Antimicrobial Susceptibility and Clonality of Clinical *Ureaplasma* Isolates in the United States

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Ureaplasma urealyticum and *Ureaplasma parvum* are pathogens involved in urogenital tract and intrauterine infections and also in systemic diseases in newborns and immunosuppressed patients. There is limited information on the antimicrobial susceptibility and clonality of these species. In this study, we report the susceptibility of 250 contemporary isolates of *Ureaplasma* (202 *U. parvum* and 48 *U. urealyticum* isolates) recovered at Mayo Clinic, Rochester, MN. MICs of doxycycline, azithromycin, ciprofloxacin, tetracycline, erythromycin, and levofloxacin were determined by broth microdilution, with MICs of the last three interpreted according to CLSI guidelines. Levofloxacin resistance was found in 6.4% and 5.2% of *U. parvum* and *U. urealyticum* isolates, respectively, while 27.2% and 68.8% of isolates, respectively, showed ciprofloxacin MICs of ≥ 4 $\mu\text{g/ml}$. The resistance mechanism of levofloxacin-resistant isolates was due to mutations in *parC*, with the Ser83Leu substitution being most frequent, followed by Glu87Lys. No macrolide resistance was found among the 250 isolates studied; a single *U. parvum* isolate was tetracycline resistant. *tet(M)* was found in 10 *U. parvum* isolates, including the single tetracycline-resistant isolate, as well as in 9 isolates which had low tetracycline and doxycycline MICs. Multilocus sequence typing (MLST) performed on a selection of 46 isolates showed high diversity within the clinical *Ureaplasma* isolates studied, regardless of antimicrobial susceptibility. The present work extends previous knowledge regarding susceptibility to antimicrobial agents, resistance mechanisms, and clonality of *Ureaplasma* species in the United States.

Ureaplasmas are bacteria belonging to the class *Mollicutes*. They are small, self-replicating organisms, capable of cell-free existence (1). Their small genomes and limited biosynthetic abilities are responsible for many of their biological characteristics and requirements for complex growth media for cultivation *in vitro* (1). Ureaplasmas of medical importance are subclassified into two distinct species, *Ureaplasma parvum* and *Ureaplasma urealyticum*; the former is more frequently recovered than the latter (1).

U. urealyticum and *U. parvum* are part of the human microbiota but are also involved in urogenital tract infection and associated with adverse pregnancy outcomes and bacteremia alongside complications such as bronchopulmonary dysplasia and meningitis in newborns (1–5). Recently, *Ureaplasma* species have been associated with fatal hyperammonemia among lung transplant patients (6). Furthermore, some authors suggest that *Ureaplasma* infections may be involved in other unexplained hyperammonemia syndromes (7).

Antimicrobial options for treating *Ureaplasma* infections are limited. Since this genus lacks peptidoglycan, ureaplasmas are not affected by β -lactams or other antimicrobial agents acting on this target. Moreover, they are not susceptible to sulfonamides or trimethoprim since they do not synthesize folic acid. However, members of this genus are frequently susceptible to antimicrobials that interfere with protein synthesis (macrolides and tetracyclines) and to DNA replication inhibitors (fluoroquinolones) (1, 8). Some reports of antimicrobial resistance in ureaplasmas have been published. Resistance to fluoroquinolones has been attributed to mutations in *gyrA*, *gyrB*, *parC*, and *parE*, macrolide resistance has been attributed to mutations in the 23S rRNA subunit or in the ribosomal protein L4 or L22 gene, and tetracycline resistance has been attributed to the presence of *tet(M)* (9–14).

Reports on the antimicrobial susceptibility of *Ureaplasma* are limited, especially reports separated by species. Moreover, because the multilocus sequence typing (MLST) scheme for this species has been recently developed, there are only a few studies in which the population distribution of this genus has been analyzed. Therefore, the aim of this study was to determine the susceptibility and clonality of contemporary isolates of *Ureaplasma* from the United States recovered at Mayo Clinic, Rochester, MN (including isolates from Mayo Medical Laboratories patients).

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MATERIALS AND METHODS

Specimens and isolates. Over a 5-month period (October 2015 to February 2016), all patient specimens of any origin testing positive by PCR at Mayo Clinic (Rochester, MN, USA) for *U. parvum* or *U. urealyticum* (15) were cultured in SP4 medium with urea (Hardy Diagnostics, Santa Maria, CA). A total of 250 clinical isolates (202 *U. parvum* and 48 *U. urealyticum* isolates) were recovered and characterized. The source of the samples was diverse and included urine (35.6%), vagina (31.2%), cervix (22.4%), semen (4.8%), bronchoalveolar lavage fluid (1.6%), urethra (1.2%), tra-

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TABLE 1 MIC distribution of *Ureaplasma* isolates separated by species

Species and antimicrobial ^a	No. (%) of isolates with the indicated MIC (μg/ml)									MIC ₅₀ (μg/ml)	MIC ₉₀ (μg/ml)	% susceptible ^b
	≤0.125	0.25	0.5	1	2	4	8	16	>16			
<i>U. parvum</i> (n = 202)												
LEV	14 (6.9)	64 (31.7)	85 (42.1)	23 (11.4)	3 (1.5)	8 (4)	4 (2)	1 (0.5)		0.5	1	93.6
CIP		4 (2)	9 (4.4)	38 (18.8)	96 (47.5)	35 (17.3)	7 (3.5)	12 (5.9)	1 (0.5)	2	4	NA
TET	161 (79.7)	29 (14.4)	8 (4)	3 (1.5)			1 (0.5)			≤0.125	0.25	99.5
DOX	194 (96)	7 (3.5)			1 (0.5)					≤0.125	≤0.125	NA
ERY	8 (4)	32 (15.8)	63 (31.2)	66 (32.7)	31 (15.3)	1 (0.5)	1 (0.5)			0.5	2	100
AZM	8 (4)	41 (20.3)	66 (32.7)	57 (28.2)	28 (3.9)	1 (0.5)	1 (0.5)			0.5	2	NA
<i>U. urealyticum</i> (n = 48)												
LEV		8 (16.7)	15 (31.2)	17 (35.4)	6 (12.5)	2 (4.2)				1	2	95.8
CIP				1 (2.1)	14 (29.2)	23 (47.9)	8 (16.7)	2 (4.2)		4	8	NA
TET	17 (35.4)	19 (39.6)	9 (18.7)	3 (6.2)						0.25	0.5	100
DOX	48 (100)									≤0.125	≤0.125	NA
ERY	1 (2.1)	5 (10.4)	2 (4.2)	17 (35.4)	22 (45.8)	1 (2.1)				1	2	100
AZM	1 (2.1)	5 (10.4)	5 (10.4)	17 (35.4)	20 (41.7)					1	2	NA

^a LEV, levofloxacin; CIP, ciprofloxacin; TET, tetracycline; DOX, doxycycline; ERY, erythromycin; AZM, azithromycin; n, number of isolates.

^b NA, not applicable (no CLSI breakpoint).

cheal aspirates (1.2%), sputum (0.4%), vulva (0.4%), and other urogenital sources (1.2%).

Antimicrobial susceptibility testing. Doxycycline, azithromycin, ciprofloxacin, tetracycline, erythromycin, and levofloxacin (Sigma-Aldrich, St. Louis, MO, USA) MICs were determined in duplicate by broth microdilution according to the Clinical and Laboratory Standards (CLSI) guidelines (16), using a range of antimicrobial concentrations from 0.125 to 16 μg/ml in 10B broth (Remel, Lenexa, KS, USA). Only the MICs of the last three antimicrobials were interpreted since there are no CLSI breakpoints for the first three agents. *U. urealyticum* American Type Culture Collection (ATCC) 33175 was used as a quality control (QC) strain in each broth microdilution assay.

Identification of genes encoding tetracycline and fluoroquinolone resistance. Bacterial DNA extraction from culture broth was performed using a DNeasy blood and tissue kit (Qiagen, Valencia, CA). The *tet(M)* gene was assessed by PCR, using previously described primers and cycling conditions, in all *Ureaplasma* isolates studied (11). For levofloxacin-resistant strains, PCR of the quinolone resistance-determining regions (QRDRs) of *gyrA*, *gyrB*, *parC*, and *parE* was performed as previously described (9, 11). Both *tet(M)* and QRDR amplicons were purified using ExoSAP-IT (USB Corporation, Cleveland, OH, USA) and bidirectionally sequenced using an ABI 3730xl instrument (Applied Biosystems, Foster City, CA). The *gyrA*, *gyrB*, *parC*, and *parE* sequences were analyzed using Clone Manager software (Sci-Ed Software, Cary, NC) and compared with those of the reference strains *U. parvum* ATCC 700970 and *U. urealyticum* ATCC 33699 (GenBank accession numbers AF222894 and CP001184, respectively) (9–11).

MLST. Multilocus sequence typing (MLST) was performed according to the original scheme using primers targeting four housekeeping genes, *ftsH*, *rpl22*, *valS*, and *thrS* (17), on 46 isolates (30 *U. parvum* and 16 *U. urealyticum* isolates), including all levofloxacin-resistant isolates, all *tet(M)*-positive isolates, and a group of susceptible isolates representative of the different study periods and specimen sources. The eBURST package, version 3 (<http://eburst.mlst.net/>), was used based on allelic profiles with 1,000 resamplings for bootstrapping to establish clonality and determine potential relationships between isolates.

Nucleotide sequence accession numbers. The following five new MLST locus alleles were deposited in the GenBank database under the indicated accession numbers: one for *ftsH* (KU710257), one for *rpl22* (KU710255), two for *valS* (KU726275 and KU710256), and one for *thrS* (KU710258).

RESULTS

Prevalence of resistance in *Ureaplasma* by species. Of the 202 *U. parvum* and 48 *U. urealyticum* isolates tested, levofloxacin resistance (MIC of >2 μg/ml) was found in 6.4% and 5.2%, respectively. All isolates had levofloxacin MICs at least one dilution lower than the ciprofloxacin MICs. Although there are no CLSI breakpoints for ciprofloxacin, 27.2% of *U. parvum* and the 68.8% of *U. urealyticum* isolates showed MICs of ≥4 μg/ml (the breakpoint for levofloxacin and moxifloxacin). All *U. urealyticum* isolates and all but one *U. parvum* isolate were susceptible to tetracycline (MICs of ≤2 μg/ml) and displayed equal or lower doxycycline MICs. Coresistance to levofloxacin was observed in the single tetracycline-resistant *U. parvum* isolate. All *Ureaplasma* isolates were susceptible to erythromycin and had equal or lower azithromycin MICs. Detailed results of MIC distributions, MIC₅₀ and MIC₉₀ values, and percentages of susceptibility by species of *Ureaplasma* are shown in Table 1.

Molecular characterization of antimicrobial resistance. Sequence comparison between the QRDRs of reference strains and the QRDRs of study levofloxacin-resistant isolates revealed no mutations in *gyrA*, *gyrB*, or *parE* in any isolate; however, previously described *parC* quinolone resistance-associated mutations were found. The most frequent mutation detected was Ser83Leu, which was present in 12 (92.1%) and 1 (50%) of the levofloxacin-resistant *U. parvum* and *U. urealyticum* isolates, respectively. The remaining levofloxacin-resistant *U. parvum* isolate harbored a Glu87Lys mutation, while no QRDR mutations were found in a single levofloxacin-resistant *U. urealyticum* isolate. Three levofloxacin-susceptible isolates (two *U. urealyticum* isolates and one *U. parvum* isolate) which showed ciprofloxacin MICs of ≥4 μg/ml were also amplified and sequenced, with no mutations found.

Screening of the *tet(M)* determinant, which is associated with tetracycline resistance, was carried out, and 10 (4.9%) of the *U. parvum* isolates were positive, while no *U. urealyticum* isolates harbored this gene. Interestingly, the 10 positive isolates included

TABLE 2 Microbiological features of representative *Ureaplasma* isolates of the study

Species and isolate no.	Specimen source ^a	MIC (μg/ml) ^b							QRDR ^c				MLST				
		LEV	CIP	TET	DOX	ERY	AZM	tet(M)	gyrA	gyrB	parC	parE	ftsH	rpl22	valS	rprS	ST ^d
<i>U. parvum</i>																	
IDRL-10860	Urine	4	16	≤0.125	≤0.125	0.5	0.5	—	WT	WT	S83L	WT	1	1	1	1	ST1
IDRL-10857	Vagina	4	16	0.5	0.25	2	2	—	WT	WT	S83L	WT	35	1	1	1	ST95
IDRL-10940	Vagina	8	16	≤0.125	≤0.125	0.5	0.5	—	WT	WT	S83L	WT	8	1	1	1	ST56
IDRL-10790	Urethra	4	16	≤0.125	≤0.125	0.5	0.5	—	WT	WT	S83L	WT	8	1	1	1	ST56
IDRL-10904	Cervix	4	16	8	2	0.5	0.5	+	WT	WT	S83L	WT	2	1	1	1	ST2
IDRL-11151	Cervix	4	16	≤0.125	≤0.125	0.5	0.5	—	WT	WT	S83L	WT	1	1	1	1	ST1
IDRL-11156	Urine	4	16	≤0.125	≤0.125	2	1	—	WT	WT	S83L	WT	8	1	1	1	ST56
IDRL-11160	Urine	16	>16	≤0.125	≤0.125	1	0.5	—	WT	WT	S83L	WT	2	1	22 ^e	1	ST108 ^f
IDRL-11170	Vagina	8	16	≤0.125	≤0.125	0.25	0.25	—	WT	WT	S83L	WT	1	2	1	1	ST22
IDRL-11178	Vagina	4	16	≤0.125	≤0.125	0.5	0.5	+	WT	WT	S83L	WT	1	1	1	1	ST1
IDRL-11211	Vagina	4	16	≤0.125	≤0.125	1	0.5	—	WT	WT	S83L	WT	1	1	23 ^e	1	ST109 ^f
IDRL-11260	Vagina	8	16	≤0.125	≤0.125	2	2	—	WT	WT	S83L	WT	1	12	1	1	ST103
IDRL-11232	Cervix	8	16	≤0.125	≤0.125	1	1	—	WT	WT	E87K	WT	20	2	1	1	ST111 ^f
IDRL-11141	Vagina	0.5	2	0.25	≤0.125	1	1	+	ND	ND	ND	ND	2	2	1	2	ST105
IDRL-11142	Vagina	0.5	2	0.25	≤0.125	0.5	0.5	+	ND	ND	ND	ND	2	2	1	1	ST4
IDRL-11149	Urine	0.5	2	0.25	≤0.125	1	1	+	ND	ND	ND	ND	8	1	1	1	ST56
IDRL-11152	Cervix	0.5	2	0.25	≤0.125	0.5	0.5	+	ND	ND	ND	ND	2	2	1	1	ST4
IDRL-11177	Urine	0.5	2	≤0.125	≤0.125	0.5	0.5	+	ND	ND	ND	ND	8	1	1	1	ST56
IDRL-11179	Vagina	0.5	2	≤0.125	≤0.125	0.5	0.25	+	ND	ND	ND	ND	2	1	1	2	ST38
IDRL-11252	Vagina	0.25	0.5	≤0.125	≤0.125	0.25	0.25	+	ND	ND	ND	ND	2	14 ^e	1	1	ST110 ^f
IDRL-11268	Vagina	0.5	2	≤0.125	≤0.125	2	2	+	ND	ND	ND	ND	2	2	1	1	ST4
IDRL-10852	Vagina	0.25	2	≤0.125	≤0.125	2	2	—	ND	ND	ND	ND	2	2	1	1	ST4
IDRL-10835	Cervix	2	8	≤0.125	≤0.125	0.5	0.5	—	WT	WT	WT	WT	1	1	1	1	ST1
IDRL-10774	BAL	0.25	2	≤0.125	≤0.125	0.5	0.5	—	ND	ND	ND	ND	1	2	1	1	ST22
IDRL-10874	Vagina	0.5	2	≤0.125	≤0.125	0.5	0.25	—	ND	ND	ND	ND	1	1	1	1	ST1
IDRL-10923	Cervix	0.25	1	≤0.125	≤0.125	0.25	0.25	—	ND	ND	ND	ND	2	1	1	1	ST2
IDRL-11125	Cervix	0.25	2	≤0.125	≤0.125	1	1	—	ND	ND	ND	ND	2	14 ^e	1	1	ST110 ^f
IDRL-11207	Vagina	1	4	≤0.125	≤0.125	1	0.5	—	ND	ND	ND	ND	1	2	1	1	ST22
IDRL-11257	TA	0.5	2	≤0.125	≤0.125	0.25	0.25	—	ND	ND	ND	ND	1	1	1	1	ST1
IDRL-11271	Cervix	0.5	2	≤0.125	≤0.125	2	2	—	ND	ND	ND	ND	2	1	1	1	ST2
<i>U. urealyticum</i>																	
IDRL-10763	BAL	4	16	0.25	≤0.125	2	2	—	WT	WT	S83L	WT	5	3	4	5	ST7
IDRL-11217	Urine	4	16	0.25	≤0.125	2	2	—	WT	WT	WT	WT	5	3	4	4	ST47
IDRL-10967	Vagina	2	8	0.5	≤0.125	2	2	—	WT	WT	WT	WT	5	3	4	4	ST47
IDRL-11184	Vagina	2	8	0.5	≤0.125	2	2	—	WT	WT	WT	WT	5	3	4	4	ST47
IDRL-11235	TA	0.5	4	0.25	≤0.125	2	2	—	ND	ND	ND	ND	42 ^e	3	4	18 ^e	ST112 ^f
IDRL-11135	Urine	1	4	0.5	≤0.125	2	2	—	ND	ND	ND	ND	6	3	4	11	ST54
IDRL-10787	Cervix	2	8	0.25	≤0.125	1	1	—	ND	ND	ND	ND	5	3	4	4	ST47
IDRL-10928	Urine	0.5	2	0.25	≤0.125	0.25	0.25	—	ND	ND	ND	ND	4	11	4	4	ST113 ^f
IDRL-11163	Cervix	0.5	4	≤0.125	≤0.125	1	1	—	ND	ND	ND	ND	4	3	4	4	ST9
IDRL-11213	Vagina	0.5	4	0.25	≤0.125	2	2	—	ND	ND	ND	ND	41	3	4	11	ST101
IDRL-11269	Cervix	0.5	2	0.5	≤0.125	2	2	—	ND	ND	ND	ND	6	3	4	11	ST54
IDRL-11295	Urine	0.5	2	≤0.125	≤0.125	1	1	—	ND	ND	ND	ND	42 ^e	3	4	18 ^e	ST112 ^f
IDRL-11298	Urine	0.5	2	≤0.125	≤0.125	2	1	—	ND	ND	ND	ND	5	3	4	4	ST47
IDRL-11299	Urine	0.5	2	≤0.125	≤0.125	1	0.5	—	ND	ND	ND	ND	4	3	4	4	ST9
IDRL-11306	Urine	0.5	2	≤0.125	≤0.125	1	0.5	—	ND	ND	ND	ND	5	3	4	5	ST7
IDRL-11308	Urine	2	8	0.25	≤0.125	1	0.5	—	ND	ND	ND	ND	4	3	4	4	ST9

^a BAL, bronchoalveolar lavage fluid; TA, tracheal aspirate.^b LEV, levofloxacin; CIP, ciprofloxacin; TET, tetracycline; DOX, doxycycline; ERY, erythromycin; AZM, azithromycin.^c WT, wild type; ND, not done.^d ST, sequence type.^e New alleles; numbering starts from the last alleles reported by Zhang et al. (17) and Schneider et al. (9).^f New STs; numbering starts from the last profiles reported by Zhang et al. (17) and Schneider et al. (9).

the single tetracycline-resistant *U. parvum* isolate, as well as 9 isolates for which the tetracycline and doxycycline MICs were low.

Detailed results of the genes responsible for antimicrobial resistance and the MICs for a selection of isolates are summarized in Table 2.

MLST and clonality. MLST revealed 14 clones among the 30 *U. parvum* isolates tested. ST1 and ST56, which belong to the same clonal complex (CC) and have three shared loci, were the most frequent clones, with six and five isolates, respectively, in each. Among the 16 *U. urealyticum* isolates studied, seven profiles were

FIG 1 Population distribution of 46 *Ureaplasma* isolates established with eBURST. Three clusters (cluster I, consisting of *U. parvum* isolates; clusters II and III, consisting of *U. urealyticum* isolates) and the remaining single clone are indicated. The number of isolates is shown for those sequence types with more than one isolate.

found with ST47 (five isolates) and ST9 (three isolates), which belong to the same CC, being the most common. Five and six new alleles and ST profiles, respectively, were discovered. Detailed results are shown in Table 2. The eBURST package revealed three main clusters (I to III) and a single nongroupable clone (two isolates). Cluster I comprised the totality of *U. parvum* isolates, while most of *U. urealyticum* isolates were distributed in clusters II and III (Fig. 1). No correlation between ST and antimicrobial resistance was observed (Table 2).

DISCUSSION

Ureaplasma species have been recognized as important pathogens in recent years, not only for their potential pathogenicity linked to urogenital tract or intrauterine infections but also for their ability to produce systemic diseases in newborns and immunosuppressed patients (1–4, 6, 18). The limited therapeutic options available to combat infections by these microorganisms make it important to understand their susceptibility to potentially active antimicrobials such as the fluoroquinolones, tetracyclines, and macrolides. There are few data available on antimicrobial susceptibility of *Ureaplasma*, especially in terms of individual species. Prior data have been produced using the Mycoplasma IST2 kit (bioMérieux, Marcy l'Étoile, France), which has been shown to compare poorly with the reference broth microdilution method (9, 12). Also, inconsistencies can occur due to the use of varying interpretative criteria (9, 12). One of the reasons for the limited data available about *Ureaplasma* susceptibility relates to difficulties in cultivation and in the performance of broth microdilution (especially in regard to preparation of the inoculum). Although CLSI guidelines recommend testing each drug in duplicate, we suggest that this may be not necessary. Of the six antimicrobials tested against the 250 isolates (a total of 1,500 tests), only nine differences in the MICs between duplicates were found, and they

were never greater than the single dilution variability inherent to any broth microdilution method. By eliminating the testing of duplicates, costs could be reduced, and the protocol could be simplified; this method would thus be rendered more applicable for use in clinical microbiology laboratories.

The fluoroquinolone resistance rate in *Ureaplasma* species varies widely among different countries and studies. For instance, in a recent study carried out in Switzerland, Schneider et al. reported a rate of nonsusceptibility to ciprofloxacin of 19.4% (9), while in a recent study in Italy, 41% of isolates were ciprofloxacin resistant (19). Levofloxacin was not tested in these studies. However, in another study carried out using clinical *Ureaplasma* isolates recovered from neonates in England and Wales between 2007 and 2013, a much lower ciprofloxacin resistance rate of 1.5% was reported, with no levofloxacin resistance found. Regarding the United States, very limited data have become available regarding the frequency of fluoroquinolone resistance in *Ureaplasma* species since the first description of a resistant strain in 2006 (13). Before our work, a single study was carried out to evaluate the occurrence and molecular mechanisms of fluoroquinolone resistance in clinical *Ureaplasma* isolates from the United States and reported a levofloxacin resistance rate of 5% (ciprofloxacin was not tested) (20). These results are similar to those from our study in which we found levofloxacin resistance rates of 6.4% and 5.2% in *U. parvum* and *U. urealyticum*, respectively. Interestingly, although there was no overall difference in the percentages of resistance between the species, fluoroquinolone MIC₅₀ and MIC₉₀ values were one dilution higher for *U. urealyticum* than for *U. parvum*. Furthermore, the percentage of isolates with ciprofloxacin MICs of ≥ 4 $\mu\text{g/ml}$ was higher for *U. urealyticum* (68.8%) than for *U. parvum* (27.2%).

Sequence analysis of the QRDRs in levofloxacin-resistant isolates revealed that the mechanism of resistance was due to muta-

tions in *parC*, with a Ser83Leu substitution being most frequent, followed by a Glu87Lys substitution (Table 2). Both substitutions have been previously reported, with the first being the most frequent mutation responsible for fluoroquinolone resistance in *Ureaplasma* species worldwide (9–12, 21). A single levofloxacin-resistant *U. urealyticum* isolate and the three levofloxacin-susceptible isolates with high ciprofloxacin MICs analyzed did not harbor QRDR mutations. An absence of QRDR mutations in fluoroquinolone-resistant isolates has been previously reported, suggesting that undescribed resistance mechanisms may exist in these isolates (9).

It is interesting that no macrolide resistance was found among the *Ureaplasma* isolates studied; this is consistent with the low rate of resistance to this antimicrobial group previously reported in several countries, including the United States (9, 12, 22).

A single doxycycline-resistant isolate (*U. parvum*) was found although 4.9% of the isolates of this species were positive for the *tet(M)* gene. These data contrast with the 33% tetracycline resistance rate previously reported in the United States in *Ureaplasma* species (22). The presence of *tet(M)* in tetracycline-susceptible isolates had been previously documented (11, 12, 23). Some *tet(M)* variants may exhibit inducible resistance, and therefore it may be necessary to screen by both broth microdilution to assess phenotypic susceptibility and molecular methods to detect *tet(M)* variants (12).

MLST showed a high diversity within the clinical *Ureaplasma* isolates studied, regardless of antimicrobial susceptibility. Isolates were grouped in three clusters and demonstrated high correlation between the groups and species for *U. parvum* (cluster I) and *U. urealyticum* (clusters II and III), as previously observed in studies from China and Switzerland (9, 17). Most of the clones found in this study were described in Chinese and Swiss populations; furthermore, some (ST1, ST2, ST4, ST9, ST47, ST54, and ST101) were associated with fluoroquinolone resistance in Switzerland (9). Since the *Ureaplasma* MLST scheme has been developed only recently, further studies are necessary to expand the knowledge about clonality in this species. Together with the information provided by the previous two studies, our results suggest that, as in other species, some clonal lineages of *Ureaplasma* species could have increased epidemic potential that may be associated with pathogenicity or spread of antimicrobial resistance.

In conclusion, the present work extends previous knowledge regarding susceptibility to antimicrobial agents, resistance mechanisms, and clonality in *Ureaplasma* species in the United States, knowledge which could contribute to optimized treatment of infections caused by these pathogens and better understanding of their epidemiology.

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