Study gauges impact of genotyping on gonorrhea treatment

Amy Carpenter Aquino

April 2019—Genotypic testing for ciprofloxacin susceptibility in *Neisseria gonorrhoeae* has been proved to be effective in guiding physician treatment in a single-center study at UCLA Health.

Jeffrey D. Klausner, MD, MPH, a clinical professor in the Department of Medicine, Division of Infectious Diseases, and the Department of Epidemiology, David Geffen School of Medicine and the Fielding School of Public Health, University of California, shared the details of the study at last year's Association for Molecular Pathology meeting and in a recent interview.

Dr. Klausner

"The overall concept is that with rapid detection of *Neisseria gonorrhoeae*, and detection of key antimicrobial resistant genes, we can enable doctors to do targeted treatment, which will reduce antibiotic selection pressure and decrease the emergence of resistance," Dr. Klausner said.

The British Association for Sexual Health and HIV endorsed this approach when it issued a gonorrhea guideline update in January, Dr. Klausner says. "They specifically recommend the use of molecular tests to predict susceptibility of antibiotics in *Neisseria gonorrhoeae* infections." The new guideline removes the recommendation for dual therapy with azithromycin and encourages the use of ciprofloxacin in certain cases when a molecular test predicts susceptibility.

Dr. Klausner describes the "wake-up case" as that of a heterosexual man who presented to a sexual health clinic in the United Kingdom in December 2014 with a two-week history of urogenital symptoms (Fifer H, et al. *N Engl J Med.* 2016;374[25]:2504–2506). The patient had returned 10 days earlier from a trip to Japan, where his Japanese female partner had been treated for gonorrhea.

The patient's nucleic acid amplification tests were positive for *N. gonorrhoeae* in a urine specimen and pharyngeal swab, and he had an *N. gonorrhoeae*-positive urethral culture. He received the standard dual treatment for *N. gonorrhoeae* infection in the U.K. at the time, which was a 500-mg injection of ceftriaxone plus one gram orally of azithromycin. (The Centers for Disease Control and Prevention currently recommends a similar dual therapy of a 250-mg injection of ceftriaxone with one gram orally of azithromycin.)

Phenotypic testing revealed that the patient's *N. gonorrhoeae* strain was resistant to cefuroxime, ciprofloxacin, and tetracycline. The patient had follow-up pharyngeal NAATs on days 15, 79, and 98; all tested positive for *N. gonorrhoeae*. A culture taken on day 98 was also positive.

The patient was then treated with double doses of the initial treatment: a one-gram injection of ceftriaxone and two grams orally of azithromycin. "Finally, nearly four months later, his pharyngeal swab tested negative," Dr. Klausner said.

Antimicrobial susceptibility testing by Etest of the pharyngeal isolate found resistance to ceftriaxone, cefixime, cefotaxime, azithromycin, penicillin, tetracycline, and ciprofloxacin. It was susceptible only to spectinomycin (which

is not available in the United States).

Whole genome and conventional sequencing of the organism found multiple gene alterations. “They found what we call a mosaic *pen*A gene, which has been classified as X,” Dr. Klausner said. “People have classified about three dozen different mosaic *pen*A types associated with an altered penicillin-binding protein 2, with a decreased target affinity to ceftriaxone.” Other gene alterations were an *mtr*R promoter deletion of one adenine (which increases MtrCDE efflux of ceftriaxone and azithromycin) and a change in *pen*B (which decreases the PorB influx of ceftriaxone and azithromycin).

Third-generation cephalosporin resistance in *N. gonorrhoeae* is rising in the U.S., as noted by CDC data published in the *New England Journal of Medicine* in 2012 (Bolan GA, et al. 366[6]:485–487). In 2011, Dr. Klausner said referring to the data, isolates from states in the West, from five percent of men who have sex with men, had higher levels of elevated MICs for decreased susceptibility to cefixime.

Drug-resistant *N. gonorrhoeae* has been declared one of the three most urgent infectious public health threats. “That’s a critical thing because it alerts funders and policymakers to this issue,” Dr. Klausner said. He was in Washington, DC, last summer, and “the one thing politicians knew about STDs,” he said, “was that there is untreatable gonorrhea out there,” which has resulted in more funding and research opportunities.

N. gonorrhoeae antibiotic resistance is a pattern that has persisted for decades, since the sulphonamides of the 1930s. But because the return on investment for a single-dose antibiotic for the infection has not been “particularly motivating” for drug developers, Dr. Klausner said, new strategies are needed. He has pursued one such strategy for the past decade.

In *N. gonorrhoeae*, there are multiple targets for different antimicrobials and multiple mechanisms of resistance. Dr. Klausner zeroes in on where fluoroquinolones work on the gyrA enzyme, which is coded by the *gyr*A gene. “As we develop better tools and understanding, we can learn more about how we can use the prediction from sequencing to look at antimicrobial susceptibility.”

N. gonorrhoeae is notorious for its promiscuity and ability to collect DNA elements, either plasmids or chromosomal genes from neighboring organisms, he said. “The oropharynx normally has a high level of *Neisseria* commensals. It’s normal in the throat to have a *Neisseria* cinerea, *Neisseria* subflava, other *Neisseria* species, and the thought is that by taking antibiotics, those other species may develop resistance.” When a patient acquires a gonococcal infection, there is easy transformation of those resistant elements to the new *N. gonorrhoeae* that have just landed in the throat, “a particularly good place for growth and the passage of resistant elements.”

CDC data from 2016 on antimicrobial susceptibility of *N. gonorrhoeae* isolates showed that 73 percent of isolates are ciprofloxacin susceptible, with 27 percent resistant. “I began thinking, we treat this bug with a sledgehammer by treating every infection with ceftriaxone plus azithromycin,” Dr. Klausner said. “If we actually knew the susceptibility profile of the organism at the time of treatment, we could probably be smarter. We could reduce the selection pressure on the organism by the use of different antibiotics.”

DNA gyrase A is the target of ciprofloxacin. “In a wild-type gyrase, the ciprofloxacin binds to that enzyme, inhibits the enzymatic activity, prevents a normal development of DNA, and the organism is nonviable,” Dr. Klausner said. In the mutated enzyme, the ciprofloxacin cannot bind or act and the organism is resistant. Different work has shown that a single point mutation at Ser-91 is associated with this mutation. “We’re lucky that one single point mutation was both necessary and sufficient,” he said. “While there are other mutations, such as in *par*C and *gyr*B, the singular presence of the alteration in Ser-91 can predict both sensitivity and resistance.”

His researcher’s review of more than 1,000 isolates and about 10 different studies found that the summary of the wild-type *gyr*A to predict fluoroquinolone susceptibility was 98 percent sensitive and nearly 99 percent specific.

Dr. Klausner’s group and Mark Pandori, PhD, formerly of the San Francisco Public Health Laboratory, developed in 2006 a reverse transcription PCR assay to study fluoroquinolone susceptibility. Using different probes and melting

points generated from that process, “we were able to clearly differentiate a resistant isolate from a susceptible isolate.”

Their early data looked at the MIC of about 100 different *N. gonorrhoeae* isolates to ciprofloxacin, and the excellent differentiation by their *gyr*A assay, Dr. Klausner said, showed a very high range of MICs in the wild-type and mutant isolates (Siedner MJ, et al. *J Clin Microbiol*. 2007;45[4]:1250–1254). “We had one missed call where the organism was determined to be wild type but actually was resistant,” he said.

Phase one of the study Dr. Klausner reported on at the AMP meeting was assay verification. They used urine triplicates seeded with wild-type or mutant *gyr*A isolates at a concentration of 1,000 CFU/mL, from the CDC and the University of Washington. “We found no difference in the performance across the different triplicates,” Dr. Klausner said. This phase included cross-reactivity studies because of a primer concern that there could be cross-reaction with other common Neisseria species in the throat. “We did not see that. The primers were developed to be targeted toward *Neisseria gonorrhoeae*, not other *Neisseria* species” (Hemarajata P, et al. *J Clin Microbiol*. 2016;54[3]:805–808).

In phase two of the study, Dr. Klausner and colleagues introduced the assay for routine clinical use at UCLA Health, which serves more than 500,000 patients at two hospitals and in more than 150 primary care clinics and other outpatient settings. “In November 2015, we began to do reflex *gyr*A genotyping on all *Neisseria gonorrhoeae* nucleic-acid–positive clinical specimens at the UCLA Health microbiology laboratory.” *Gyr*A results were reported to providers in the electronic health record within 24 to 48 hours of receipt of the specimen. The report showed the positive *N. gonorrhoeae* result and the presence or absence of the wild-type *gyr*A gene. A standard disclaimer said the *gyr*A assay was not FDA approved but had good performance results in accordance with CLIA requirements.

The study’s objectives were to examine the impact of *gyr*A genotyping on *N. gonorrhoeae* treatment at UCLA Health and to evaluate patient outcomes among those with a wild-type gyrA genotype. In a retrospective review portion of the study, Dr. Klausner’s team examined records for all laboratory-confirmed cases of *N. gonorrhoeae* between Jan. 1, 2015 and Sept. 8, 2017. They also collected test-of-cure data among patients with wild-type *N. gonorrhoeae* infections treated with ciprofloxacin.

“During this 32-month study, there were more than 500 *Neisseria gonorrhoeae* infected patients,” Dr. Klausner said. Some patients had multiple anatomic site-specific infections or repeat infections. Forty percent of infected patients were treated empirically on about the same day. “That is one limitation to the assay,” he said, since standard practice is to treat gonorrhea immediately before obtaining test results if a patient is symptomatic.

For patients who were not empirically treated, the average time from specimen collection to treatment was up to five days, which provides a good window for performing reflex testing and sending results to the provider to inform treatment choices, he said.

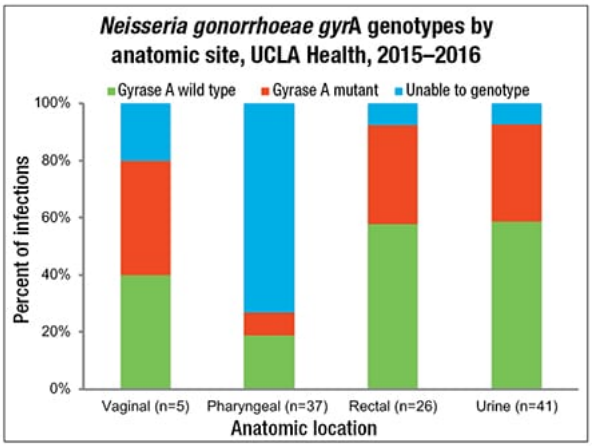
“When we looked at 655 infections that underwent *gyr*A genotyping, we had results for 43 percent that were wild type, 27 percent were mutant, and about 30 percent were indeterminate,” Dr. Klausner said. The reason for the high number of indeterminate infections is complicated, he added, and may have to do with the site of specimen source, either the rectum or pharynx. “We still see that difficulty,” he tells CAP TODAY. “Newer assays are somewhat better, but because of lower bacterial burden in the pharynx, or competition from other Neisseria species in the pharynx, the ability of the assay to characterize the *gyr*A gene is decreased.” Research is underway on the molecular assays’ performance to predict susceptibility so that they are equally successful, independent of anatomic site, “because we know that *Neisseria gonorrhoeae* infections in the throat can be spread to sex partners, and we also know that *Neisseria gonorrhoeae* infections in the throat serve as an important reservoir where drug resistance is acquired.”

One of the study’s primary outcomes showed a decline in ceftriaxone use for treatment of *N. gonorrhoeae* infections from 94 percent before *gyr*A genotyping to 76 percent after *gyr*A genotyping. “What replaced that ceftriaxone treatment, surprisingly, was ciprofloxacin,” Dr. Klausner said. “Over time, we had a nice increase in the use of ciprofloxacin among those who were nonempirically treated.”

A retrospective test-of-cure study of 25 patients with *gyr*A wild-type infections treated with ciprofloxacin showed a 100 percent cure rate, regardless of anatomic site. “The sample size is small but quite encouraging.”

To sum up, the study found that routine *gyr*A genotyping can be implemented in a large health system and that gyrA results can have an impact on the treatment of patients with *N. gonorrhoeae*. Ceftriaxone use declined with a rise in ciprofloxacin use, and the test of cure was 100 percent (Allan-Blitz LT, et al. *Clin Infect Dis*. 2017;64[9]: 1268–1270). It was a single-center study, further implementation and replicaton of the *gyr*A assay are needed, and there was no measure of the ecologic impact of the reduction in ceftriaxone use on *Neisseria gonorrhoeae* in the population, Dr. Klausner said, citing the study’s limitations.

“For me, it was important to show that this could be done,” he tells CAP TODAY. He isn’t certain his team’s assay is the best. “But it is a laboratory-developed method that is widely available, and any laboratory can follow the published protocol and replicate it.”



Final clinical validation is underway as phase three. It’s an NIH-funded clinical study of the assay with 240 *N. gonorrhoeae gyr*A wild-type culture-positive patients treated with 500 mg of ciprofloxacin. “We completed enrollment at the end of December. The results look promising,” Dr. Klausner says. “We will be able to produce a precise estimate of the efficacy of ciprofloxacin in the treatment of *Neisseria gonorrhoeae* infections, with predicted susceptibility as determined by the *gyr*A molecular assay.”

He expects genotypic testing to be a topic of discussion among groups updating gonorrhea treatment guidelines. “This is an important topic for which now there is new evidence. Molecular susceptibility testing can enable physicians to select specific antibiotics,” he says.

SpeeDx (Sydney, Australia) received the CE mark and the Australian Therapeutic Goods Administration approval for its assay to predict ciprofloxacin susceptibility in *N. gonorrhoeae*, Dr. Klausner says. Shield Diagnostics (San José, Calif.) announced in March the launch of its Target-NG, a rapid molecular test for antibiotic susceptibility in *N. gonorrhoeae*. The company says Target-NG can determine if a given gonorrhea infection is susceptible to ciprofloxacin with the same turnaround time as gonorrhea screening tests. (Dr. Klausner has advised various test manufacturers, among them Shield Diagnostics, SpeeDx, Cepheid, Hologic, and Click Diagnostics.)

Advances in molecular testing are key to stemming the growth of antimicrobial resistance, Dr. Klausner said. “New genotypic *Neisseria gonorrhoeae* diagnostics are here—highly accurate and predictive of susceptibility and treatment outcomes. And better, multi-targeted tests integrated with *Neisseria gonorrhoeae* detection and additional antibiotics are forthcoming.”

Amy Carpenter Aquino is CAP TODAY senior editor.