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Fluoroquinolone Resistance in *Ureaplasma parvum* in the United States

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We report the first case of naturally occurring fluoroquinolone resistance in *Ureaplasma* spp. from the United States. Resistance in this case probably developed as a result of mutations in the *gyrA* and *parC* genes of the DNA gyrase/topoisomerase IV complex that occurred in the presence of antimicrobial selective pressure.

A 28-year-old woman receiving care through the Cleveland Clinic with a history of chronic bladder infections and severe bladder thickening had been treated with prolonged courses of tetracycline, azithromycin, and ofloxacin. She had numerous vaginal cultures that were positive for *Ureaplasma* spp. as well as urine cultures that were positive for *Klebsiella pneumoniae*. The vaginal ureaplasma isolate, designated 48105, was submitted for antimicrobial susceptibility testing and speciation in January 2002. MICs (Table 1) determined by broth microdilution (13) showed elevated values for all fluoroquinolones, but results were no different than what would be expected for erythromycin and doxycycline (14). MICs were confirmed by repeat testing. After susceptibility test results were reported, she was treated with doxycycline. However, therapeutic response continued to be poor, and she eventually underwent cystectomy with urinary diversion and placement of a ureteral stent. Despite surgical intervention, she was subsequently admitted to other hospitals over several months for uroseptic episodes. The ureaplasma isolate was identified to the species level as *U. parvum* by PCR using species-specific probes for conserved regions of *Ureaplasma* 16S rRNA (9).

Bacterial resistance to fluoroquinolones has been attributed to substitution mutations, principally in the *gyrA* and *parC* genes and, to a lesser extent, in the *gyrB* and *parE* genes of the DNA gyrase/topoisomerase IV complex (6). The mechanism of fluoroquinolone resistance in *U. parvum* 48105 was investigated by amplification of these genes by PCR and sequencing of purified PCR products using corresponding sequences of the published ureaplasma genome as a basis for comparative analysis and identification of base substitutions (8). *U. parvum* cells were grown in 200 ml of 10B medium (13), and genomic DNA was extracted from pelleted cells using a QIAGEN DNeasy Tissue kit (QIAGEN, Inc., Valencia, CA) according to the manufacturer's instructions. PCR primers flanking *gyrA* (UU082), *gyrB* (UU081), *parC* (UU467), and *parE* (UU466) genes were designed based on the *U. parvum* serovar 3 genome sequence (8), and each gene was then amplified. DNA sequences of the PCR amplicons were determined using an Applied Biosystems 3730XL sequencer (Foster City, CA) and were primed by the PCR primers as well as oligonucleotides that annealed in the central regions of the genes. Both strands of each amplicon were sequenced.

Six mutations were identified in the *U. parvum* 48105 *parC* gene, five mutations in *parE*, and one mutation in *gyrA* that resulted in amino acid substitutions (Table 2). There were only three silent mutations observed in *gyrB*. The *gyrA* mutation that resulted in the substitution of glutamine for lysine at amino acid 103 in *U. parvum* 48105 may be near enough to the tyrosine active site of the protein at amino acid 122 to contribute to the resistance observed.

Twenty-six clinical isolates of fluoroquinolone-resistant *Ureaplasma* spp. have been described thus far from China (15) and France (1, 2). Although a variety of mutations were detected, the most frequently observed genetic alterations were replacement of serine at amino acid 83 of UU467 (*parC*) with leucine and replacement of the asparagine at amino acid 112 of UU082 (*gyrA*) with glutamic acid. These mutations occurred singly in 13 isolates and in tandem in 12. Twelve of the 13 fluoroquinolone-resistant isolates had mutations in both genes. One of the *parC* mutations observed in *U. parvum* 48105 had the same serine-to-leucine alteration at *parC* amino acid 83 that has been previously reported for several of the aforementioned French and Chinese fluoroquinolone-resistant *Ureaplasma* isolates. Contributions of the other five *parC* amino acid substitutions and all five amino acid substitutions in the

TABLE 1. MICs for fluoroquinolone-resistant *Ureaplasma parvum* 48105 compared to those of *Ureaplasma* species in general[a]

Antimicrobial	MIC for *U. parvum* 48105 (μg/ml)	MIC range for *Ureaplasma* spp. (μg/ml)
Erythromycin	2	0.02–16
Doxycycline	0.063	0.02–1[b]
Gatifloxacin	16	0.125–1
Gemifloxacin	32	0.03–0.05
Levofloxacin	32	0.2–2
Moxifloxacin	8	0.12–0.5
Ofloxacin	128	0.2–25

[a] MIC range data for *Ureaplasma* spp. are from reference 14.
[b] Doxycycline-susceptible strains only.

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TABLE 2. Characterization of mutations in *Ureaplasma parvum* 48105 resulting in amino acid changes

Mutation base number[a]	Base change	Codon change[b]
gyrA (UU081)		
99378	C to A	Glutamine to lysine (103)
parC (UU467)		
531454	TA to AT	Valine to aspartic acid (3)
531694	C to T	Serine to leucine (83)
531709	CA to GC	Alanine to glycine (88)
532348	A to G	Serine to glycine (301)
533648	G to A	Alanine to threonine (734)
533650	C to T	Alanine to threonine (735)
parE (UU466)		
529963	A to G	Asparagine to aspartic acid (151)
530259	T to A	Aspartic acid to glutamic acid (249)
530268	T to C	Serine to aspartic acid (274)
530761	GT to AC	Valine to threonine (417)
530881	G to A	Alanine to threonine (457)

[a] Numbers in parentheses in this column refer to numbered genes as they were described in the published *Ureaplasma parvum* genome (8). No changes took place in *gyrB* (UU082).

[b] Numbers in parentheses in this column refer to the amino acid number (*Escherichia coli* numbering sequence).

parE gene to fluoroquinolone resistance observed in *U. parvum* 48105 appear unlikely, since they are located outside the quinolone resistance-determining region.

K. pneumoniae was isolated from urine of this patient on multiple occasions in addition to the vaginal isolations of *Ureaplasma* spp. during the same time period. However, there were other episodes when she was extremely symptomatic in which no pathogenic bacteria other than vaginal *U. parvum* were detected. Thus, it was the impression of her clinicians that *U. parvum* was the major cause of her urological problems. Irrespective of the importance of *U. parvum* from a clinical standpoint in this case, this represents the first reported isolation of a ureaplasma from a human in North America with naturally occurring resistance to fluoroquinolones.

Ureaplasma spp. are commonly found in the lower urogenital tract of healthy women, but they have sometimes been implicated as causes of acute and chronic infections of the urinary tract (7, 10–12). Eradication of ureaplasmas from the female vagina has proven difficult, sometimes necessitating prolonged courses of antimicrobial therapy (3). Fluoroquinolones are attractive choices for treating urinary tract infections. They are bactericidal, and thus far there have been very few documented reports of naturally occurring resistance in human ureaplasmas. It is likely that fluoroquinolone resistance in *Ureaplasma* spp. may occur to some degree in view of the widespread use of these drugs for treatment of respiratory and urogenital infections. Data on antimicrobial resistance in ureaplasmas are very limited, because *Ureaplasma* spp. cultures are rarely obtained for clinical purposes and in vitro susceptibilities are almost never performed. We recently reported that 45% of *Ureaplasma* spp. were tetracycline resistant and contained *tet*(M), indicating that resistance to other drug classes is also increasing (14). Documented resistance of *Ureaplasma* spp. to fluoroquinolones in the United States underscores the importance of performing susceptibility tests on these organisms in the event of fluoroquinolone treatment failure for a condition known to be associated with them, as well as in any circumstance where eradication may be difficult and/or require prolonged administration of drugs, such as in immunocompromised hosts. Evidence that the mutations described above are responsible for the fluoroquinolone resistance in *U. parvum* 48105 is circumstantial. In recent years, site-directed mutagenesis protocols have been reported for *Mycoplasma genitalium*, a close phylogenetic relative of the ureaplasmas (4, 5). Similar methods might be effective for performing allelic replacement experiments on fluoroquinolone-resistant ureaplasmas to confirm whether the observed mutations fully account for the phenotype.

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