Antibiotic resistance among *Ureaplasma* spp isolates; cause for concern?

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Keywords. *Ureaplasma*, antibiotic resistance, antibiotic resistance surveillance, detection of antibiotic resistance

Synopsis:

There is a growing global concern regarding the rise of antibiotic resistant organisms. Many of these reports have focused on various Gram-positive and Gram-negative pathogens, with little attention to the genus *Ureaplasma*. *Ureaplasma* spp. are associated with numerous infectious diseases affecting pregnant women, neonates and the immune compromised. Treatment options are extremely limited due to high levels of intrinsic resistance resulting from the unique physiology of these organisms, and further restricted in cases of the developing fetus or neonate often limiting therapeutic options to predominantly macrolides, or rarely fluoroquinolones. The increasing presence of macrolide and fluoroquinolone resistant strains among neonatal infections may result in pan-drug resistance and potentially untreatable conditions. Here we review the requirements for accurate measurement of antimicrobial susceptibility, provide a comprehensive review of the antimicrobial resistance (AMR) for *Ureaplasma* species in the literature, and contextualize these results relative to some investigator's

reliance on commercial kits that are not CLSIlinical Laboratory Standard Institute compliant when determining AMR. The dramatic variation in the resistance patterns and impact of high levels of AMR amongst neonatal populations suggests the need for continued surveillance. Commercial kits represent an excellent tool for initial antibiotic susceptibility determination and screening. However, AMR reporting must utilize internationally-standardised methods as high titre samples, or *M. hominis*-contaminated samples, routinely give false AMR results. Furthermore, requirement for future reports to determine the underlying AMR mechanisms will determine if expanding AMR is due to spontaneous mutation, transmission of resistance genes on mobile elements or selection and expansion of resistant clones.

Introduction: *Ureaplasma* as a pathogen

A focus on the ESKAPE pathogens, multi-drug resistant *Mycobacterium tuberculosis* and drug resistant *Neisseria gonorrhoeae* predominate both the scientific literature and the media with little attention drawn to some of the less prominent pathogens. This relative lack of attention does not correlate to the absence of a problem. *Ureaplasma* are some of the smallest self-replicating organisms known to inhabit the human host. As the name suggests they possess a unique capacity to utilize urea as a primary carbon source in the generation of ATP.[1] Within the genus two human associated species exist: *Ureaplasma urealyticum* and *Ureaplasma parvum* and predominantly differ in the genomic coding capacity (0.75−0.78 Mbp versus 0.84−0.95 Mbp genomes).[2] *Ureaplasma* spp. have had a controversial history as a pathogen in part due the high colonization rate among healthy individuals with 40 – 80 % of healthy females being colonized. *Ureaplasma* are now recognized pathogens among pregnant females, neonates, sexually active individuals and the immunocompromised.[3, 4] One of the most recent reports have identified a link between individuals suffering from hyperammonemia following lung transplantation and systemic infection by *Ureaplasma* spp.[5]

Therapeutic options

Treatment of *Ureaplasma* spp. infections are complicated by high levels of intrinsic resistance to many commonly prescribed antimicrobials; for example the lack of a cell wall confers resistance to all beta-lactam and glycopeptide antibiotics whereas the lack of *de novo* synthesis of folic acid renders cells resistant to sulphonamides and diaminopyrimidines.

Only four classes of antibiotics are recognized for the treatment of *Ureaplasma* infections. These are notably those which belong to the fluoroquinolone, tetracycline, chloramphenicol and macrolide classes. When considering infections among pregnant females or neonates the number of therapeutic options are further restricted due to accumulation of tetracyclines in developing bones, "grey baby syndrome" associated with chloramphenicol and reticence in using fluoroquinolones in neonates. Therefore emergence of macrolide resistant strains threaten to severely limit treatment of *Ureaplasma* infections among these individuals, especially as *Ureaplasma* fluoroquinolone resistance is present and expanding in Europe.[6]

Administration of antibiotics has been associated with both clinical and microbiological cure in clinical presentations. In a study by Bharat *et al.*, resolution of hyperammonemia was correlated with administration of azithromycin or levofloxacin resulting in subsequent microbiological cure.[5] In a single case the patient did not respond to azithromycin treatment, but this was later attributed to the presence of a macrolide resistant strain. In some instances chloramphenicol has been used in the treatment of *Ureaplasma* induced meningitis among both adults and neonates, although potential complications surrounding toxicity in systemic use needs to be balanced with clinical outcome.[7, 8] Although favorable results have been noticed in many studies, the use of antibiotics among individuals with suspected non-gonococcal urethritis (NGU) as a result of *Ureaplasma spp.* infection is still questionable. A study by Khosropour *et al.*, noted that 57% of individuals with NGU who were initially infected with *Ureaplasma* spp. and received antimicrobial therapy with initially azithromycin (1g)

followed by doxycycline (100 mg twice daily for seven days), or *vice versa*, were still colonized after six weeks of therapy.[9]

These data suggest in many cases it is possible to manage infections caused by *Ureaplasma*, when dealing with antibiotic susceptible strains. As highlighted by this review antibiotic resistant strains of *Ureaplasma* are present within the community. The mechanisms of resistance vary accordingly depending on the antibiotic in question. Accumulation of point mutations in the 23S rRNA genes and the quinolone resistance determining regions (QRDRs) of the *parC* genes are the predominant mechanisms of resistance to macrolides and fluoroquinolones, respectively with acquisition of the gene encoding the Tet(M) ribosomal protection protein on the Tn*916*-like mobile element being associated with resistance to tetracycline.[6] The detailed mechanisms of resistance are beyond the scope of this review.

Determining antibiotic susceptibility profiles for Ureaplasma spp isolates using Clinical Laboratory Standards Institute (CLSI) guidelines and commercially available kits

Routine antimicrobial susceptibility testing (AST) for *Ureaplasma* is rarely performed due to the fastidious nature and specialized growth medium requirements. Therefore, most infections are treated empirically, utilizing molecular methods for test of cure. For this reason, AST is predominantly conducted for surveillance purposes, in the development of novel antimicrobials or clinical cases where patients fail to respond to treatment.[10]

AST has been reported for *Ureaplasma* over numerous decades. In 2001, the publication Cumitech 34, outlined not only diagnostic methods for u~~U~~reaplasmas and ~~M~~mycoplasmas, but also detailed standardized methods for AST. However, in 2011 an international collaboration to standardize AST~~M~~ for *Ureaplasma* spp, *M. hominis* and *M. ~~pneuomoniae~~pneumoniae* was published by the ~~Clinical and~~

~~Laboratory Standards Institute (~~CLSI~~)~~. CLSI M43-A highlights the requirement for standardized media (10B broth or A8 agar) quality control isolates (*U. urealyticum* [SV9] ATCC® 33175™ in the case of *Ureaplasma*) and reference ranges for determining susceptibility or resistance.

Although standardized methodologies exist there is still a lack of routine AST. One factor which may contribute to the lack of routine AST maybe the complex nature of testing regimes. Ureaplasmas are unable to grow as confluent lawns on bacteriological agar plates therefore negating the use of commonly used disk-diffusion assays, therefore broth microdilution and agar dilutions methods are favored, although these have their drawbacks. The inability to grow *Ureaplasma* to a turbid culture, owing to the self-toxic nature of metabolites produce~~d~~s as well as small cell size, means that McFarland standards are not available for standardizing inoculum size. Broth culture methods can utilize an increase in pH in the medium which increases from pH=6.5 to pH>8.0 caused by the conversion of urea to ammonium ions by *Ureaplasma*, changing the phenol red in the medium from yellow-orange to cerise red. To achieve the required 10^4 – 10^5 ~~CFU~~cfu/~~ml~~ mL inoculum for reliable susceptibility testing, cultures require predetermination of ~~CFU~~ cfu prior to AST with freezing of the culture of known inoculum so that numbers can be adjusting accordingly. This can be a lengthy process which delays reporting of the isolates antibiogram. Routine clinical laboratories cannot feasibly accommodate setting these methods, even if the complex routine medium can be obtained commercially, it is too labor intensive and requires specialized training of staff. This is where the commercially available *Ureaplasma* AST kits find their niche.

Commercial kits provide a streamlined and simplistic approach to detection of *Ureaplasma* spp and AST. These kits contain dried antibiotic powders at two breakpoint concentrations which become reconstituted upon inoculation. Although these kits can be sourced from a range of suppliers, caution must be exercised when interpreting the results because there are a number of factors that do not comply with the approved CLSI guidelines. Firstly, none of these kits utilize a dilution method of accurately quantifying the inoculum which is added to the test panel. Although some kits have separate wells that can differentiate inoculum levels of $\geq 10^4$ ~~CFU~~cfu/specimen, they utilize an undisclosed method of

inhibition as no physical dilution prior to addition to these wells occurs in the sample preparation (Table 1). It is well established that a load greater than 10^5 will give a false-resistant result.[6] Assay, such as the MIST2, gives a semi-quantitative result of either positive or $\geq 10^4$. This assay will therefore not differentiate if there is a high bacterial load of greater than the recommended 10^5 which has been documented to be as high as 10^7 in a number of samples.[11]

Secondly commercial kits cannot separate results for *Ureaplasma* and *Mycoplasma hominis* mixed cultures.[12] Due to the intrinsic resistance of *M. hominis* to macrolides it is impossible to determine if *Ureaplasma* sp in these mixed samples are susceptible~~ensitive~~ to macrolides.[13] This has led to the unfortunate false-resistance reporting by investigators that note higher rates of macrolide resistance among sample with co-isolation of both organisms[12], as they did not do follow-up investigations on *Ureaplasma* isolates purified from the *M. hominis* contamination. For reliable susceptibility testing it is essential to isolate a purified culture of test isolate.

The most important shortfall in the commercial AST kits is the use of test concentrations different from the CLSI-determined breakpoints. Interpretation guidance provided with these kits define (1) growth in growth control, with negative result in either concentration of antibiotic indicates a susceptible isolate; (2) growth in the growth control and lower antibiotic concentration but not the higher suggests intermediate susceptibility; and (3) growth in all conditions suggest full resistance. Unfortunately the concentrations in many of these kits do not match those defined by CLSI documentation: CLSI designate the erythromycin breakpoint as growth at greater or equal to 16 mg/L erythromycin suggests a resistant isolate, whereas the BioMerieux kit utilizes 4 mg/L, four-fold less than recommended. This may lead to over-reporting macrolide resistance among studies which have utilized the MIST2 kit, a topic which is discussed later. Conversely the breakpoint for tetracycline stated by CLSI has been stated as 2 mg/L whereas the lower and higher breakpoint concentrations are 4 and 8 mg/L, respectively. Although this may suggest the possibility of underreporting of tetracycline among many clinical isolates, in many cases with TetM mediated resistance results in high MIC values of greater than 32 mg/L. Exceptions to this have been noted in the situations of phenotypically susceptible~~ensitive~~ strains

which are *tetM* positive, but are only resistant following induction with antibiotic.[14, 15] This anomaly would be missed by both commercial as well as CLSI approved protocols. With respect to testing for ~~flouroquinolone~~fluoroquinolone resistance there are again inconsistencies with CLSI protocol. The primary concern is the low threshold for ciprofloxacin breakpoints at 2 mg/L. No agreed breakpoint was agreed for ciprofloxacin and it is known that a much higher concentration is required to inhibit growth of *Ureaplasma* than some of the newer third and fourth generation ~~fluroquinolones~~fluoroquinolones such as levofloxacin and moxifloxacin, respectively. Although ofloxacin is not part of the CLSI recommended repertoire of fluoroquinolones, the breakpoint is the same as suggested for levofloxacin and moxifloxacin. By taking these points into consideration it maybe that investigators identify false-negative ciprofloxacin isolates with susceptibility to either levofloxacin or moxifloxacin.

Evaluation of studies reporting antibiotic resistance

Antibiotic resistance is recognized as an international issue whereby resistant strains can be imported from countries with high levels of resistance. For this reason, we carried out a review of the literature from the past ten years (2006 – 2016) to identify the number of studies examining resistance among *Ureaplasma* spp. From this we identified 33 reports on clinical antibiotic resistance among *Ureaplasma* from a collection of single case reports as well as larger studies.[6, 8, 12, 14-43] From these reports we extracted data regarding the year of publication, country in which the study was conducted, the patient group examined, methods by which AST was determined, whether the species of *Ureaplasma* was determined, number of isolates examined and finally, where relevant, the percentage of reported isolates resistant to antibiotics stated (Table 2).

We identified, as expected, the rates of resistance varied by country and in some instances noted dramatic difference in reports from within the same country. For example a study by Huang *et al.*, 2016 examined 1951 individuals and identified 54 % to be resistant to erythromycin.[12] This is in contrast to the work by Song *et al*. and Ye *et al*., who examined 1513 and 15594 individuals with much lower rates

of resistance at 11 % and 1%, respectively.[39, 40] In some instances resistance was high to only a single class of antibiotic. For example a study by Leli *et al.*, found high levels of ofloxacin resistance (27.6%) among 152 *Ureaplasma* isolated in Italy, whereas no resistance any tetracycline or macrolide antibiotics were detected.[29] The highest levels of fluoroquinolone resistance was documented in countries such as China with figures of 53 % of isolates resistant to ofloxacin and 88 % of isolates resistant to levofloxacin.[12, 39] Resistance to tetracyclines were noted in high numbers in South Africa (73% of isolates),[38] USA (34 % of isolates)[27] and Cuba (31 % isolates).[34] Many of these isolates were additionally confirmed for the presence of the *tetM* mobile genetic element. Of greatest concern in relation to treatment of neonatal infection are the high reported levels of macrolide resistance seen in certain countries. Using erythromycin as the indicator for resistance, as suggested by the CLSI, the highest levels of resistance were seen in Hungary (85 %),[26] South Africa (80 %),[38] Turkey (54 %),[18] China (54 %),[12] Israel (46 %)[25] and Cuba (46 %).[34] Although these percentages are high in relation to countries such as the UK (0 - 2 %) or Croatia (0 – 7 %) there is a real possibility of clonal strains being introduced from countries of high resistance to those with low resistance. Alternatively these levels reported may be an over representation as a result of the in accuracies of commercial assays as described previously.

Use of the broth microdilution technique was as prevalent as the use of the Mycoplasma-IST kit (10/33 studies and 11/33 studies, respectively). However, as discussed earlier there are numerous limitations to commercial kits such as the Mycoplasma IST2, such as the incorporation of breakpoint levels which do not agree with CLSI guidelines. This may have resulted in the over-reporting resistance for some antibiotics.

Although *Ureaplasma* have been recognized as two separate species since 2000, there is still lack of discrimination at the species level. Many of the diagnostic methods used in the literature review only report the presence of *Ureaplasma* and do not differentiate to the species level, partly due to culture based commercial kits, and in some incidences report *U. urealyticum* by default due to historic taxonomic reasons. This reporting style has a negative impact on surveillance and understanding of

distribution of resistant species as well as understanding the role of the two species in clinical outcome. For example the association between *Ureaplasma* and NGU has been controversial, but studies which have looked at *Ureaplasma* as two independent species have shown that *U. urealyticum* are significantly associated with NGU with an adjusted odds ratio of 2.3 compared with *U. parvum* (adjusted OR 0.4).[44] Nucleic acid technologies exist whereby species differentiation can be determined and should be adopted for any future reporting.[45]

The role of Commercial kits in a clinical setting

While the available commercial kits for *M. hominis* and *Ureaplasma* spp. detection and antibiotic susceptibility testing (in their current formats) may not provide publishable antibiotic resistance data without follow-up investigation, these kits provide an ideal method to investigate these emerging pathogens in a busy clinical setting. Urethritis, inflammation of the urethra, is a common condition which is usually sexually acquired and commonly classified into those caused *Neisseria gonorrhoea* infection or other causes. The 2015 UK National Guideline on the management of non-gonococcal urethritis (NGU), published by the Clinical Effectiveness Group of the British Association for Sexual Health and HIV (Horner et al., 2015 doi: 10.1177/0956462415586675) list ureaplasmas as one of the most common causes (11-26%) of NGU in men, only superseded by *Chlamydia trachomatis* (11-50%) and *Mycoplasma genitalium* (6-50%). Based on the guidelines, the first line treatment in outpatient clinics is with azithromycin (single dose of 1 gram) or doxycycline (100 mg/day for 7 days). These treatment levels were demonstrated to have similar efficacy in the past, 75% and 69%, respectively, against ureaplasmas (Manhart LE, Gillespie CW, Lowens MS, et al. Standard treatment regimens for nongonococcal urethritis have similar but declining cure rates: a randomized controlled trial. Clin Infect Dis 2013; 56: 934–942.; Khosropour CM, Manhart LE, Colombara DV, et al. Suboptimal adherence to doxycycline and treatment outcomes among men with non-gonococcal urethritis: a prospective cohort study. Sex Transm Infect 2014; 90:

3–7.); however, as highlighted in table 1, inadvertent treatment of ureaplasmas is likely to decline with increasing global emergence of antimicrobial resistance. Furthermore, there is increasing evidence that treatment with a single 1 gram azithromycin dose drives development of mutations in the 23sRNA gene resulting in macrolide antimicrobial resistance in *M. genitalium*, [Bradshaw CS, Chen MY and Fairley CK. Persistence of Mycoplasma genitalium following azithromycin therapy. PLoS One [Electronic Resource]. 2008; 3: e3618.; Twin J, Jensen JS, Bradshaw CS, et al. Transmission and selection of macrolide resistant Mycoplasma genitalium infections detected by rapid high resolution melt analysis. PLoS One 2012; 7: e35593.; Ito S, Shimada Y, Yamaguchi Y, et al. Selection of Mycoplasma genitalium strains harbouring macrolide resistance-associated 23S rRNA mutations by treatment with a single 1 g dose of azithromycin. Sex Transm Infect 2011; 87: 412–414.], which would likely also develop in the closely related ureaplasmas, and this may contribute to the failure of first line therapy to treat up to 25% of patients. Treatment of these recurrent urethritis patients, requires multiple follow-up appointments and may persist for up to a month with empirical treatment of up to 4 different antibiotics (macrolides, doxycycline, metronidazole, and fluoroquinolones) before it resolves.

In the clinical setting, commercial kits provide reliable sensitive detection in 24-48 hours and give important guidance for therapeutic treatment in resistant infection. Furthermore, they require no specialist equipment, reagents or training. Examination of the characteristics of all available kits, the latest generation of commercial kits available include the Myco Well D-One kit, which utilizes the CLSI breakpoints for antibiotic concentrations, and additionally specifically identify *Gardnerella vaginalis*, *Trichomonas vaginalis* and *Candida albicans* (all relevant to common genitourinary clinical investigation). The advantage of this particular kit is that titration of microbial load by traditional methods for any positive sample, to ensure the inoculum tested was approximately 10^4 cfu, would ensure that the results were performed under CLSI-

compliant guidelines and therefore the results could be published. Ureaplasmas are also emerging as pathogens in other clinical settings as well: development of bronchopulmonary dysplasia (or chronic lung disease) in premature neonates [Viscardi and Kallapur doi: 10.1016/j.clp.2015.08.003]; presence as the sole organism identified in histologically confirmed chorioamnionitis of moderate/late preterm and term placentae [Sweeney et al. 2016; doi: 10.1093/infdis/jiv587]; underlying cause of fatal hyperammonemia in lung transplant patients (Bharat et al., 2015); wound infection or absess formation in kidney transplant patients (Loupy et al, 2008; Eilers et al., 2007); and meningitis in adults (Geissdorfer et al., 2008). Therefore, simplistic commercial kits that detect ureaplasmas and direct therapy may find expanding utility in clinical settings outside of genitourinary medicine.

Concluding remarks

This review has highlighted that there is a need for continual surveillance in order to keep track of resistance patterns. Commercial kits are an easy way for an initial screening, but indication of resistance needs to be followed up appropriately, not just reported. From this we suggest the following recommendations. 1) If a mixed *M. hominis* and *Ureaplasma* spp. culture is identified, isolation of single *Ureaplasma* colonies and repeat AST is required in order to obtain reliable data for macrolide resistance. 2) Confirm resistance with approved CLSI guidelines including quantifying the inoculum and/or 3) determine the underlying mechanism of resistance. While it is tempting to attribute the low antibiotic resistance rates in some countries, such as the UK, to vigilance in prescribing polices and prudent use, the geographic differential in antibiotic resistance is unlikely to be maintained, particularly with the degree of travel between the countries of high levels and low levels of resistance in combination with the increased prescribing of macrolide antibiotics for *N. gonorrhoeae, Chlamydia trachomatis* and *Mycoplasma genitalium* infections. The correct CLSI directed means of determining antibiotic

susceptibilities, or determine the underlying mechanisms of resistance among ~~Ureaplasmas~~ ureaplasmas must be adhered to in order to produce reliable and comparable data for international surveillance.

Funding

None to declare

Transparency declarations

None to declare

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Product	Supplier	Quantification available*	CLSI recommended antibiotics (minimum inhibitory concentration for resistance)				
			Levofloxacin (≥≥4 mg/L)	Moxifloxacin (≥≥4 mg/L)	Tetracycline (≥≥2 mg/L)	Erythromycin (≥≥16 mg/L)	Telithromycin (≥≥8 mg/L)
MIST2	BioMérieux	10^4 and $\geq 10^4$	1 and 4	N/A	4 and 8	1 and 4	N/A
Complement Mycofast revelutioN	ELiTech	10^3, 10^4 and $\geq 10^5$	1, 2 and 4**	0.25 and 2**	1, 2, 4 and 8**	8 and 16**	N/A
SIR Mycoplasma	BioRad	$10^2 - 10^4$, $10^4 - 10^5$ and $>10^5$	N/A	N/A	4 and 8	8 and 16**	N/A
Mycoplasma system plus	Liofi~~o~~lchem SLR	$<10^4$, $<10^5$ and $>10^5$	N/A	N/A	4 and 8	1 and 4	N/A
MYCO WELL D-ONE	CPMI	10^4 and $\geq 10^5$	2 and 4**	2 and 4**	N/A	8 and 16**	N/A

Table 1. Compliance of commercial rapid diagnostic and antimicrobial susceptibility testing kits in comparison with Clinical Laboratory Standards Institute (CLSI) guidelines for *Ureaplasma* spp.. The antibiotics present represent those determined suitable for testing by the CLSI along with minimum inhibitory concentrations (MIC). N/A, not applicable. *All assays quantify by colony count independent methods. **CLSI compliant MIC ranges included for this antibiotic.

Author (reference)	Year of publication	Country	Patient group and sample type	Method of susceptibility testing	Species determined	Number of isolates examined	Isolates resistant (Percent)												
							~~Dox~~DOX	T~~et~~ET	~~Min~~MIN	CIP~~i~~~~p~~	~~Ofx~~OFX	LVX~~ev~~	~~Mox~~MOX	~~Azi~~AZI	CLR~~la~~~~ri~~	~~Ery~~ERY	~~Rox~~ROX	~~Jos~~JOS	~~Pri~~PRI	~~Clin~~CLI
Beeton [15]	2016	England and Wales	Endotracheal aspirates from neonates, cervical swabs and patients with immunological disorders	Broth microdilution	Yes	130	2	2	-	2	-	0	0	0	-	0	-	-	-	-
Huang [12]	2016	China	Mix of fertile and infertile men	Mycoplasma IST	No	1951	5	-	-	94	-	88	-	39	31	54	50	5	-	-
Schneider [43]	2015	Switzerland	Genital samples	Mycoplasma IST2 and Broth microdilution	Yes	103	0	0	-	19.4	9.7	-	-	1	4.9	1.9	-	0	0	-
Kawai [42]	2015	Japan	Vaginal and placental swabs and endotracheal aspirates from neonates	Broth microdilution	Yes	28	-	-	-	93	-	57	-	-	-	-	-	-	-	-
Messano [41]	2014	Italy	Male urethral swabs	Mycoplasma IST2	No	115	2	2	-	36	16	-	-	2	2	5	-	0	0	-

Song* [40]	2014	China	Mix of male urethral and female cervical swabs	Mycoplasma IST2	No	1513	0 - 3	1 - 4	-	64 – 93	44 - 77	-	-	0 - 6	3 - 8	6 - 11	-	0 - 1	0 - 1	-
Ye [39]	2014	China	Female urogenital swabs	Mycoplasma IST2	No	15594	2	3	-	75	53	-	-	0.1	0.1	1	-	0	0	-
Redelinghuys [38]	2014	South Africa	Females attending antenatal clinic self-collected vaginal swabs	Complement Mycofast revelutioN	Yes	44	-	73	-	-	-	41	2	-	-	80	-	-	-	100
Vargovic [37]	2014	Croatia	Male and female urogenital samples	SIR Mycoplasma	No	507	3	5	-	-	22	-	-	8	-	7	-	-	-	99
Hunjak [36]	2014	Croatia	Female urogenital samples	Mycoplasma IST 2	Yes	424	0	0	-	35	5.2	-	-	0.3	0	0	-	0	-	-
Pignanelli [35]	2014	Italy	Women with cervicitis	Mycoplasma IST 2	No	899	2	3	-	40	6	-	-	6	15	19	-	4	2	-
Diaz [34]	2013	Cuba	Women with vaginal discharge	Mycoplasma System Plus	No	154	17	31	16	-	64	-	-	30	63	46	-	-	-	18
Ponyai [33]	2013	Hungary	Swabs from male and female patients with non-gonococcal non-chlamydial urethritis	SIR Mycoplasma	No	373	2	4	-	-	25	-	-	10	-	81	-	-	-	75
Dhawan [32]	2012	India	Males and females from a STD outpatients clinic	Broth microdilution	Yes	35	9	-	-	-	23	-	-	29	-	-	-	14	-	-

Mardassi [31]	2012	Tunisia	Mixed patient group	Broth microdilution	Yes	22	-	23	-	-	-	-	-	-	-	-	-	-	-	-
Govender [30]	2012	South Africa	Female vaginal swabs from women at a termination of pregnancy clinic	Broth microdilution	Yes	15	13	33	-	-	7	-	-	13	-	27	-	0	-	-
Leli [29]	2012	Italy	Male and female urogenital samples (72 % were native, 28% immigrant)	Complement Mycofast revelutioN	No	152	0	-	-	66	28	-	-	0	-	0	0	0	0	-
Zhu [28]	2012	China	Females with genital tract infections	A commercial Mycoplasma strip	No	1538	2	-	2	-	33	20	-	15	6	-	33	12	-	-
Xiao [27]	2012	USA	Variety of clinical isolates between 1997 - 2011	Broth microdilution	Yes	257	-	34	-	-	-	5	-	-	-	1	-	-	-	-
Farkas [26]	2011	Hungry	Male and female urogenital swabs	SIR Mycoplasma	No	247	5	6	-	-	21	-	-	12	-	85	-	10	-	79
Samra [25]	2011	Israel	Various GUM samples	Broth microdilution	No	63	3	3	-	-	-	0	0	13	0	46	25	-	-	-
Goulenok [24]	2011	France	Systemic lupus erythematosus	Not state	No	1 (Case study)	Suse~~ns~~	Suse~~ns~~	-	-	-	-	-	-	-	-	-	-	-	-

			patient without Hypogammaglobulinemia																	
Mihai [23]	2011	Romania	Endocervical swabs from infertile women	Mycoplasma IST2	No	372	2	6	-	52	16	-	-	8	9	16	-	2	3	-
Biran [22]	2010	France	Term neonate with CSF infection	Not stated	Yes	1 (Case study)	-	-	-	Res	-	-	Susens	-	-	-	-	-	-	-
Lucke [21]	2010	Switzerland	Sternal wound infection	Mycoplasma IST2	Yes	1 (Case study)	Susens	Susens	-	Res	Int	-	-	Susens	Susens	Susens	-	Susens	Susens	-
Krausse [20]	2010	Germany	Mixed patient group and sample	Agar dilution and E-test	No	179	1	3	3	16	2	-	-	7	5	21	6	2	-	43
Beeton [6]	2009	UK	Neonatal lavage fluid	Broth microdilution	Yes	61	2	2	-	2	-	-	-	2	2	2	-	-	-	-
Kechagia [19]	2008	Greece	Vaginal swabs from women aged 18-62	Mycoplasma IST2	No	111	0	5	-	86	20	-	-	9	7	37	-	0	9	-
Geissdörfer [8]	2008	Germany	Adult male with Ureaplasma meningitis	Mycoplasma IST2	Yes	1 (Case study)	-	-	-	Res	Int	-	Int	-	-	-	-	-	-	-
Dégrange [14]	2008	France	Patients in Bordeaux, France	SIR Mycoplasma	No	276	2	2	2	-	-	-	-	-	-	-	-	-	-	-
Karabay [18]	2006	Turkey	Women with abnormal vaginal discharge		No	193	2	14	-	41	58	-	-	-	-	54	-	2	8	-

Xie* [17]	2006	China	Samples from outpatients clinic	Mycoplasma IST2	No	804	4 to 11	5 to 12	-	82 to 89	24 to 67	-	-	15 to 23	17 to 28	11 to 64	-	0 to 3	0 to 5	-
Duffy [16]	2006	USA	Chronic bladder infection	Broth microdilution	Yes	1 (Case study)	Susens	-	-	-	Res	Res	Res	-	-	Susens	-	-	-	-

Table 2. Summary of global antibiotic resistance among *Ureaplasma* isolates from 2006 to 2016.

~~Dox~~ DOX – Doxycycline, ~~Tet~~ TET – Tetracycline, ~~Min~~ MIN – Minocycline, CIP~~ip~~ – Ciprofloxacin, OFX~~fx~~ – Ofloxacin, L~~ev~~VX – Levofloxacin, ~~Mox~~ MOX – Moxifloxacin, ~~Azi~~ AZI – Azithromycin, CLR~~lari~~ – Clarithromycin, ~~Ery~~ ERY – Erythromycin, ~~Rox~~ ROX – Roxithromycin, ~~Jos~~ JOS – Josamycin, ~~Pri~~ PRI – Pristinamycin, CLI~~lin~~ – Clindamycin. Res = Resistant, Int = Intermediate and Sus~~ens~~ = ~~Sensitive~~Susceptible. - Not determined . *Incidence of resistance was broken down by year with the lowest and highest percentages recorded.