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Ureaplasma spp. isolated from genital samples in Switzerland: susceptibility patterns, resistance genes, and sequence type distribution

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INTRODUCTION

- *Ureaplasma urealyticum*, *Ureaplasma parvum*, and *Mycoplasma hominis* are causative agents of urogenital tract infections such as non-chlamydial and non-gonococcal urethritis, prostatitis, cervicitis, and pelvic inflammatory disease.
- Antibiotic resistance in *U. urealyticum*, *U. parvum* and *M. hominis* (MH) poses an increasing issue. However, data regarding antibiotic susceptibility is limited to several countries, whereas information about clonality is available only from China (Zhang *et al*., Eur J Clin Microbiol Infect Dis, 2014).
- To our knowledge, data on the antimicrobial susceptibility of genital mycoplasmas isolated in Switzerland are completely lacking. More importantly, information concerning the spread of specific clones at the international level is urgently needed. Therefore, the aim of this study was to fill these gaps by analyzing the *Ureaplasma* isolates detected among Swiss people.

MATERIALS AND METHODS

- 140 genital samples collected in two laboratories from unique patients in Bern during 2014.
- ID and AST were obtained using the mycoplasma IST 2 kit (bioMérieux) and sequencing of 16S rDNA.
- Serovars were obtained with a PCR-based method (Kong *et al*., J Clin Microbiol, 2000) and clonality was analyzed with MLST and expanded MLST (eMLST) as described previously (Zhang *et al*., Eur J Clin Microbiol Infect Dis, 2014; Zhang *et al*., Plos One, 2014).
- Quinolone and macrolide resistance were studied by sequencing *gyrA/B*, *parC/E*, as well as genes encoding 23S rRNA and L4/22 ribosomal proteins.
- Phylogenetic analysis of the detected sequence types (STs) was performed using the concatenated sequences of the four MLST housekeeping genes with MEGA 5.0 software implementing the neighbor-joining method with bootstrap tests (1,000 replicates).

RESULTS: Clonal distribution, subtypes, antibiotic phenotypes and molecular characterization of 25 *Ureaplasma* isolates

Table 1. Clonal distribution, subtypes, antibiotic phenotypes, and molecular characterization of 25 *Ureaplasma* isolates

Strain ID	Sample	Sex/Age	Species[a]	MLST analysis							Antimicrobial susceptibility tests by using the IST 2 kit [d]									MICs (µg/ml) [e]		QRDR genes analysis				Macrolide resistance traits		
				ST	*ftsH*	*rpl22*	*valS*	*thrS*	*ureG* [h]	*mba* [h]	CIP	OFL	CLA	ERY	AZI	JOS	PRI	TET	DOX	CIP	AZI	GyrA	GyrB	ParC	ParE [f]	23S rRNA	L4	L22
6	GEN	F/22	UUA	100 [b]	40 [c]	11 [c]	4	4	5	6	R	I	I	I	S	S	S	S	S	0.5, S	≤0.064, S	wt	wt	wt	wt	both wt	wt	wt
41	SEM	M/35	UUA	Un	4	3	4	NA	5	10	S	S	S	S	S	S	S	S	S	-	-	-	-	-	-	-	-	-
55	CER/VAG	F/34	UUA	101 [b]	41 [c]	3	4	11	5	10	I	S	S	S	S	S	S	S	S	≤0.25, S	≤0.064, S	wt	wt	wt	wt	-	-	-
58	CER/VAG	F/34	UUA	54	6	3	4	11	5	10	I	I	S	S	S	S	S	S	S	≤0.25, S	≤0.064, S	wt	wt	wt	Thr417Val	-	-	-
66	CER/VAG	F /41	UUA	100 [b]	40 [c]	11 [c]	4	4	5	2	I	I	S	S	S	S	S	S	S	≤0.25, S	≤0.064, S	wt	wt	wt	wt	-	-	-
72	VAG	F/22	UUA	47	5	3	4	4	5	6	I	I	S	S	S	S	S	S	S	≤0.25, S	≤0.064, S	wt	wt	wt	Thr417Val	-	-	-
76	GEN	F/20	UUA	9	4	3	4	4	5	6	R	I	I	I	S	S	S	S	S	≤0.25, S	≤0.064, S	wt	wt	wt	wt	both wt	wt	wt
96	CER/VAG	F /33	UUA	101 [b]	41 [c]	3	4	11	5	10	R	I	R	I	I	S	S	S	S	≤0.25, S	≤0.064, S	wt	wt	wt	wt	both wt	wt	wt
109	VAG	F/17	UUA	102 [b]	6	2	2	11	5	29	R	I	S	S	S	S	S	S	S	≤0.25, S	≤0.064, S	wt	wt	wt	wt	-	-	-
1	CER	F/26	UPA	2	2	1	1	1	2	7	R	I	I	S	S	S	S	S	S	0.5, S	≤0.064, S	wt	wt	wt	Val417Thr	both wt	wt	wt
52	SEM	M/34	UPA	1	1	1	1	1	2	2	I	S	S	S	S	S	S	S	S	≤0.25, S	≤0.064, S	wt	wt	wt	wt	-	-	-
53	SEM	M/19	UPA	38	2	1	1	2	2	2	S	S	S	S	S	S	S	S	S	-	-	-	-	-	-	-	-	-
59	CER/VAG	F/36	UPA	103 [b]	1	12 [c]	1	1	3	2	S	S	S	S	S	S	S	S	S	-	-	wt [g]	wt [g]	wt [g]	wt [g]	-	-	-
67	VAG	F/36	UPA	4	2	2	1	1	2	2	I	S	S	S	S	S	S	S	S	1, S	≤0.064, S	wt	wt	wt	wt	-	-	-
70	CER/VAG	F/32	UPA	104 [b]	1	1	1	2	2	1	S	S	S	S	S	S	S	S	S	-	-	wt [g]	wt [g]	wt [g]	wt [g]	both wt [g]	wt [g]	wt [g]
71	CER	F/26	UPA	58	9	1	1	1	1	36 [c]	I	S	S	S	S	S	S	S	S	2, I	0.5, I	wt	wt	wt	Val417Thr	-	-	-
78	SEM	M/29	UPA	104 [b]	1	1	1	2	2	1	R	I	S	S	S	S	S	S	S	≤0.25, S	≤0.064, S	wt	wt	wt	wt	-	-	-
98	CER/VAG	F/48	UPA	4	2	2	1	1	2	2	S	S	S	S	S	S	S	S	S	-	-	-	-	-	-	-	-	-
103	CER/VAG	F/28	UPA	4	2	2	1	1	2	2	S	S	S	S	S	S	S	S	S	-	-	-	-	-	-	-	-	-
114	CER/VAG	F/37	UPA	105 [b]	2	2	1	2	2	2	S	S	S	S	S	S	S	S	S	-	-	-	-	-	-	-	-	-
126	CER/VAG	F/40	UPA	106 [b]	2	13 [c]	1	1	3	2	I	S	S	S	S	S	S	S	S	≤0.25, S	≤0.064, S	wt	wt	wt	wt	-	-	-
127	CER/VAG	F/29	UPA	107 [b]	2	1	9	1	2	7	S	S	S	S	S	S	S	S	S	-	-	-	-	-	-	-	-	-
134	CER	F/33	UPA	104 [b]	1	1	1	2	2	1	S	S	S	S	S	S	S	S	S	-	-	-	-	-	-	-	-	-
138	CER/VAG	F/41	UPA	4	2	2	1	1	2	2	S	S	S	S	S	S	S	S	S	-	-	-	-	-	-	-	-	-
141	SEM	M/33	UPA	22	1	2	1	1	3	2	R	I	S	S	S	S	S	S	S	4, R	≤0.064, S	wt	wt	Ser83Leu	wt	-	-	-

Note. GEN, genital specimen not better defined; VAG, vaginal swab; CER, cervical swab, SEM, semen; M, male; F, female; ST, sequence type; CIP, ciprofloxacin; OFL, ofloxacin; CLA, clarithromycin; ERY, erythromycin; AZI, azithromycin; JOS, josamycin; PRI, pristinamycin; TET, tetracycline; DOX, doxycycline; Un, undetermined; NA, not amplified; R, resistant; I, intermediate; S, susceptible; wt, wild-type protein; -, not performed
[a] Species identification was obtained by sequencing the 16S rDNA; [b] New Sequence types (STs); [c] New alleles; [d] AST results obtained implementing the Mycoplasma IST 2 kit (bioMérieux) and interpreted according to the manufacturer's criteria; [e] MICs obtained in broth microdilution according to CLSI (tests repeated twice). Since interpretative criteria for CIP and AZI are not available from CLSI, MICs were interpreted according to the IST 2 kit criteria ; [f] This is the first report of substitution Thr417Val in UUA; [g] These isolates were used as negative control for all PCR/DNA sequencing for QRDR and macrolide resistance genes; [h] Alleles for eMLST; eST type not available

- Numerous new STs in both UUA and UPA were identified.
- The main Chinese clones were present: ST4 was the most prevalent among UPA isolates, but ST1, ST2, ST22, ST38, and ST58 were also detected. With regard to UUA, unique ST9, ST47, and ST54 were identified.
- Only 5 of 15 CIP-nonsusceptible isolates (based on the IST 2 kit) had mutations in the QRDRs. This previously observed phenomenon was ascribed to mechanisms not yet recognized; however, the IST 2 kit might overrate the FQ resistance (high MICs that are actually in the susceptible range with the microdilution method).
- In the QRDR, only the well-described Ser83Leu substitution in ParC conferred resistance to CIP, whereas both Thr417Val (for UUA) and Val417Thr (for UPA) ParE substitutions did not significantly influence the MICs.
- None of the four isolates nonsusceptible to macrolides possessed substitutions in L4, L22 or the 23S rRNA copies. This may be explained by the fact that: (i) our four isolates were actually susceptible to macrolides according to the CLSI criteria, and (ii) all macrolide-nonsusceptible strains were truly sensitive when tested with the microdilution method.

RESULTS: ID and AST of 103 UUA/UPA isolates

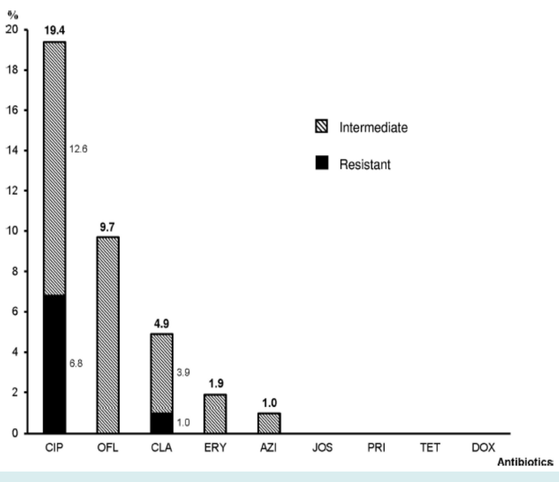

Figure 1. Overall prevalence of non-susceptible *U. urealyticum*/*parvum* strains (UUA/UPA; n=103). ID and AST results were achieved implementing the Mycoplasma IST 2 kit (bioMérieux). The interpretative criteria provided by the manufacturer were applied.

RESULTS: Phylogenetic analysis of the 17 different STs

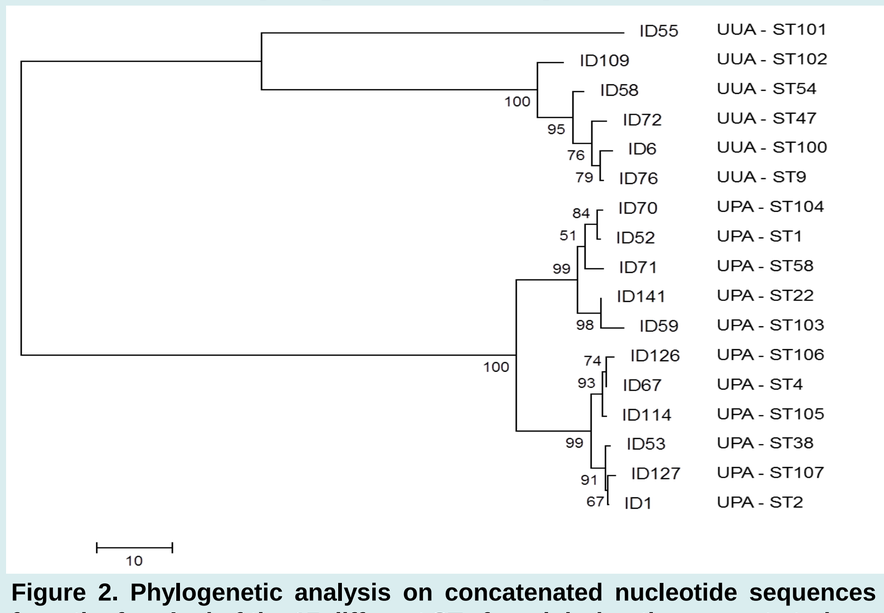

Figure 2. Phylogenetic analysis on concatenated nucleotide sequences from the four loci of the 17 different STs found during the present study.

The phylogenetic analysis performed on 6 UUA and 11 UPA isolates indicated that most of the novel STs found are strongly related to the ancestors (ST1 for UPA and ST47 for UUA) and to the very prevalent Chinese variants (ST1, ST2, ST4, ST22, and ST9).

CONCLUSIONS:

- **This is the first study analyzing susceptibility of *Ureaplasma* spp. isolates detected in Switzerland and the clonal distribution outside China.**
- **Resistance rates are low compared to other surrounding countries, but the empirical use of quinolones is compromised.**
- **Conflicting results from the IST 2 kit and standard broth microdilution were observed for CIP and AZI (i.e., most of the isolates routinely reported as nonsusceptible to these antibiotics were actually fully sensitive)**
- **We hypothesize that some hyperepidemic STs (e.g., ST4) spread worldwide via sexual intercourse.**
- **Unfortunately, our study does not provide adequate clinical data to establish the extent of infection or colonization and the potential link with specific STs of *U. urealyticum* and *U. parvum* among our patients: Large combined microbiological and clinical studies should address these important aspects in the near future.**