Genetic basis of natural resistance to erythromycin in *Mycoplasma hominis*

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Sir,

Only a few studies have been reported, so far, on the mechanisms of antibiotic resistance in mycoplasmas, and those have frequently involved *Mycoplasma pneumoniae*. Little information is available in the literature on the mechanisms of macrolide resistance found in other species of mycoplasmas or ureaplasmas. In a previous study it was reported that high-level resistance to erythromycin was found in a strain of *Ureaplasma urealyticum* and associated with a six-fold reduction in intracellular influx and accumulation with a reduction in antibiotic binding to ribosomes, compared with that of a susceptible strain.1 More recently, in *M. pneumoniae*, acquired resistance to erythromycin has been associated with point mutations (A → G transition) in the domain V loop of the 23S rRNA.2 Nevertheless, resistance to one antibiotic does not always mean resistance to all antibiotics within the macrolide–lincosamide–streptogramin (MLS) groups; this is especially true for mycoplasmas. *Mycoplasma hominis*, which is naturally resistant to erythromycin in vitro, but susceptible to 16-membered macrolides ( josamycin or miocamycin) and lincosamycin, whereas this is not true of *U. urealyticum*. While the genetic basis for MLS resistance has been extensively studied for other bacteria and *M. pneumoniae*, it has not been explored in *M. hominis*. As reviewed by Cundliffe,3 specific residues within domain V of 23S rRNA are involved in the action of MLS antibiotics and chloramphenicol. Chemical footprinting studies demonstrated that the reactivity of certain purines within domain V was specifically altered by macrolides, lincosamides, streptogramin B, chloramphenicol or azalides; in fact, strong antibiotic protection is associated with A2058 and A2059. These findings prompted us to investigate the natural resistance of *M. hominis* to erythromycin and its natural susceptibility to 16-membered macrolides and/or to lincosamides.

Three different strains were investigated for the purpose; *M. hominis* strain PG21, *M. hominis* strain CT-PAF and *M. hominis* CT-Mh1. Briefly, strains were grown in standard mycoplasma media, and DNA was extracted and purified by standard methods. The primers for PCR amplifications were constructed by aligning known sequences of the domain V of 23S rRNA gene in closely related species (forward primer 5′-CTATAACGGTCC- TAAGGTAG-3′; reverse primer 5′-GGTCTCTCGT- ACTAGAAAG-3′). PCR amplification was performed by standard method, and the cycling programme was: one cycle at 98°C for 10 min; 30 cycles at 95°C for 30 s, 57°C for 30 s, and 72°C for 30 s; and a final elongation step at 72°C for 10 min. Amplified fragments were purified on agarose gel and sequenced by the dye termination method. The sequences were deposited at GenBank under accession numbers AF101242, AF131860 and AF131073. The comparison of these sequences with those of *Escherichia coli* and some other species has shown a G→A transition at 2057 (*E. coli* coordinates) in the central loop of domain V of the 23S rRNA (Figure). This suggested that MLS type resistance in those three strains can be correlated with such a mutation, which is known to result in a similar pattern of resistance in other organisms. BLAST analysis showed that such a transition was present in two other mycoplasma species, *Mycoplasma flocculare* (23S rRNA gene sequence, GenBank accession number: L22210) and *Mycoplasma hyopneumoniae* (23S rRNA gene sequences, GenBank accession number: X68421). Moazed & Noeller4 incubated 70S ribosomes together with antibiotics and showed direct protection of both A2058 and A2059 by both erythromycin and carbomycin (a 16-membered macrolide) against derivatization by DMS. Moreover, as shown by Douthwaite & Aagaard,5 clindamycin protected both A2058 and A2059, whereas lincomycin protected only A2058. This explains why our strain showed resistance against erythromycin owing to G2057A and was susceptible to lincomycin and clindamycin. In fact, unlike other sites, such as 2058 or 2059, position 2057 did not appear protected against chemical modification by bound antibiotic. The remaining nucleotide sequence did not reveal any transition at position C2611. The phenotype associated with such a transition is frequently associated, in other bacteria, with chloramphenicol resistance and clindamycin susceptibility;6 in fact, in a separate experiment we demonstrated that all three
strains were also cloramphenicol resistant (MIC > 16 mg/L) although they retained their susceptibility to clindamycin and lincomycin. We can conclude that the natural resistance to both erythromycin and cloramphenicol in M. hominis can be associated with that mutation and may also explain erythromycin resistance in other mycoplasmas such as M. hyopneumoniae. 7

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References