by RT-PCR experiments. Thus, doxycycline appears to enhance the *tet*(M) transcription in this isolate. No mutation was observed in the 3430 16S rRNA, and the IS PCR was still positive after 10 passages. These observations suggested that the *tet*(M) regulation in isolate 3430 might involve transcriptional attenuation, as described for *Enterococcus faecalis* (16).

In summary, this study showed an increase of tetracycline resistance in clinical isolates of *M. hominis* and reported the presence of two isolates that are *tet*(M) positive but susceptible to tetracyclines. These isolates were misidentified as susceptible and are difficult to identify as resistant unless one looks for the presence of the *tet*(M) determinant systematically. Knowing the behavior of such strains in the presence of tetracyclines in vivo is a matter to debate. The observed inducibility of resistance in one of these two isolates suggests that the tetracycline-susceptible isolates which carry *tet*(M) should be reported to be as resistant to all tetracyclines, despite the lack of clinical data showing that such isolates would fail therapy. Finally, this is the first report of an IS*30*-related family in *M. hominis*, confirming the presence of this IS family in mycoplasmas.

Nucleotide sequence accession number. The nucleotide sequence reported for the IS*Mhom1* element has been deposited under GenBank accession no. DQ973625.

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