

New Mutation in 23S rRNA Gene Associated with High Level of Azithromycin Resistance in *Neisseria gonorrhoeae*[▽]

Although azithromycin (1 g orally in a single dose) is not recommended for treating gonorrhea, it is still used in some parts of the world, and it is widely used either to treat *Chlamydia trachomatis* infections or, in combination with expanded-spectrum cephalosporins, to treat simultaneous infections with both chlamydia and gonococci (7). A higher dose of 2 g of new formulations of azithromycin with a lower incidence of gastrointestinal intolerance may be more effective (9), but recent descriptions of gonococcal strains showing azithromycin resistance or high-level azithromycin resistance (AzHLR; MIC of azithromycin, >256 mg/liter) would make that treatment untenable if strains of this type were to emerge elsewhere and spread (1, 2, 6).

Decreased susceptibility of *Neisseria gonorrhoeae* to azithromycin is due mainly to the overproduction of an *mtrCDE*-encoded efflux pump induced by *mtrR* mutations (10) but also to the presence of the *mef* gene, whose code for an efflux pump has been associated with resistance of gonococci to macrolides (3).

Recently, a gonococcal strain isolated in Argentina for which the MIC of azithromycin was >2,048 mg/liter was described. Neither mutations in the promoter region of the *mtrR* gene explaining the AzHLR nor the presence of the *mef* gene was observed in this strain (2).

Mutations in the peptidyltransferase loop in domain V of 23S rRNA alleles have also been associated with macrolide resistance in *N. gonorrhoeae* (5), and now the Argentinian strain with AzHLR has been analyzed for the presence of these mutations within domain V. A two-step PCR method was followed (5) to analyze three gonococcal strains isolated in Argentina showing different levels of azithromycin susceptibility: the strain with AzHLR (azithromycin MIC, >2,048 mg/liter) mentioned above (strain 1782), one isolate (strain 2498) for which the azithromycin MIC was 8 mg/liter (indicating resistance), and one susceptible gonococcal strain (3783; azithromycin MIC, 0.06 mg/liter). The sequences obtained in this study were compared with those (Gene Bank accession numbers AF450074 to AF450081) corresponding to azithromycin-resistant gonococcal strains and associated with mutations at this level (5).

The susceptible Argentinian strain did not show mutations in the sequenced fragment from any of the four copies of the 23S rRNA *rrl* gene. In strain 2498, for which the MIC was 8 mg/liter, all four alleles were found to contain C2599T, which has been associated with this level of resistance (5). The strain showing AzHLR did not have this particular mutation but presented an A2143G mutation (*N. gonorrhoeae* numbering) in all four alleles. This mutation, which corresponds to A2059G in *Escherichia coli*, has not been reported to occur in gonococci, but it has been associated with macrolide resistance in *Helicobacter pylori*, several *Mycobacterium* species, *Mycoplasma pneumoniae*, *Streptococcus pneumoniae*, and *Propionibacterium* species and

more recently in *Treponema pallidum* (4, 8). Adenosine 2058 or neighbor nucleotides (such as A2059) are the key for macrolide interaction on the ribosome (8), and A2143G is strongly suggested to be responsible for the AzHLR in gonococcal strain 1782, isolated in Argentina.

The finding observed for the strain for which the MIC was 8 mg/liter is also interesting: all alleles showed a C2599T mutation, but a dinucleotide (TT) insertion in the promoter region of the *mtrR* gene, which has also been associated with reduced susceptibility to azithromycin (2), was also found. Ng et al. (5) observed a spontaneous *N. gonorrhoeae* mutant strain with the same mutation in four *rrl* alleles and a T insertion in the promoter region of the *mtrR* gene for which the MIC was 4 mg/liter. As far as we know, Argentinian strain 2498 is the first clinical isolate with both mechanisms of resistance, and it illustrates how gonococcal strains are continuously adapting under antibiotic selection pressure.

Nucleotide sequence accession numbers. The sequences obtained in this analysis have been deposited in GenBank under accession numbers GQ389765 to GQ389775 and GU361858.

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