Letters to the Editor

Induced Macrolide Resistance in *Mycoplasma genitalium* Isolates from Patients with Recurrent Nongonococcal Urethritis[▽]

Mycoplasma genitalium accounts for as much as 15 to 20% of cases of male nongonococcal urethritis (NGU) (7). It is also implicated in female cervicitis and increasingly so in pelvic inflammatory disease (3). Since it is a fastidious microorganism that is very difficult to culture, nucleic acid amplification tests (NAAT) have become invaluable in its detection and microbiological evaluation.

Currently recommended treatments of NGU include multidoses of doxycycline (100 mg twice daily for 7 days) or a single dose of azithromycin (1 g). With regard to *M. genitalium*-positive NGU, a recent randomized study has shown multidose treatment with doxycycline to be inferior to single-dose azithromycin (cure rates of 45% versus 87%, respectively) (8). However, even single-dose azithromycin can be associated with treatment failure rates of up to 28% (2). An extended 5-day course of azithromycin (500 mg on day 1, followed by 250 mg on days 2 to 5) has a much higher cure rate of up to 96% (1) but becomes less efficacious when used after initial treatment failure with single-dose azithromycin (6).

Jensen et al. (4) have shown that macrolide resistance in *M. genitalium* can be induced by insufficient therapy with single-dose azithromycin and found the resistance to be strongly associated with three single-base mutations (A2058C, A2058G, A2059G) in region V of the 23S rRNA gene (4). They developed a conventional PCR to detect all of these mutations.

In September 2009, we started *M. genitalium* NAAT testing of genital samples from selected patients with recurrent NGU at the Christchurch Sexual Health Centre, Christchurch, New Zealand. The PCR was performed as described by Jensen et al. (5) with the 2× Universal TaqMan gene expression master mix (Applied Biosystems, Life Technologies, Foster City, CA). Between September 2009 and October 2010, 52 samples were collected and *M. genitalium* was detected in 11 (21%) samples. The nucleic acid extracts were stored at –80°C until October 2010, when we retrospectively performed PCR and DNA sequencing on these extracts to look for the single-base mutations mentioned above, using the method described by Jensen et al. (4).

Of the 11 nucleic acid extracts, we were able to amplify and sequence the 23S rRNA genes of 9 extracts. PCR of the other 2 nucleic acid extracts did not yield an amplicon upon repeat testing, probably due to nucleic acid degradation during storage. Of the 9 sequenced nucleic acid extracts, 4 extracts contained the A2059G mutation, whereas none contained mutations at position 2058.

We retrospectively reviewed the notes for the relevant patients (those with extracts containing the mutation) and found that all four patients had previously been treated multiple times for sexually transmitted infections. We were able to determine that three of these patients had received single-dose azithromycin between 34 and 58 days preceding the positive test for *M. genitalium*; the fourth patient had also received single-dose azithromycin in the few weeks preceding a positive test, but we were unable to determine the exact time frame. All four patients had received an extended course of azithromycin following the diagnosis of *M. genitalium* urethritis: two experi-

enced treatment failure, whereas the other two were subsequently cured, based on the fact that *M. genitalium* DNA was no longer detectable.

Of the 5 patients whose *M. genitalium* extracts did not contain any of the macrolide resistance mutations (of the 9 extracts whose rRNA genes we were able to amplify and sequence), 3 had experienced successful empirical therapy with single-dose azithromycin (2 patients) or extended-dose azithromycin (1 patient); 1 patient was lost to follow up. The remaining 1 patient had received treatment with single-dose azithromycin, but upon subsequent testing, *M. genitalium* DNA was detectable and in this case contained the A2059G mutation, and the patient was included in our group above. This nicely demonstrates the induction of macrolide resistance by single-dose azithromycin treatment.

To our knowledge, this is the first report from New Zealand describing macrolide resistance in *M. genitalium*. Despite a very limited number of samples, we have evidence that the A2059G mutation conferring induced macrolide resistance is present at a high frequency in *M. genitalium* extracts from patients with recurrent NGU at the Christchurch Sexual Health Centre. This brings into question whether or not single-dose azithromycin should be the first-line treatment of choice for *M. genitalium*-positive urethritis. At the Christchurch Sexual Health Centre, the current practice is to prescribe an extended course of azithromycin for all cases of *M. genitalium*-positive urethritis (primarily because moxifloxacin is currently not readily accessible in New Zealand). We also note with interest that in two patients with the resistant isolate, infection was still cleared with an extended course of azithromycin. This would suggest that macrolide resistance conferred by the A2059G mutation was not absolute. Clearly, the subject of macrolide resistance in *M. genitalium* requires more study.

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