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Quinolone Resistance–Associated Mutations in *Mycoplasma genitalium*: Not Ready for Prime Time

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The 2019 Centers for Disease Control and Prevention report of top antibiotic resistance threats in the United States included 2 sexually transmitted pathogens.[1] *Neisseria gonorrhoeae*, which has been of concern for many years, was again listed as an urgent threat, and *Mycoplasma genitalium* is one of three organisms included in a new category, the Watch List. There is heightened concern over *M. genitalium* because of how rapidly it is moving toward becoming an untreatable infection. Macrolide resistance exceeds 50% in many settings,[2] and resistance to fluoroquinolones is being increasingly reported.[3] Third-line pristinamycin,a streptogramin, proved to be less efficient when used on a broader scale[4] than in the initial observational study,[5] although true resistance has not been documented *in vitro*. The organism seems to be susceptible to tetracyclines in vitro, but clinical efficacy is only 30% to 40%.[6]

Resistance-guided therapy, which leverages assays with the capacity to detect macrolide resistance,[7] was developed to combat the rapidly diminishing efficacy of azithromycin and preserve moxifloxacin for as long as possible. Under this approach, patients are empirically treated with doxycycline followed by high-dose azithromycin in cases of macrolide-sensitive infection, or by moxifloxacin in cases of macrolide-resistant infection. Using resistance-guided therapy, cure rates for both azithromycin and moxifloxacin exceed 90% and selection of additional macrolide resistance is less than 5%.[8]

Given the success of this approach, there has been considerable speculation about whether incorporating detection of quinolone resistance could similarly slow the expansion of quinolone resistance. However, this has proven more challenging than anticipated. In this issue of the journal, Conway and colleagues[9] explore the relationship between detection of quinolone resistance–associated mutations (QRAM) identified by the SpeeDx MG + parC (beta 2) assay and moxifloxacin treatment outcomes in a case series of 96 *M. genitalium*–positive persons. The assay used by Conway et al detects groups of mutations in the *parC* gene of *M. genitalium* and defines QRAM as S83 R/I combined or D87/Y/N/H combined; S83N is detected separately, as this mutation alone does not seem to increase minimum

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inhibitory concentrations (MICs),[10] but is located in the core region for resistance. All of the patients in Conway's cohort had macrolide resistance, and QRAMs were detected in 7 (7%) of 96. Moxifloxacin treatment outcomes were available for 3 of the 7 persons with QRAM, and the correlation between the presence of the ParC mutations and treatment outcomes was poor. Two of the 3 patients experienced microbiologic cure; one had a D87 mutation and the other an S83R/I mutation. Only the third patient had a treatment outcome consistent with the QRAM profile. This person had an S83R/I mutation and experienced moxifloxacin treatment failure.

The results of the study by Conway et al highlight the challenges in determining fluoroquinolone resistance in *M. genitalium*. Given the difficulty in culturing *M. genitalium*,[11] minimum inhibitory concentrations are only rarely performed in the context of research and cannot be used to guide therapy. Antimicrobial resistance is therefore inferred based on the detection of specific mutations. Macrolide resistance is strongly associated with mutations in the 23S rRNA at positions 2058 and 2059 (*Escherichia coli* numbering). All of these macrolide resistance mutations (MRMs) have been clearly associated with treatment failure after azithromycin, and only scattered cases of clearance in their presence have been reported. Unlike with *N. gonorrhoeae*, there does not seem to be a middle ground of "decreased susceptibility" that can be overcome with higher doses of antibiotic. As a result, detection of any one of the MRM is an excellent proxy for macrolide resistance and azithromycin treatment failure.

In contrast, the significance of mutations linked to moxifloxacin treatment failure is less clear. Fluoroquinolone treatment failures in *M. genitalium* have been linked primarily to mutations in the *parC* gene, which encodes topoisomerase IV, and the level of resistance may be increased in the presence of mutations in the *gyrA* gene, which encodes DNA gyrase. Numerous mutations in these regions have been detected, but those most frequently associated with high MIC values or documented treatment failure consist of S83I and D87Y[12] or D87N[13] (J.S. Jensen, unpublished data) in ParC. S83R has been associated with moxifloxacin treatment failure in one study[14] but not in another.[15] Because the SpeeDx MG + parC (beta 2) assay used by Conway et al detects groups of ParC mutations, it is not possible to distinguish between several of the ParC mutations, and not all may play a role in treatment failure. It also does not assess mutations in the *gyrA* gene, and co-occurrence of S83I and a GyrA M95I or D99N mutation was more strongly associated with moxifloxacin treatment failure than S83I alone[15] in one study and seems to lead to higher MIC values in a limited number of cultured isolates.[10]

In addition to the likely role of *gyrA* mutations, there are numerous other factors that make it challenging to determine the extent to which a given mutation predicts quinolone resistance and subsequent treatment failure. Among these are the advent of sequential therapy for *M. genitalium* and individual-level variability in pharmacodynamics of the antibiotic. The relationship between MRM and azithromycin treatment failure was identified when azithromycin alone was recommended as first-line therapy for *M. genitalium* infections. However, sequential therapy has now become the norm in UK[16] and Australian guidelines.[17] Pretreatment with doxycycline, which is the first step in sequential therapy, will cure up to 40% of infections as monotherapy, and the residual doxycycline together with moxifloxacin

may have a synergistic effect for some ParC mutations. Determining the importance of QRAM in this context is quite challenging. In cases where a patient with a specific QRAM experiences cure, it is unclear whether that is due to the doxycycline or to poor correlation between the mutation and treatment outcomes. Furthermore, even the same ParC mutations may lead to different MICs. Some isolates with the S83I mutation may have MICs as low as 1 mg/L[10] being very close to the expected breakpoint and others as high as 16 mg/L. Some D87 mutations may also have MICs low enough to be treatable in a proportion of the cases. Dosing recommendations do not vary by body weight, meaning that lower-weight persons may have more concentrated antibiotic levels and experience cure more frequently than larger, heavier persons. Despite reported treatment successes, these mutations are all clearly associated with elevated MICs *in vitro*, making the term QRAM appropriate.

The poor correlation of treatment outcomes with specific QRAM in the study by Conway et al may also be influenced by imperfectly sensitive and specific diagnostic assays. One of the cured patients still had discharge at the time of the test of cure, and that patient may have had a false-negative result. *M. genitalium* sometimes drops below the level of detection and then recrudesces several weeks later[18,19]; that may have happened in this man and may also explain some apparent cures after azithromycin in MRM infections.

Accurately identifying quinolone resistance determinants will require significant effort. Larger studies that collect more detailed information on the potential for reexposure will be critical. This was one of the key conclusions of the Conway article, and it warrants emphasis. Only 5 of the 7 patients with QRAMs had clinical information available, and treatment outcome data were available for only 3 of these. This is unfortunately consistent with many other small studies, making it impossible to determine the role of less frequently occurring mutations, reexposure, and false-positive or false-negative test results. The largest study to date included 88 persons with ParC mutations, and only 15 of these had GyrA mutations.[15] It will be essential to design larger studies that incorporate tests of cure, organism load data, and epidemiologic data on the potential for reexposure to differentiate true treatment failure from reinfection. We must encourage culture to increase the number of clinical strains with characterized mutations and perform MICs against different antimicrobials. Although MICs will likely never play a key role in clinical decision making, they will be key to understanding the role of mutations and allow for testing of new antimicrobials and combinations of old ones. Finally, global surveillance to monitor changes in the prevalence of macrolide and quinolone resistance over time will be essential.

There is little dispute about the value of determining the macrolide resistance profile to guide therapy. It works because moxifloxacin is still a viable alternative antibiotic. However, even if we are able to identify the key fluoroquinolone resistance determinants, there will be no clinical benefit without additional therapeutic options. At our present state of knowledge, the use of tools to detect QRAMs is limited to research and to patients with moxifloxacin treatment failure to exclude reinfection and save expensive, difficult to source antimicrobials for those that really need them. Until then, we must remain good stewards of the antibiotics that we have, refraining from screening and reconsidering tests of cure until we have definitive evidence of harm caused by asymptomatic infections, and using the right antibiotic in the right dosage to limit the total amount.

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