Multiple Drug–Resistant *Chlamydia trachomatis* Associated with Clinical Treatment Failure

Jyoti Somani,[1] Vinod B. Bhullar,[2] Kimberly A. Workowski,[1] Carol E. Farshy,[2] and Carolyn M. Black[2]

[1]*Division of Infectious Diseases, Emory University School of Medicine, and* [2]*Division of AIDS, STD, and Tuberculosis Laboratory Research, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia*

In vitro susceptibility testing and genotyping were done on urogenital isolates of *Chlamydia trachomatis* from 3 patients, 2 of whom showed evidence of clinical treatment failure with azithromycin and one of whom was the wife of a patient. All 3 isolates demonstrated multidrug resistance to doxycycline, azithromycin, and ofloxacin at concentrations >4.0 μg/mL. Recurrent disease due to relapsing infection with the same resistant isolate was documented on the basis of identical genotypes of both organisms. This first report of clinically significant multidrug-resistant *C. trachomatis* causing relapsing or persistent infection may portend an emerging problem to clinicians and public health officials.

Chlamydia trachomatis, a nonmotile, gram-negative obligate intracellular bacterium, is primarily a human pathogen that causes inclusion conjunctivitis, lymphogranuloma venereum, and urogenital tract disease. Genital tract infection with *C. trachomatis* is asymptomatic in 50%–80% of men and women [1, 2]. In men, symptomatic *C. trachomatis* infection may manifest as urethritis or epididymitis, whereas in women it often presents as cervicitis, urethritis, salpingitis, or endometritis. Asymptomatic or "silent," chronic infection in women has been recently recognized as a significant cause of infertility [3]. *C. trachomatis* infection is the most commonly reported infectious disease in the United States. This may be in part because of its well-known ability to cause asymptomatic infection, thus creating a reservoir that facilitates widespread transmission among multiple partners [4–7]. Until recently, asymptomatic infection and the lack of a simple and sensitive screening test have been barriers to the accurate detection of *C. trachomatis* infection. With the development of nucleic acid amplification technology, efforts to define the epidemiology of *C. trachomatis* infection have been renewed.

A well-documented feature of chlamydial infection has been its high rate of recurrence among sexually active populations [8]. However, determining whether the high rate of recurrent disease is due to reinfection or to persistent infection with the same organism has been difficult [9]. Immunity to chlamydial infections is type specific; thus, once an initial infection is resolved, reinfection is believed to be the result of exposure to chlamydial strains that differ in type from the initial infecting strain [10]. In contrast, persistent infections are those in which *Chlamydia* has entered a metabolically quiescent and noninfectious state; such infections have been demonstrated in both mouse models and cell culture [11–13]. Unless the interval between infections is too short to mount an immune response, persistent infection can presumably be distinguished from reinfection by demonstrating that the chlamydial strains from both the initial and subsequently detected infections have identical major outer membrane protein (MOMP) gene sequences. However, chlamydial genotyping data that aid distinction between reinfection and persistent infection have been limited in reported studies of recurrent infections. *Chlamydia*-specific DNA and antigens have been found in the upper genital tracts of infertile women, but attempts to culture *C. trachomatis* from these specimens have generally been unsuccessful, suggesting that persistent infection may not be uncommon [13–15].

Of particular concern in this era of increasing antibiotic resistance is whether persistent infection is a consequence of increasing resistance to standard antimicrobial agents. Although *C. trachomatis* has been historically sensitive to the tetracyclines, macrolides, and fluoroquinolones, recent reports have noted increasing in vitro resistance [16]. However, although in vitro antimicrobial resistance to tetracycline and erythromycin has been described [17], the clinical significance of these findings is unknown.

We describe 2 patients with *C. trachomatis* infections that persisted after standard treatment and that demonstrated multidrug resistance. To our knowledge, these are the first reported cases of clinically significant *C. trachomatis* infection resistant to ofloxacin and azithromycin. In addition, for one of the pa-

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Reprints or correspondence: Dr. Carolyn M. Black, Mailstop C17, Centers for Disease Control and Prevention, 1600 Clifton Rd. NE, Atlanta, GA 30333 (cxb2@cdc.gov).

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tients, we documented recurrent disease due to relapse with the same resistant isolate, on the basis of genotyping of both organisms. We also describe the wife of one of the patients, who was infected with the same multidrug-resistant strain. We believe these cases may signify an emerging problem with resistant *C. trachomatis* infections, which could have far-reaching implications for subsequent patient management.

Materials and Methods

Case reports. Patient 1 was a 17-year-old pregnant woman from Wyoming who was seen on 30 May 1997 for her first prenatal visit at 30 weeks of pregnancy. Routine screening for *C. trachomatis* was positive by ligase chain reaction (LCR) testing of urine. She was given erythromycin on 6 June 1997, but her treatment was changed to amoxicillin, 500 mg 3 times daily, on 7 June 1997 because of gastrointestinal intolerance, and she completed the recommended 7-day course of therapy. Results of the LCR test of urine were positive on 20 June 1997. She denied being sexually active since becoming pregnant and affirmed compliance with treatment. On 3 July 1997 she was given 1 g of azithromycin. Repeat LCR tests of urine were positive on 18 and 28 July 1997. She delivered a normal infant on 23 August 1997, at which time a cervical swab specimen was tested by culture and found to be positive for *C. trachomatis.* Further clinical information on the mother and child were not available. Because of the persistence of her chlamydial infection, her isolate was sent to the Centers for Disease Control and Prevention (CDC) for susceptibility testing (table 1).

Patient 2 was a 29-year-old heterosexual man from Atlanta who had recurrent episodes of *C. trachomatis* urethritis that were documented by DNA probe on 13 October 1995, 6 March 1996, 6 October 1996, and 2 and 30 April 1997. Before April 1997, he had clinically responded to standard doxycycline therapy (100 mg orally twice daily for 7 days), with resolution of symptoms. His wife (patient 3) was treated with doxycycline at each episode of his infection, but specimens collected from her were negative by DNA probe throughout this time period (October 1995 to April 1997). On 2 April 1997, he received 1 g of azithromycin for symptomatic urethritis, but he returned with persistent symptoms on 30 April 1997. On 12 May 1997, he described intermittent dysuria and discharge but had no objective evidence of urethritis. Urine and urethral specimens collected on 12 May 1997 were positive for *C. trachomatis* by polymerase chain reaction (PCR). Human immunodeficiency virus (HIV) antibody tests, serologic testing for syphilis, and culture for *Neisseria gonorrhoeae* were all negative. He was reevaluated on 21 May 1997 for persistent dysuria, and a urethral Gram's stain revealed urethritis (21 May 1997). Urine and urethral specimens collected on 21 May 1997 were tested at the CDC by PCR and culture of *C. trachomatis* and were assayed for antimicrobial susceptibility. *C. trachomatis* culture and PCR results were positive, and the isolate showed resistance to doxycycline, azithromycin, and ofloxacin (table 1). Patient 2 was treated with azithromycin on 21 May 1997, with subsequent resolution of his symptoms. Patient 3 was empirically treated with azithromycin on the same day (21 May 1997) by another clinician on the basis of known contact exposure to her husband but had no *C. trachomatis* diagnostic testing done at that time. Follow-up urine specimens from both patients and the urethral swab from patient 2 were negative for *C. trachomatis* by PCR on 23 June 1997. Six months later, patient 3 noted increasing vaginal discharge and sought medical attention in January 1998. At this time, both she and her husband (patient 2) denied any history of marital infidelity during the previous 6 months. Pelvic examination revealed cervicitis, and culture of cervical specimens was positive for *C. trachomatis*. HIV testing, serologic testing for syphilis, and culture of cervical specimens for herpes simplex virus and *N. gonorrhoeae* were negative. Also at this time, her husband (patient 2) complained of slight dysuria. A urethral swab specimen from him was also culture positive for *C. trachomatis*. Genotypes of both isolates and antibiotic susceptibilities were determined at the CDC. Both patients were treated with azithromycin; subsequent PCR testing of urine was negative 4 weeks and 3 months later. Further clinical information and laboratory evaluation of these patients were not available.

PCR. A commercially available PCR test (Amplicor PCR; Roche Diagnostics, Indianapolis) was used to detect *C. trachomatis* in urine and urethral and cervical swab specimens submitted to the CDC. The PCR test was done according to the manufacturer's protocol.

Antibiotic susceptibility testing. Antimicrobial susceptibility testing with doxycycline, azithromycin, and ofloxacin was done on *C. trachomatis* strains isolated by culture in BGMK cells from urethral or cervical swab specimens that had been transported in M4 transport medium (MicroTest, Snellville, GA). In vitro antimicrobial susceptibility testing was done as described elsewhere [18–20] with minor modifications [16]. Briefly, susceptibility testing was done in cell culture with BGMK cells grown to 90% confluence

Table 1. Antibiotic susceptibilities of *Chlamydia trachomatis* isolates from 3 patients and of positive and negative control isolates.

Isolate, date obtained	Doxycycline MIC_{90}	Doxycycline MIC	Doxycycline MCC	Azithromycin MIC_{90}	Azithromycin MIC	Azithromycin MCC	Ofloxacin MIC_{90}	Ofloxacin MIC	Ofloxacin MCC
Patient 1, 8/23/1997	ND	0.3	>4.0	ND	0.5	>4.0	ND	4.0	>4.0
Patient 2									
5/21/1997	ND	0.125	>4.0	ND	1.0	>4.0	ND	2.0	>4.0
1/1998	0.03	>4.0	>4.0	0.125	0.5	>4.0	0.125	>4.0	>4.0
Patient 3, 1/1998	0.03	>4.0	>4.0	0.125	>4.0	>4.0	0.125	>4.0	>4.0
Susceptible control	0.008	0.015	0.03	0.25	0.5	1.0	0.25	0.5	0.5
Resistant control	0.06	>4.0	>4.0	0.5	>4.0	>4.0	0.5	>4.0	>4.0

NOTE. MIC, MIC_{90} (90% of MIC), and MCC (minimum chlamydicidal concentration) values are given in μg/mL. Dates are given as month/day/year or month/year. ND, not determined.

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in 48-well microtiter plates (Costar, Cambridge, MA). Each well was inoculated with 300 μL of the test isolate, diluted in tissue culture medium (Iscove's modified Dulbecco's medium; Life Technologies GIBCO BRL, Gaithersburg, MD) to yield ∼10,000 inclusion-forming units (ifu)/well (or ∼40 ifu/×400 field). This inoculum resulted in infection of ∼20% of the host cells in the monolayer. Microtiter plates were centrifuged at 1750 *g* for 1 h, after which the supernatants were aspirated. Antimicrobial agents were obtained as standard powders for in vitro susceptibility testing and were reconstituted according to the manufacturers' instructions: doxycycline and azithromycin from Pfizer Laboratories (Groton, CT) and ofloxacin from Ortho Pharmaceuticals (Raritan, NJ). Antimicrobial agents were prepared by 2-fold dilution in Iscove's modified Dulbecco's medium containing 1.8 μg/mL cycloheximide, 584 mg/L L-glutamine, and 10% fetal calf serum and then were added to each well to give a final concentration range of 0.008–4.0 μg/mL. Plates were incubated at 35°C in 5% CO_2 for 48–72 h. After incubation, wells were fixed with methanol and stained with a *Chlamydia* genus–specific monoclonal antibody reagent (Pathfinder; Kallestad Diagnostics, Austin, TX) for identification of the inclusions to determine the MIC and MIC_{90} of the antimicrobial agents for these isolates. The minimum chlamydicidal concentration (MCC) of antimicrobial agents for these isolates was determined after a subsequent passage of the contents of duplicate unstained wells to a fresh monolayer in antibiotic-free medium. Because we have observed previously that the earliest and most sensitive measure of an inhibitory effect of antimicrobials on *C. trachomatis* is a dramatic alteration of the morphology and size of the inclusions, the MIC of each agent was defined as the concentration of antibiotic at which no inclusions of typical morphology were identified on direct fluorescent antibody staining after incubation in cell culture [16]. The MIC_{90} was defined as the concentration of antibiotics at which 90% of typical inclusions were inhibited after incubation in cell culture. The MCC was defined as the lowest concentration of drug that permitted no inclusions to be formed on passage in an antibiotic-free medium.

Resistant and susceptible controls, consisting of *C. trachomatis* laboratory strains with previously characterized susceptibilities (one fully susceptible [N-8685] and one resistant to doxycycline, azithromycin, and ofloxacin [CDC-TU-486]), were included in each assay. N-8685 is a serovar D strain that was originally obtained from the University of Washington. N-8685 is routinely used as a susceptible control strain in our laboratory because it exhibits MIC and MCC activities against doxycycline, azithromycin, ofloxacin, and clindamycin that are representative of ∼40 laboratory strains and clinical isolates in a collection at the CDC of strains of the 3 most prevalent serovars (D, E, and F). Some of these isolates were in the asymptomatic infection group previously described by our laboratory in a study of >40 *C. trachomatis* isolates that were evaluated for susceptibilities to commonly used antibiotics, including doxycycline, azithromycin, and ofloxacin [16]. The resistant control strain CDC-TU-486 is a clinical isolate from an 18-year-old woman with cervicitis who was seen at a teen clinic in Atlanta in 1996. She was treated with azithromycin but then was lost to follow-up, so we have no knowledge of whether symptoms resolved or treatment failed.

Each assay was repeated with a lower inoculum size (5000 ifu/well or 10 ifu/×400 field) and with 6.0-mL glass vials containing a 12-mm coverslip used in place of 48-well tissue culture cluster plates to confirm results. Patient isolates determined to be resistant and negative controls without identifiers were sent to the laboratory of R. Jones (Indiana University School of Medicine, Indianapolis) for independent confirmation of the antimicrobial susceptibility results.

Genotyping. *C. trachomatis* DNA samples were prepared from 500 μL of the culture transport medium from endocervical (patients 1 and 3) or urethral (patient 2) swab specimens by centrifugation for 10 min at 12,000 *g* followed by resuspension of the pellet in a lysis buffer containing 10 m*M* Tris-HCl at pH 8.3, 0.05% Triton X, and proteinase K (final concentration, 100 μg/mL) for 1 h. Proteinase K was then inactivated by incubation at 95°C for 30 min. Processed samples were amplified by a nested PCR assay developed in our laboratory at the CDC. The PCR assay used primers specific to the *C. trachomatis* MOMP gene (*omp*1). For the primary amplification, 25 μL of the lysate was added to 75 μL of a reaction mix with a final concentration of 10 m*M* Tris-HCl at pH 8.3, 50 m*M* KCl, 200 μ*M* of each dNTP, 25 pmol of each primer (CT90UF: 5′-GGACATCTTGTCTGGCTTTAACT-3′ and CT220DR: 5′-GCGCTCAAGTAGACCGATATAGTA-3′), and 2.5 U of Taq polymerase (Perkin Elmer, Foster City, CA). Amplification was done in a thermocycler (Perkin Elmer 9600) under the following conditions: 94°C for 5 min, followed by 40 cycles of 94°C for 30 s, 52°C for 1 min, and 72°C for 1 min and a final extension at 72°C for 5 min. For the nested PCR reaction, 5 μL of the first-round PCR product was added to 95 μL of a reaction mix prepared as described above except with the substitution of the primer pair (CT40UF: 5′-ATAGCGAGCACAAAGAGAGC-3′ and CT80DR: 5′-CCAGAAACACGGATAGTGTTATTA-3′). Amplification was done as described above. PCR products were analyzed on a 1.2% agarose gel and were stained with ethidium bromide to confirm amplification and DNA fragment size. Amplified products were purified with a kit (PCR purification kit; Qiagen, Chatsworth, CA). Sequencing was done on an automated sequencer by use of a dRhodamine Terminator Cycle Sequencing kit (model 377; Perkin Elmer Biosystems, Foster City, CA), according to the manufacturer's instructions. Edited sequences were aligned and analyzed with the GCG software package (Genetic Computer Group, Madison, WI). Genotypes were determined by comparison of our sequences with reference *C. trachomatis omp*1 sequences in the GenBank database.

Serologic testing. Serum samples were tested by microimmunofluorescence (MIF) testing as described elsewhere [21], with commercially available *C. trachomatis* antigen pools representing D–K and the L_{1-3} serovars (Washington Research Foundation, Seattle). To label specifically bound anti-chlamydial antibody in the test serum, we used fluorescein isothiocyanate–conjugated goat anti–human IgG monoclonal antibody. Positive and negative reference control sera were included in each assay, and each test was repeated to confirm the result.

Results

The MIC and MCC activities of multiple antibiotics for the chlamydial isolates from our 3 patients are described in table 1. The MIC and MCC activities for all drugs tested were similar

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for both the high (10,000 ifu/well) and low (5000 ifu/well) inoculum sizes. In all but 1 case (patient 1, azithromycin MIC), MIC and MCC activities of all drugs tested were markedly higher for patient isolates than for a susceptible control *C. trachomatis* strain. Doxycycline MICs were >4.0 μg/mL for isolates from patient 2 and 3 but only 0.015 μg/mL for the susceptible control strain. The azithromycin MICs ranged from 0.5 μg/mL to >4.0 μg/mL for patient isolates, compared with 0.5 μg/mL for the susceptible control strain. All isolates were also resistant to ofloxacin, with MICs of 2.0 and >4.0 μg/mL for patient isolates and 0.5 μg/mL for the control strain. MIC and MCC activities of isolates from patients 2 and 3 were tested blindly by an independent laboratory and found to be similar to our results.

The clinical isolate from patient 1 was determined to be *C. trachomatis* subtype (or serovar) E on the basis of the deduced amino acid sequence of the MOMP. Additional clinical material could not be obtained from patient 1. All clinical isolates from patient 2 and his wife (patient 3) were determined to be subtype F. The *omp*1 genotypes of the isolates from patients 2 and 3 were found to be identical. Furthermore, genotypes of the organisms obtained from patient 2 at initial diagnosis and during his relapse in January 1998 were identical. Interestingly, at the time of relapse, MIF testing of serum samples revealed a titer of anti-chlamydia IgG of ⩽1 : 16 for patient 2 and 1 : 256 for his wife. Measurement of IgM yielded negative results for both.

Discussion

These 3 patients, 2 of whom failed to respond clinically to antibiotic treatment, represent the first well-documented cases of infection with multidrug-resistant *C. trachomatis* isolates. In addition to alerting us to the likelihood of emerging resistance of *C. trachomatis*, clinical findings from patient 2 in particular suggest that *C. trachomatis* infection may remain in a latent state, evident by the negative intervening PCR testing, and then relapse months later, causing symptomatic disease.

The characteristics of antibiotic resistance of *C. trachomatis* differ significantly from those of other bacteria in several ways. First, because chlamydiae are intracellular pathogens, antimicrobial susceptibility must be determined by their ability to proliferate within a host cell in the presence of varying concentrations of antibiotic. Second, unlike the case for most bacteria, when *C. trachomatis* organisms are found to be resistant to typically effective antibiotics such as tetracycline, the resistance is not absolute. In fact, *C. trachomatis* displays what is known as “heterotypic resistance” in vitro; that is, the chlamydial population contains both susceptible and resistant organisms. Thus, although it is possible that all organisms within a population may be capable of expressing resistance, only a small proportion do so at any one time. The marked differences that were observed between the MIC_{90} and MIC for isolates from patients 2 and 3 illustrate this concept, as does the difference between the MIC and MCC for patient 1 (table 1). Removal of the antibiotic from the medium during testing for the MCC allows the small percentage of organisms that were resistant to the first exposure to antibiotic (MIC) to then multiply and form inclusions. In our laboratory, heterotypic resistance exhibited by some *C. trachomatis* strains would have been missed unless both MIC and MCC testing were done (CDC, unpublished data). Similar to our findings, Jones et al. [17] reported a tetracycline-resistant isolate of *C. trachomatis* for which only ~1% of the population demonstrated resistance. More recently, Lefevre et al. [22] reported an infection with tetracycline-resistant *C. trachomatis* in a woman who was found to have asymptomatic inflammation on cervical cytologic testing. Their antimicrobial susceptibility studies suggested that only a small proportion (<1%) of organisms were resistant. Although resistant isolates do form inclusions at high concentrations of tetracycline, there are far fewer inclusions formed than at the lower concentrations, suggesting that only a small proportion of organisms within the population express resistance. Furthermore, in strains that exhibit heterotypic resistance, we see many aberrant inclusions, and the proportion of atypical to typical inclusions gradually increases along with a decrease in the overall number of inclusions until all inclusions are aberrant or absent; this may explain the large difference in isolates from patients 2 and 3 between the MIC_{90} (some inclusions are still typical) and the MIC (no inclusions, or all inclusions are atypical). We believe this to be a result of the fact that the resistance exhibited by individual organisms within the chlamydial population is heterogenous (defined as heterotypic resistance).

Jones et al. [17] noted that the heterotypic resistance shown by isolates of *C. trachomatis* was apparent only with large inocula ($>5.0 \times 10^3$ ifu/well). This is because with inocula of $<5 \times 10^3$ ifu, the number of inclusion-forming units that remain viable after exposure of heterotypically resistant strains to the antibiotic is so small that they can easily be missed during visualization under a microscope for MIC determinations. We used inocula of both 5×10^3 and 10×10^3 ifu in our study and found no difference in MIC and MCC activities for any strains tested. However, the susceptible control strain failed to show any resistance at either inoculum size; thus, it is not the inoculum size itself that produces the apparent resistance.

The mechanism responsible for heterotypic resistance in *C. trachomatis* is not known. It is possible that the multidrug resistance that we observed is phenotypic in nature rather than genotypic, because the molecular targets of azithromycin, doxycycline, and ofloxacin are quite different, and it is unlikely that a single or limited number of gene mutation(s) could be responsible for simultaneous resistance to these diverse agents. We suspect that, rather than being direct resistance, heterotypic resistance may be a by-product of some undefined alteration of the growth rate or life cycle, resulting in a longer phase or intermediate stage that is more refractive to antimicrobial

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agents. Alternatively, heterotypic resistance may be mediated by some kind of mechanism that excludes the drug from the chlamydial cell or inclusion (e.g., an efflux pump). Future studies are needed to test these hypotheses.

It is possible that the phenomenon of heterotypic resistance is not new but remains largely undetected, because test-of-cure is not routinely done for chlamydial infections and a clinician is not likely to suspect persistence because the rate of "recurrent" infections due to reexposure is so high. Whereas in vitro antimicrobial resistance of *C. trachomatis* has been recognized since as early as 1980, its clinical significance has been unclear, because patients have responded to the antimicrobial agent nevertheless [17, 23]. Jones et al. [17] reported 5 *C. trachomatis* isolates that exhibited resistance to tetracycline, doxycycline, erythromycin, and clindamycin but were sensitive to ofloxacin and ciprofloxacin. Of the 5 patients, 3 had negative follow-up cultures (2 after treatment with minocycline, 1 with doxycycline) and 2 were lost to follow-up. Additionally, in vitro *C. trachomatis* resistance from female genital tract isolates has been described since the early 1990s. Recently, however, we have observed a higher level of resistance, with many typical inclusions seen at first exposure to high concentrations of drug (MIC) instead of only after passage of the strain in antibiotic-free medium (MCC) (authors' unpublished data). It is only recently, however, that the technology for molecular typing of chlamydial strains has evolved to the point that we can distinguish those recurrent infections that are most likely to be persistent. Further study is needed to support or refute the notion that heterotypic resistance of *C. trachomatis* is emerging and is related to increases in clinical treatment failures.

The recovery of *C. trachomatis* isolates demonstrating high-level resistance to multiple antimicrobial agents, including doxycycline, azithromycin, and ofloxacin, along with demonstrated clinical failure of antibiotic treatment, with questionable organism eradication in our 2 cases, may portend an emerging problem. In France, a tetracycline-resistant isolate was recovered from a woman who had persistent infection after doxycycline treatment [22]. However, susceptibility testing to azithromycin or ofloxacin was not reported. The extent that failed treatment causes persistent infection with antibiotic-resistant organisms is unknown. Certainly, the technical expertise and time required to determine antimicrobial susceptibilities for *C. trachomatis* hinder efforts to understand the extent of this problem. Furthermore, routine test-of-cure is not the standard of care. Further efforts are needed to determine the scope of this problem. Although routine testing for cure following treatment of *C. trachomatis* infection and more aggressive workup of recurrent *C. trachomatis* disease, including testing isolates for susceptibility, are not practical in most clinical settings, these measures and the possibility of resistance should be considered when treatment with standard therapy has failed.

Recurrent chlamydial genital tract infection is a common and well-documented phenomenon. Its etiology is likely multifactorial, including treatment noncompliance, repeated exposure leading to reinfection, and only partially protective immunity following infection. Recrudescence of a latent *C. trachomatis* infection may also be a cause of recurrent disease, as has been demonstrated recently. Dean et al. [12] identified 7 women who had multiple positive chlamydial cultures from episodes of recurrent infection with the same serovar over several years. *omp*1 genotyping suggested long-term persistence of the women's original strains. Latent infection in other chlamydial diseases, such as trachoma and pneumonia, has also been shown to occur [24–27]. In addition, the phenomenon of persistent *C. pneumoniae* infection has been reported elsewhere, with increased azithromycin MICs and MCCs for the clinical isolate despite patient recovery [28].

It is unclear why a multiresistant *C. trachomatis* infection appeared to resolve, at least as indicated by the very sensitive PCR assay of urine, in patient 2. It may be that the phenomenon of heterotypic resistance to antibiotics seen in cell culture occurs in vivo, so that only a small percentage of infecting organisms are resistant. Perhaps the majority of the infectious organisms are susceptible to the antibiotic, and the remaining resistant organisms are sufficiently few to be either undetectable by the diagnostic test or, in most but not all cases, eradicated by the host's immune response. In either case, it is plausible that a small number of residual organisms remain in a nonreplicative form until certain conditions restore the organism to its infectious form. Although this scenario is speculative in humans, studies with a mouse model have shown reactivation with viable *C. trachomatis* following apparent clearance of primary infection when the animals were given an immunosuppressive agent [11]. Additionally, in vitro evidence suggests that low doses of interferon-γ can induce *C. trachomatis* organisms into a nonreplicative state; interestingly, the organisms can revert back to infectious, viable forms when the interferon-γ is removed [29]. Furthermore, and most relevant to our report, a number of studies have reported that various antimicrobial agents, including penicillin, chlortetracycline, erythromycin, and sulfonamides, can also induce persistence of chlamydial infection in vitro (reviewed in [13]). In these studies, the chlamydiae produced aberrant noninfectious forms in the presence of the antibiotic that reverted to typical and infectious forms once the antibiotic was removed from the culture medium. The variety of different molecular targets of these antimicrobial agents suggests that persistence can be generated by more than one mechanism. The host's immune response also may affect clearance of *C. trachomatis* infection [30]. However, a much greater understanding of the elements of persistent *C. trachomatis* infection and the resultant immune response is necessary [13, 31]. The negative MIF serology of patient 2 is a surprising finding, although difficult to interpret. MIF serology, although the most sensitive of the serologic tests for chlamydia, has not been widely studied in men [31, 32]. Among women, positive MIF antibody titers have been shown to correlate with chronic

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upper genital tract infection [32–34]. In a study of 387 men attending a sexually transmitted disease clinic, 91.3% of men whose culture of urethral specimens was positive were also positive for IgG by MIF, and 80.4% of culture-negative men were positive for IgG [35]. IgM seroreactivity was much less common in both groups of men. It may be that patient 2 was unable to mount an effective immune response; however, subsequent serologic testing would be useful to delineate any future immune response.

There are no data regarding management of clinically resistant *C. trachomatis* infection. In vitro data suggest that resistance to ofloxacin imparts resistance to other fluoroquinolones, such as ciprofloxacin. Although many of the newer quinolones, including trovafloxacin, sparfloxacin, grepafloxacin, and tosufloxacin, have equal or greater MICs for *C. trachomatis*, they need to be tested against an ofloxacin-resistant strain [36, 37]. Perhaps a prolonged course of therapy with a standard agent such as doxycycline or azithromycin would be effective against resistant *C. trachomatis* disease, because such therapy has been efficacious against *C. pneumoniae* infection in cases of relapse [38].

In conclusion, we present the first 2 cases of clinically significant multidrug-resistant *C. trachomatis* infection causing relapsing or persistent infection. The additional case we present is the wife of one of these patients, who also had a multidrug-resistant infection but in whom we could not document persistent infection. These cases may suggest an emerging problem to clinicians and public health officials. We believe that increased surveillance for both in vitro resistance and treatment failure should be implemented in select settings to determine the role of heterotypic resistance in transmission and maintenance of *C. trachomatis* infections.

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