

# Les mycoplasmes urogénitaux : des agents d'IST ?

**Sabine Pereyre**

**USC EA 3671 Mycoplasma and chlamydial infections in humans  
INRA - University of Bordeaux  
Bordeaux University Hospital**

**National French Reference Center for bacterial IST**

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 université  
de **BORDEAUX**

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SCIENCE & IMPACT

 **CHU**  
Hôpitaux de  
Bordeaux

# Characteristics of mycoplasmas

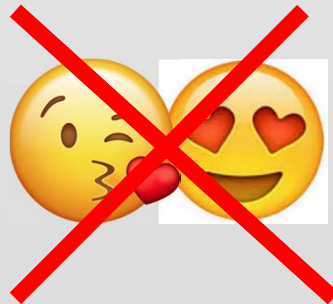
- **Smallest free-living eubacteria** (500 to 2200 kbp)
- **Lack of cell wall** (« *Mollis cutis* » or Mollicutes)
- **18 human species:** respiratory or urogenital tract
- **5 human pathogenic species:**

- *M. pneumoniae* → respiratory tract infections
  - *M. hominis*
  - *U. urealyticum*
  - *U. parvum*
  - *M. genitalium*
- urogenital tract infections  
Sexually transmitted infections (Mg)

# *Ureaplasma* spp. and *M. hominis*

- **Commensals of the urogenital tract**
  - More frequent in women
  - *Ureaplasma* spp. (30%) >> *M. hominis* (<10%)
  - Variable according to different parameters
    - ✓ age, sexual activity, race, pregnancy, socio-economic level
- **Opportunistic pathogens**
  - Challenge to interpret their presence in the lower genital tract

- **Not IST agents**



# Mycoplasmal urogenital infections

Disease	<i>M. hominis</i>	<i>Ureaplasma</i> spp.	<i>M. genitalium</i>
<b>Male genital disease</b>			
Nongonococcal urethritis	-	+	+
Epididymitis, prostatitis	-	±	±
<b>Gynecologic infections</b>			
Bacterial vaginosis	+	-	±
Cervicitis	-	-	+
PID	+	-	+
<b>Adverse pregnancy outcomes</b>			
	+	+	±
<b>Neonatal infections</b>			
	±	+	?
<b>Extragenital infections</b>			
	+	+	±

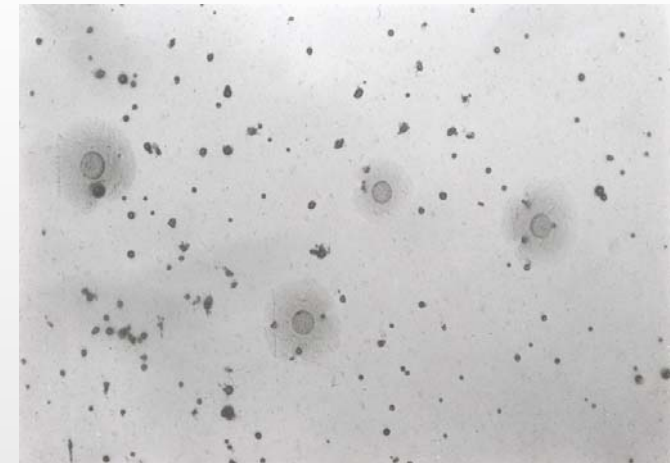
+, Confirmed association  
 ±, Non confirmed association  
 -, No association  
 ?, Unknown

# Laboratory detection

- **Culture : *M. hominis* and *Ureaplasma* spp.**
  - Agar or liquid broth
  - Commensal → Quantitative cultures required

Specimens	Threshold
<b>Sterile sites or specimens</b>	No threshold Detection = infection
<b>Men</b>	
Urethral specimens, sperm	$U \geq 10^4$ CCU/ml
1 <sup>er</sup> void urines	$U \geq 10^3$ CCU/ml
<b>Women (cervico-vaginal specimens)</b>	
	- Mh $\geq 10^4$ CCU/ml - U : no threshold, detection non significant
<b>Neonates</b>	
Endotracheal specimens	$U \geq 10^4$ CCU/ml

U, *Ureaplasma* spp. Mh, *M. hominis*; CCU, color changing unit



- **PCR : specimens from sterile sites**
  - Higher sensitivity

# Urogenital mycoplasmas and antibiotics

- **Intrinsic resistance related to:**

- **the *Mollicutes* class:** ATB targeting the cell wall ( $\beta$ -lactams, glycopeptides, fosfomicin) and rifampicin (mutation in *rpoB* gene)

- **certain species and macrolides and related ATB:**

- ✓ *M. hominis* resistant to 14- and 15-membered macrolides

- ✓ *Ureaplasma* spp. resistant to lincosamides

- **Active antibiotics**

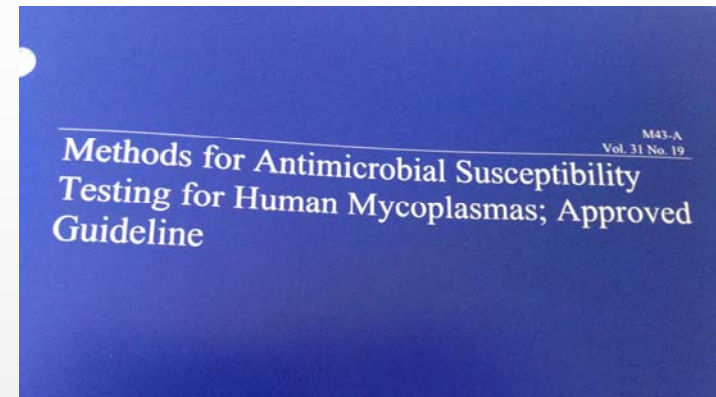
- **Macrolides and related ATB:** macrolides, lincosamides, streptogramin combinations, ketolides (**MLSK**)

- Fluoroquinolones

- Tetracyclines

# Antibiotic susceptibility testing

- Phenotypic techniques
  - Broth microdilution
  - Agar dilution
  - E-tests (*M. hominis*)
- CLSI recommendations  
M43-A 31(19), 2011
- In routine, commercialized kits  
*M. hominis*, *Ureaplasma* spp. only



- Molecular techniques to detect tetracycline, fluoroquinolone or macrolide resistance

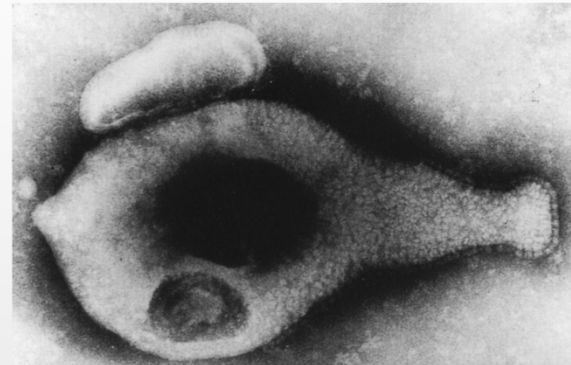
# Acquired resistance to antibiotics in *Ureaplasma* spp. and *M. hominis*

- ✓ **Resistance to tetracyclines +++**
  - acquisition of *tet(M)* gene
  - **15 % Mh, 7,5 % U (France, 2010-2015)**
  
- ✓ **Resistance to macrolides**
  - Mutations in domain V of 23S rRNA
  - Very rare cases in Mh and U
  
- ✓ **Resistance to fluoroquinolones**
  - mutations of gyrase and topoisomerase IV genes
  - Patients previously treated by FQ
  - **3 % Mh and 1% U (France, 2010-2015)**



# *Mycoplasma genitalium*

- **1980: *Mycoplasma genitalium* isolated from 2 of 13 men with nongonococcal urethritis (NGU)**
  - Very slow growth (>50 days)
  - Very few isolates available



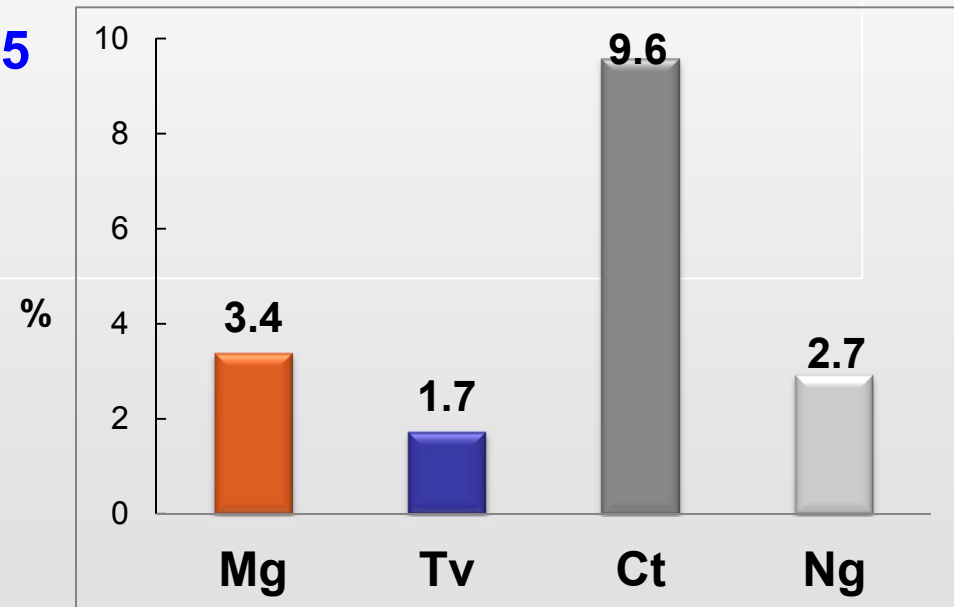
*Tully, Int J Syst Bacteriol 1983*

- **1990's: development of PCR assays**
- **1995: smallest genome known (580 kbp, 485 genes)**
  - The 2<sup>nd</sup> bacterial genome fully sequenced (Himmelreich, 1995)
  - Minimal requirements of life, concept of minimal cell

# *M. genitalium* prevalence

- **Community-based populations 1–3%**
  - Carriage frequently asymptomatic
- **STI testing centers populations (high risk) 4 – 38%**
- **Prevalence in France 2014-2015**

Urogenital specimens submitted for *C. trachomatis* (Ct) and *N. gonorrhoeae* (Ng) detection (2594 patients)

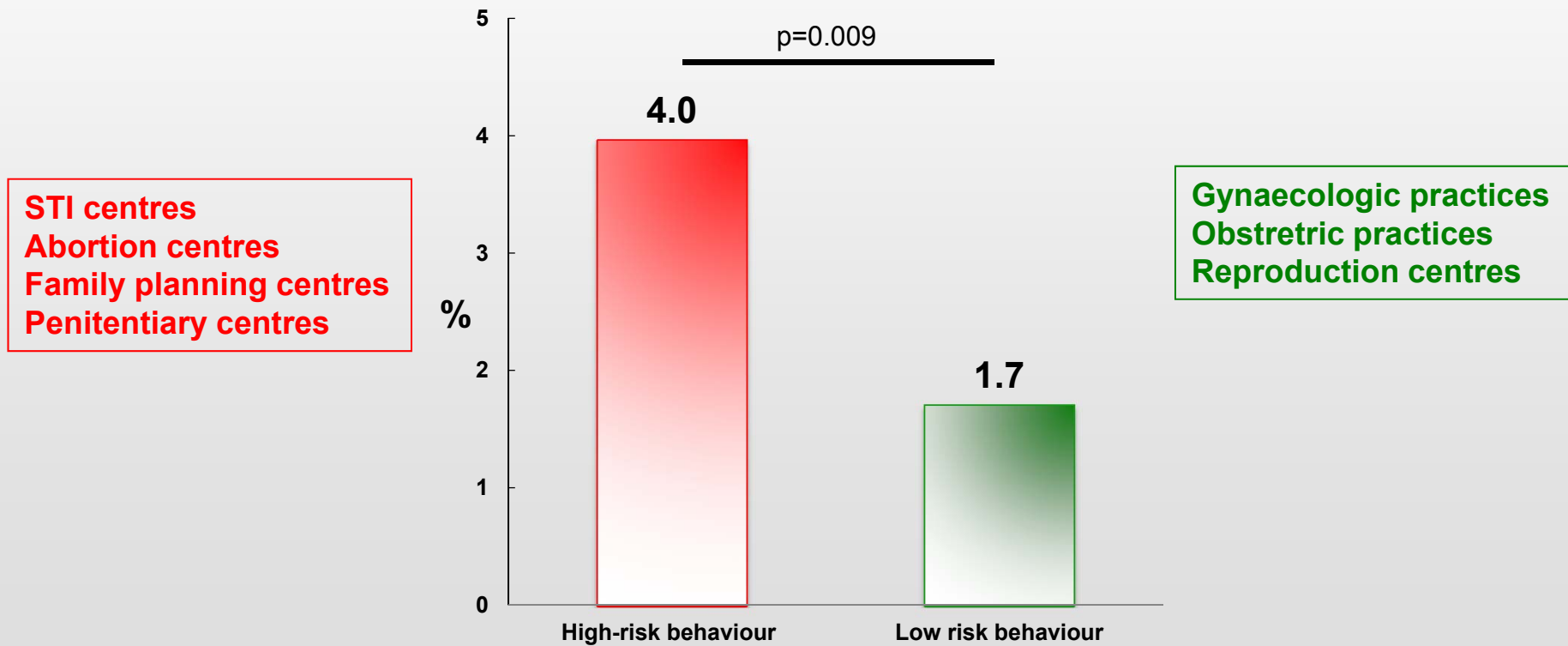


Pereyre *et al.* Clin. Microbiol. Infect. 2016

# *M. genitalium* prevalence in France

- **By sample collection sites**

Urogenital specimens submitted for *C. trachomatis* and *N. gonorrhoeae* detection in France 2014-2015



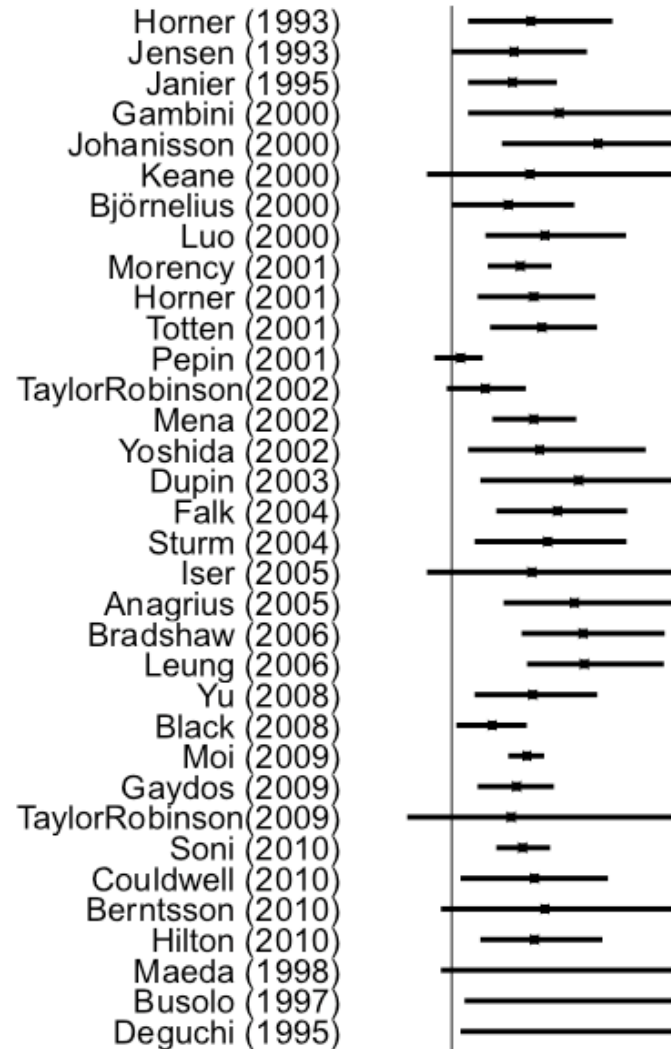
## *M. genitalium*: disease association

Men	Women
<b>Non gonococcal urethritis (NGU)</b>	<b>Urethritis</b>
<b>Balanoposthitis</b>	<b>Cervicitis</b>
<b>Epididymitis Prostatitis</b>	<b>Endometritis, Salpingitis (PID)</b>
<b>Proctitis (MSM)</b>	<b>Adverse pregnancy outcomes</b>
	<b>Female infertility</b>
	<b>Increased HIV transmission</b>

# Association between *M. genitalium* and male NGU

Male Urethritis

Odds Ratio (95% CI)



**34 studies**  
**1993-2010**

Europe, America, Asia, Oceania

**Pooled OR = 5.5**  
**(4.3-7.0)**

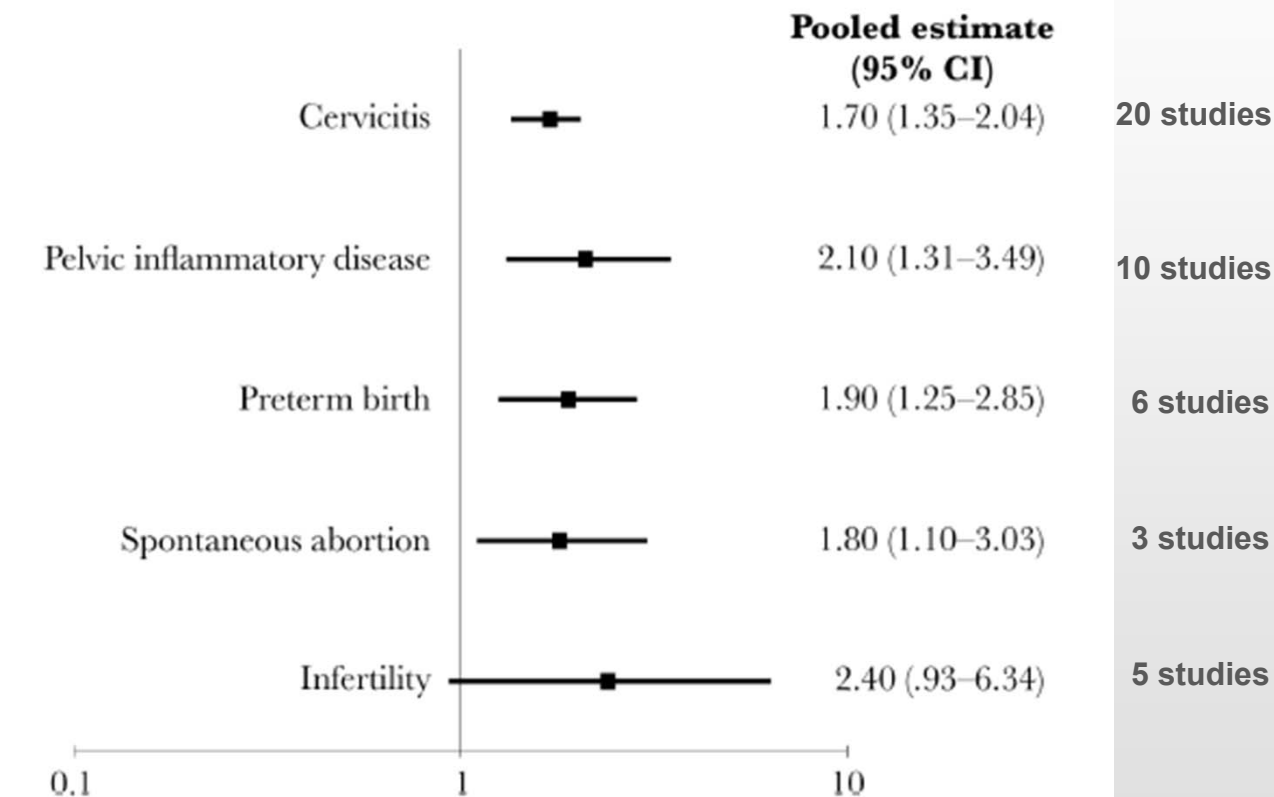
**2<sup>nd</sup> cause of NGU after**  
***C. trachomatis***

Odds Ratio (log scale)

## *M. genitalium*: disease association

Men	Women
<b>Non gonococcal urethritis (NGU)</b>	<b>Urethritis</b>
<b>Balanoposthitis</b>	<b>Cervicitis</b>
<b>Epididymitis Prostatitis</b>	<b>Endometritis, Salpingitis (PID)</b>
<b>Proctitis (MSM)</b>	<b>Adverse pregnancy outcomes</b>
	<b>Female infertility</b>
	<b>Increased HIV transmission</b>

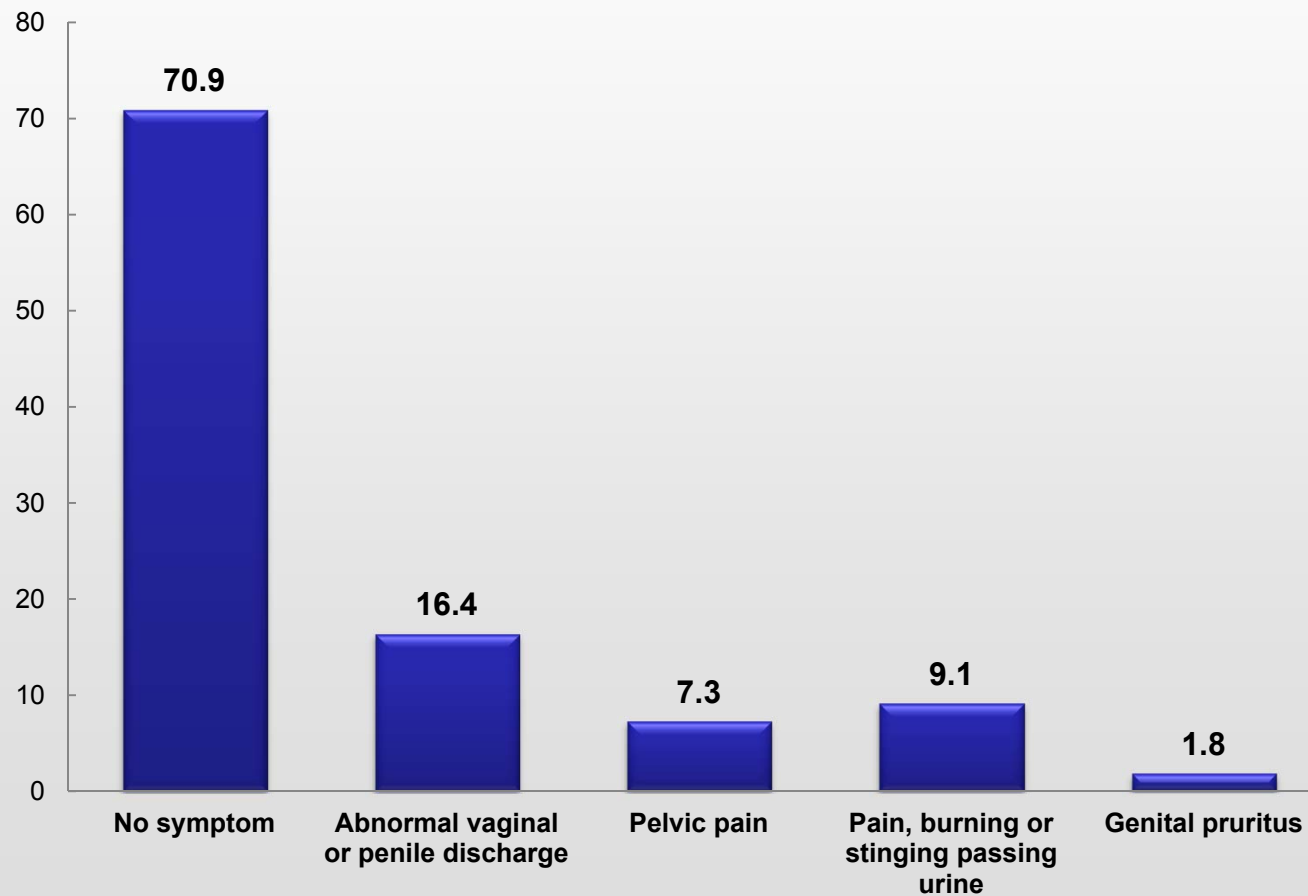
# Association between *M. genitalium* and female disease Meta-analysis 1980-2014



**Figure 1.** Summary effect sizes from meta-analysis of the association between *Mycoplasma genitalium* infection and 5 female reproductive tract disease syndromes Adapted from Lis et al [7]. Abbreviation: CI, confidence interval.

# Clinical symptoms in *M. genitalium* infections

*M. genitalium*-positive urogenital specimens submitted for *C. trachomatis* and *N. gonorrhoeae* detection in France 2014-2015





# An STI agent



- **Sexually transmission is established**
  - **Among heterosexual contacts, women are twice as likely as men to be infected** (aOR=2.18, *Slifirski, Emerg. Infect. Dis. 2017*)
  - **Transmission is probably lower than that for *C. trachomatis***
    - ✓ *Consistent with lower infectious load of Mg*
    - ✓ *men with symptomatic NGU may be more infectious than men with asymptomatic infection*
- **Among MSM, rectal infection is more common than urethral infection**
  - **MSM: rectal positivity: 42% vs urethral positivity : 8%** (*Slifirski, Emerg. Infect. Dis. 2017*)
- **Transmission through oral sex is likely to be rare**
  - **as carriage of Mg in the oropharynx is not frequent**

# Diagnostic of *M. genitalium* infections

- **Only direct diagnosis**, no serology kit commercialized
- **Culture extremely fastidious** (co-culture with Vero cells required)  
No routine MIC determination
- **Nucleic acid amplification tests:**
  - A lot of in-house PCRs, real-time PCR ++, TMA
  - MgPa adhesin gene (*mgpB*), 16S rRNA
  - Monoplex and multiplex tests commercialized, some CE-marked

## Commercially available mono and multiplex NAATs for *M. genitalium*

Manufacturer	Kit	Technique	Pathogens targeted
Hologic	<i>Mycoplasma genitalium</i> Aptima assay	TMA	<i>M. genitalium</i>
Roche/TIB MolBiol	LightMix <i>Mycoplasma genitalium</i>	qPCR	<i>M. genitalium</i>
Progenie molecular	MYGE-U, MYGE-G	qPCR	<i>M. genitalium</i>
SpeedX	ResistancePlus MG	qPCR	<i>M. genitalium</i> and macrolide resistance
BioGX (BD MAX)		qPCR	<i>M. genitalium</i> and urogenital mycoplasmas
Diagenode	S-DIAMGTV	qPCR	<i>M. genitalium</i> , <i>Trichomonas vaginalis</i>
Fast-track Diagnostics	Several kits	qPCR	<i>M. genitalium</i> and several STI pathogens and urogenital mycoplasmas
Sacace	Several kits	qPCR	<i>M. genitalium</i> alone or multiplexed with several STI pathogens and/or urogenital mycoplasmas
Seegene	Several kits	qPCR	<i>M. genitalium</i> and several STI pathogens and urogenital mycoplasmas

- **No reimbursement**
- **Need for external quality assessment programs**

# Indication for Mg testing

DOI: 10.1111/jdv.13849

JEADV

REVIEW ARTICLE

## 2016 European guideline on *Mycoplasma genitalium* infections

J.S. Jensen,<sup>1\*</sup> M. Cusini,<sup>2</sup> M. Gombert,<sup>3</sup> H. Moi<sup>4,†</sup>

<sup>1</sup>Microbiology and Infection Control, Statens Serum Institut, Copenhagen, Denmark

<sup>2</sup>Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

<sup>3</sup>Moscow Scientific and Practical Centre of Dermatovenereology and Cosmetology, Moscow, Russia

<sup>4</sup>Olafa Clinic, Oslo University Hospital, Institute of Medicine, University of Oslo, Oslo, Norway

## Symptoms

- Symptoms or signs of urethritis in men
- Mucopurulent cervicitis
- Cervical or vaginal discharge with risk factor of STI
- Intermenstrual or post coital bleeding
- Acute pelvic pain and/or PID
- Acute epididymo-orchitis in a male <50 yo

# Indication for Mg testing

## Symptoms

## Risk factors

- Symptoms in a regular sexual partner

- Persons with high-risk sexual behavior (<40 yo, >3 new sexual contacts in the last year)

The public health value of testing asymptomatic persons for Mg has not been established. Decision on testing should be informed by local epidemiology when available

- Sexual contact of persons with STI or PID, with Mg-infected persons

- Before termination of pregnancy or other procedures, that break the cervical barrier

- Regular testing of MSM including anal sampling

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<sup>4</sup>Oslo Clinic, Oslo University Hospital, Institute of Medicine, University of Oslo, Oslo, Norway

# *M. genitalium* and tetracyclines

- **Relative potency *in vitro***

MIC ranges (µg/ml)

<i>Antibiotics</i>	<i>M. genitalium</i>	<i>M. hominis</i>	<i>Ureaplasma spp.</i>
<i>Tetracyclines**</i>			
Doxycycline	≤0.01-0.3	0.1-2	0.02-1
Minocycline	≤0.01-0.2	0.03-1	0.06-1

- **BUT, low eradication rate**
  - **Microbiological cure : between 30 and 40 %**

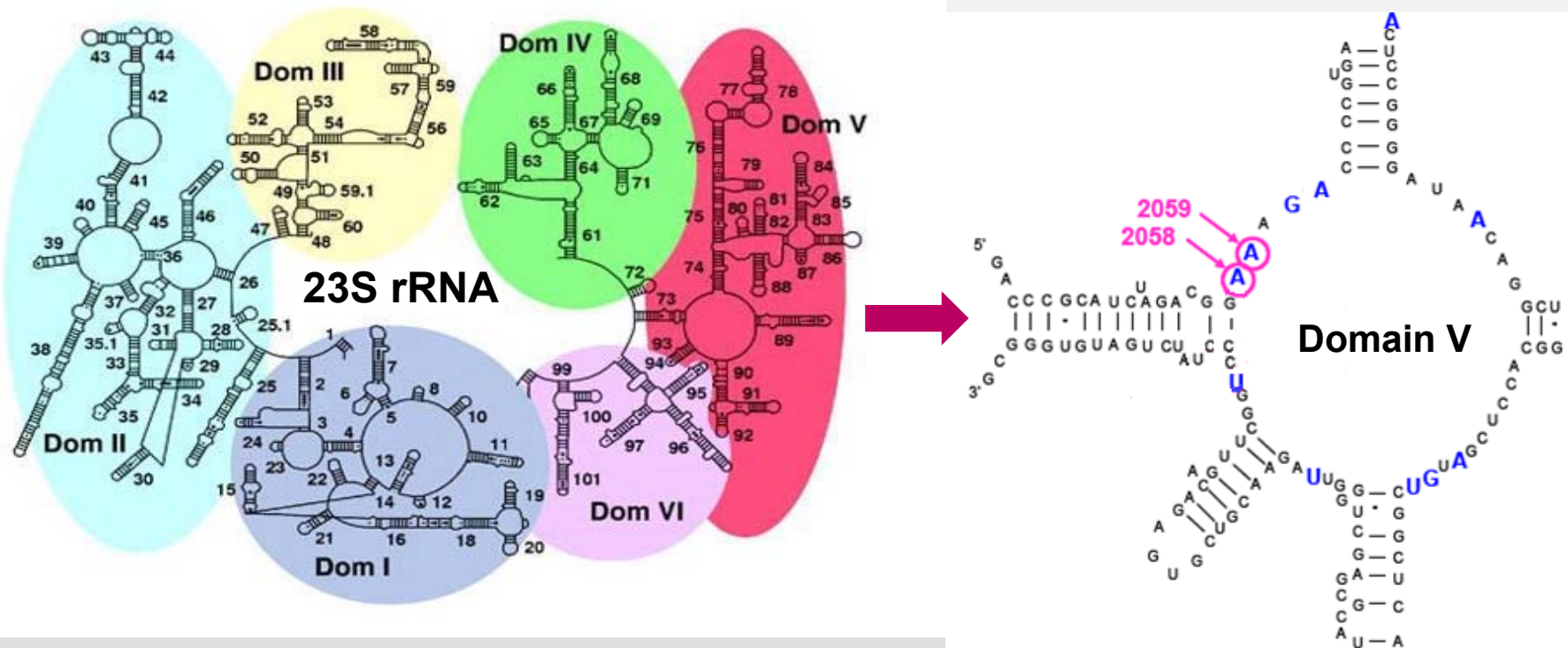
# *M. genitalium* and macrolides

- Low MICs

<i>Antibiotics</i>	<i>M. genitalium</i>	<i>M. hominis</i>	<i>Ureaplasma spp.</i>
MLSK group			
Erythromycin	≤0.01	32->1 000	0.02-16
Roxithromycin	<0.01	>16	0.1-2
Clarithromycin	≤0.01-0.06	16->256	≤0.004-2
Azithromycin	≤0.01-0.03	4->64	0.06-4
Josamycin	0.01-0.02	0.05-2	0.03-4
Clindamycin	0.2-1	≤0.008-2	0.2-64
Pristinamycin	≤0.01-0.02	0.1-0.5	0.1-1
Quinupristin/ Dalfopristin	0.05	0.03-2	0.05-0.5
Telithromycin	≤0.015	2-32	≤0.015-0.25
Solithromycin	≤0.000000063- 0.000125	0.002-0.008	0.002-0.063

# Macrolide resistance in *M. genitalium*

- Mutations in domain V of 23S rRNA
  - Single operon encoding 16S and 23S rRNA
  - A2058G/C/T, A2059G/C/T (*E. coli* numbering)



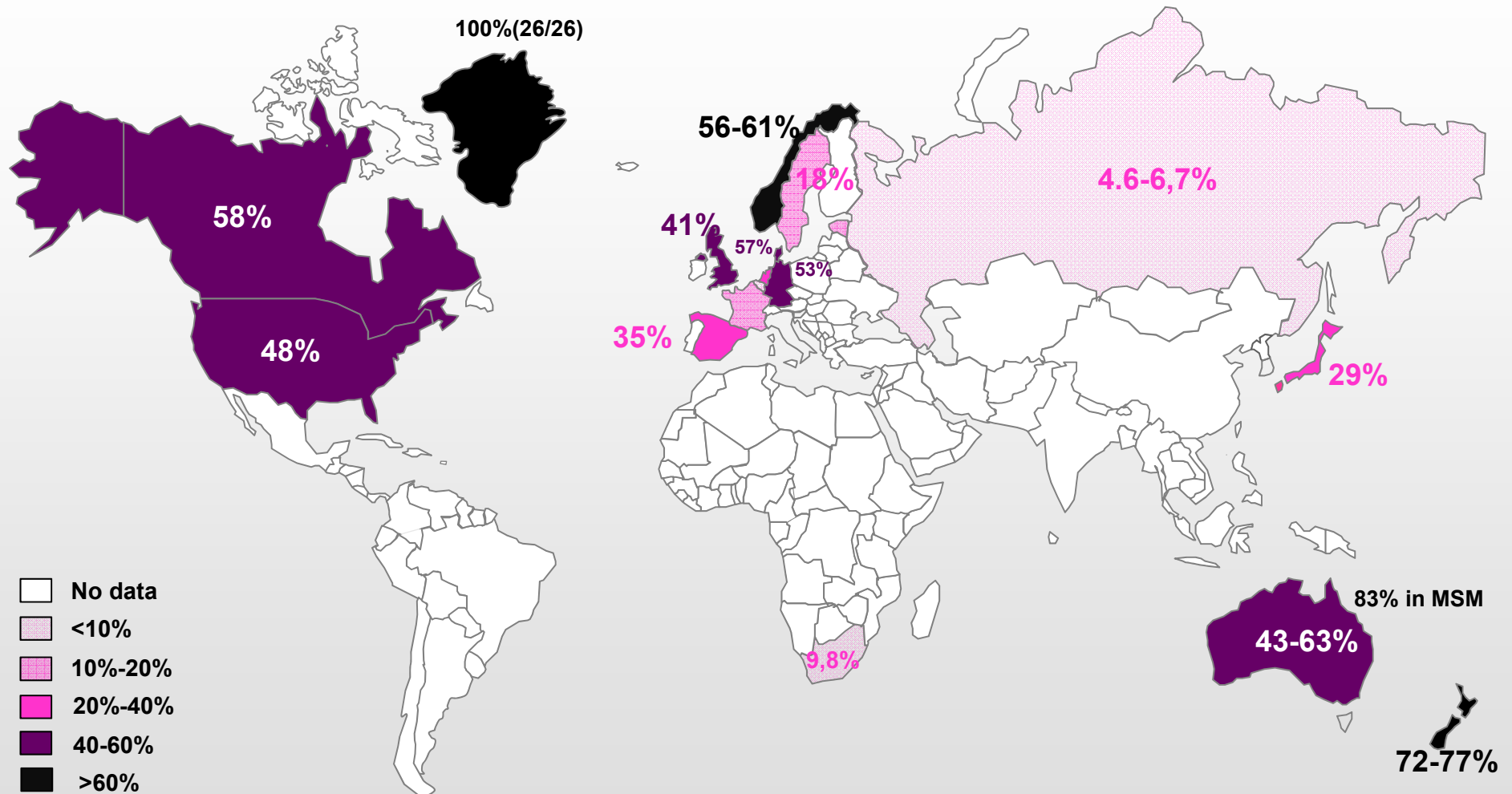


# Detection of macrolide resistance-associated mutations

- **Amplification and 23S rRNA sequencing**
  - Time-consuming, not adapted to routine
- **In-house methods**
  - **FRET real-time PCR** (Touati *et al.* J. Clin. Microbiol. 2014)
  - **HRM (High Resolution Melting curve analysis)** (Twin *et al.* PloS One 2012)
  - **PCR and pyrosequencing** (Salado-Rasmussen *et al.* Clin. Infect. Dis. 2014)
  - **Taqman PCR** (Wold *et al.* J. Eur. Acas. Dermatol. Venereol. 2015), (Kristiansen *et al.* J. Clin. Microbiol. 2016)
  - **Single probe PCR and melting curve analysis** (Gossé *et al.* J. Clin. Microbiol. 2016)
- **Commercial kits**
  - **ResistancePlus™ MG kit (SpeeDx, Australia) : multiplex real-time PCR Detection of Mg and 5 mutations** (Le Roy, J. Clin. Microbiol. 2017)

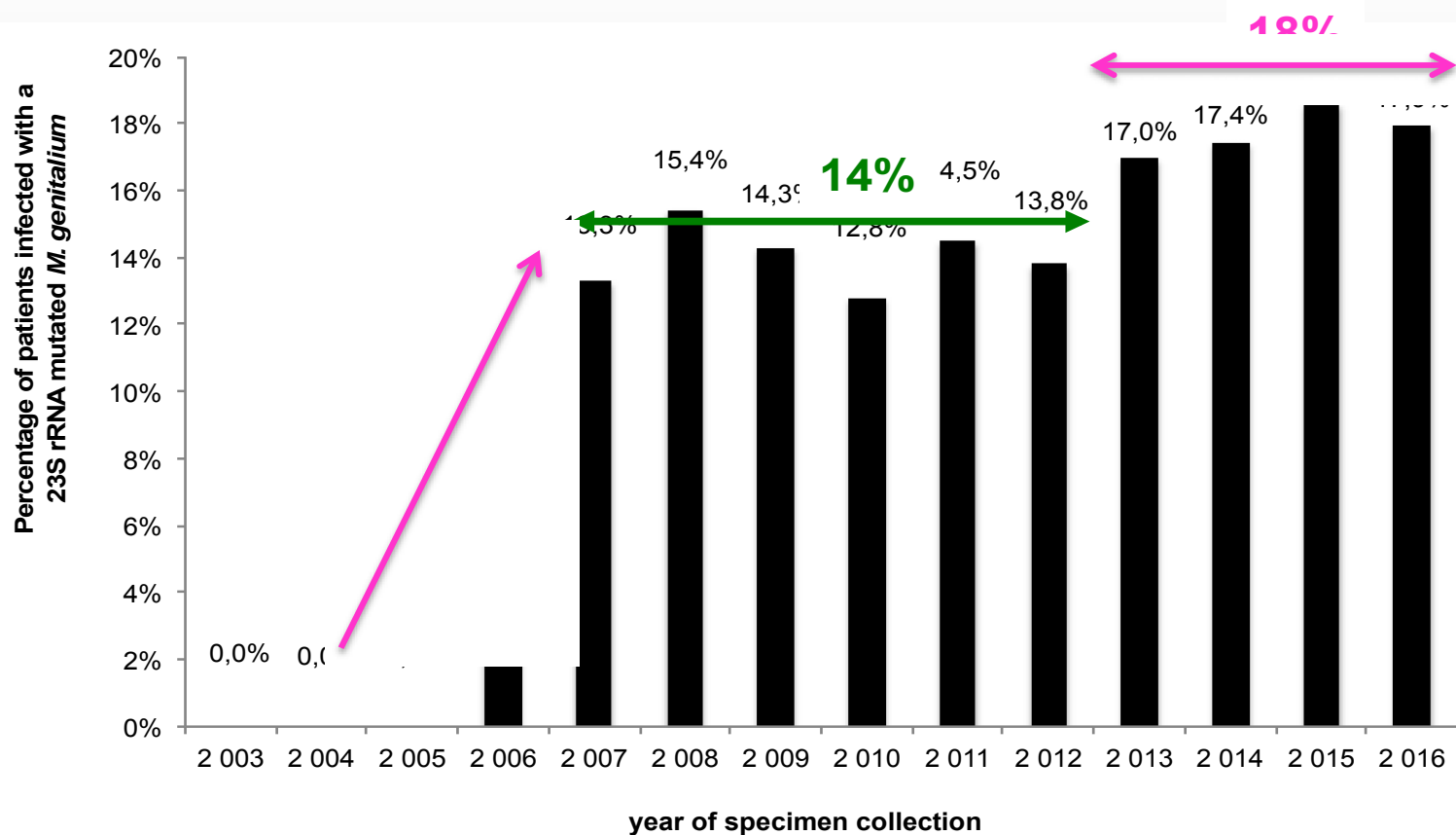


# Prevalence of macrolide resistance in *M. genitalium*



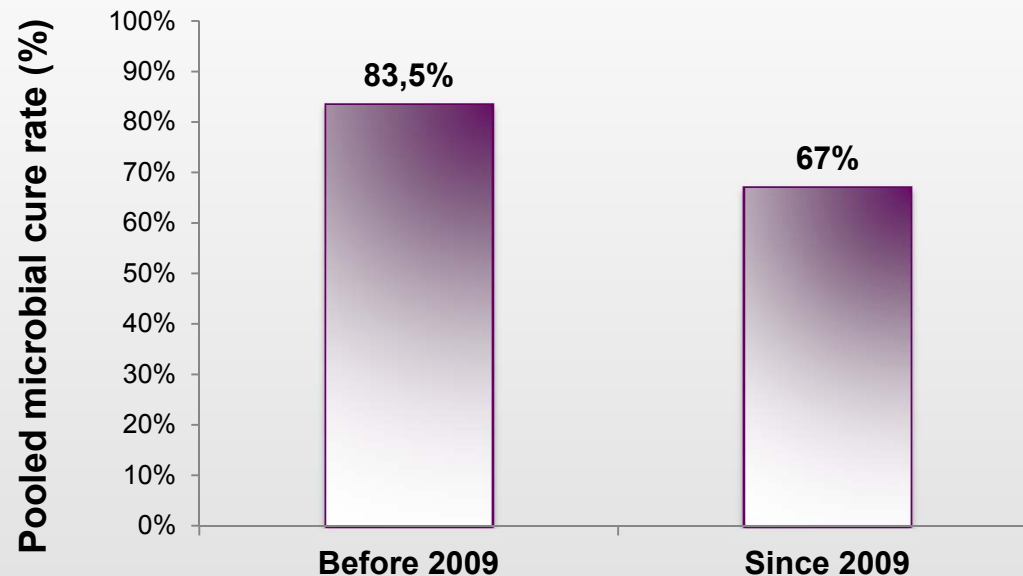
Anagnrius, *PloS one* 2013; Tagg, *J. Clin. Microbiol.* 2013; Pond, *Clin. Inf. Dis.* 2014; Salado-Rasmussen, *Clin. Inf. Dis.* 2014; Kikuchi, *J. Antimicrob. Chemother.* 2014; Hay, *Sex. Transm. Dis.* 2015; Gushin, *BMC Infect. Dis.* 2015; Nijhuis, *J. Antimicrob. Chemother.* 2015; Gesink, *Can. Fam. Physician*, 2016; Getman, *J. Clin. Microbiol.* 2016; Gossé, *J. Clin. Microbiol.* 2016; Shipitsina, *Plos One*, 2017; Basu, *J. Clin. Microbiol.* 2017; Tabrizi, *J. Clin. Microbiol.* 2017; Barbera, *Sex. Transm. Dis.* 2017; Dumke, *Diagn Microbiol infect Dis*, 2016; Coorevits, *J. Glob. Antimicrob. Resist.* 2017; Anderson, *J. Clin. Microbiol.* 2017; Unemo, *Clin. Microbiol. Infect.* 2017.

# Macrolide resistance in *M. genitalium* Bordeaux, France



# *M. genitalium* treatment studies : Azithromycin 1g

- **Meta-analysis on the efficacy of AZM 1g for Mg treatment** (*Lau Clin. Infect. Dis.* 2015)  
21 studies, 1490 participants, mostly male NGU



- **AZM 1g single dose is no more the the 1<sup>st</sup> line treatment**
  - ➔ Therapeutic failure if patient infected with a mutated strain
  - ➔ **Selection of resistant mutants during AZM treatment**

## ***M. genitalium* treatment studies : Azithromycin 1.5 g/5 days**

- **Extended 1.5 g AZM (500 mg d1, 250 mg d2-4)**  
**85% effective and associated with lower risk of inducing AZM R**  
*Anagnius PLoS One 2013, Bjornelius Sex Transm Infect 2008, Falk J Antimicrob Chemother 2015, Gundevia STI 2015*
- **Patients failing azithromycin 1g single dose cannot be treated successfully with extended 1.5 g AZM**  
*Jernberg Sex Transm Infect 2008, Jensen Clin Infect 2009*
- **Moxifloxacin 400 mg for 7-10 d in case of AZM failure... but...**  
*Jensen JEADV 2016, Horner, STD and AIDS, 2016; www.cdc.gov/std/tg2015*

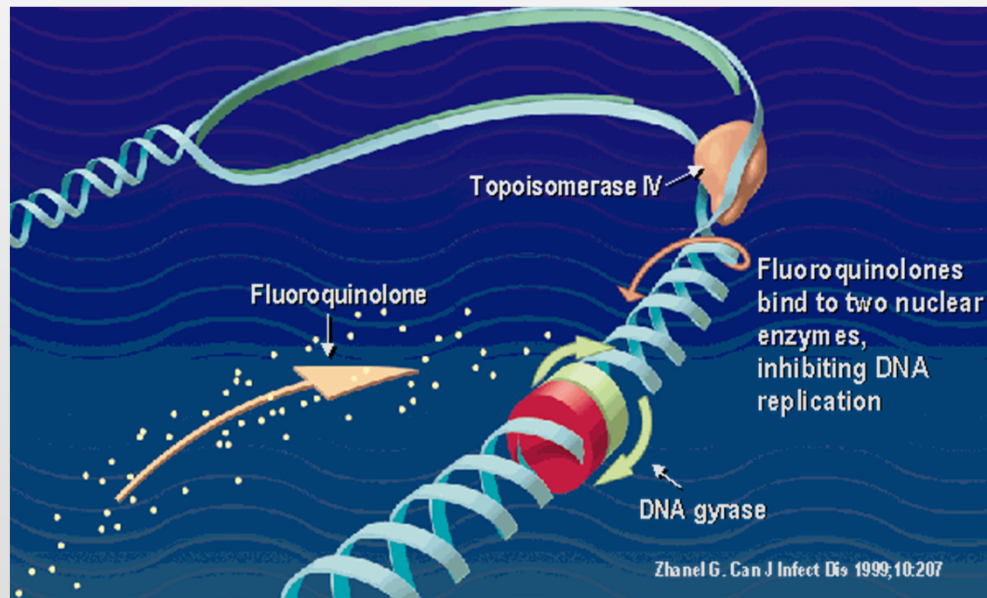
# *M. genitalium* and fluoroquinolones

- Only moxifloxacin has low MICs

<b>Antibiotics</b>	<b><i>M. genitalium</i></b>	<b><i>M. hominis</i></b>	<b><i>Ureaplasma spp.</i></b>
<b>Fluoroquinolones</b>			
Ciprofloxacin	2	0.1-4	0.1-16
Ofloxacin	1-2	0.1-4	0.2-4
Levofloxacin	0.5-1	0.1-2	0.2-2
Moxifloxacin	0.03-0.06	0.06-0.125	0.125-1
Sitafloxacin (Japan only)	0.125		

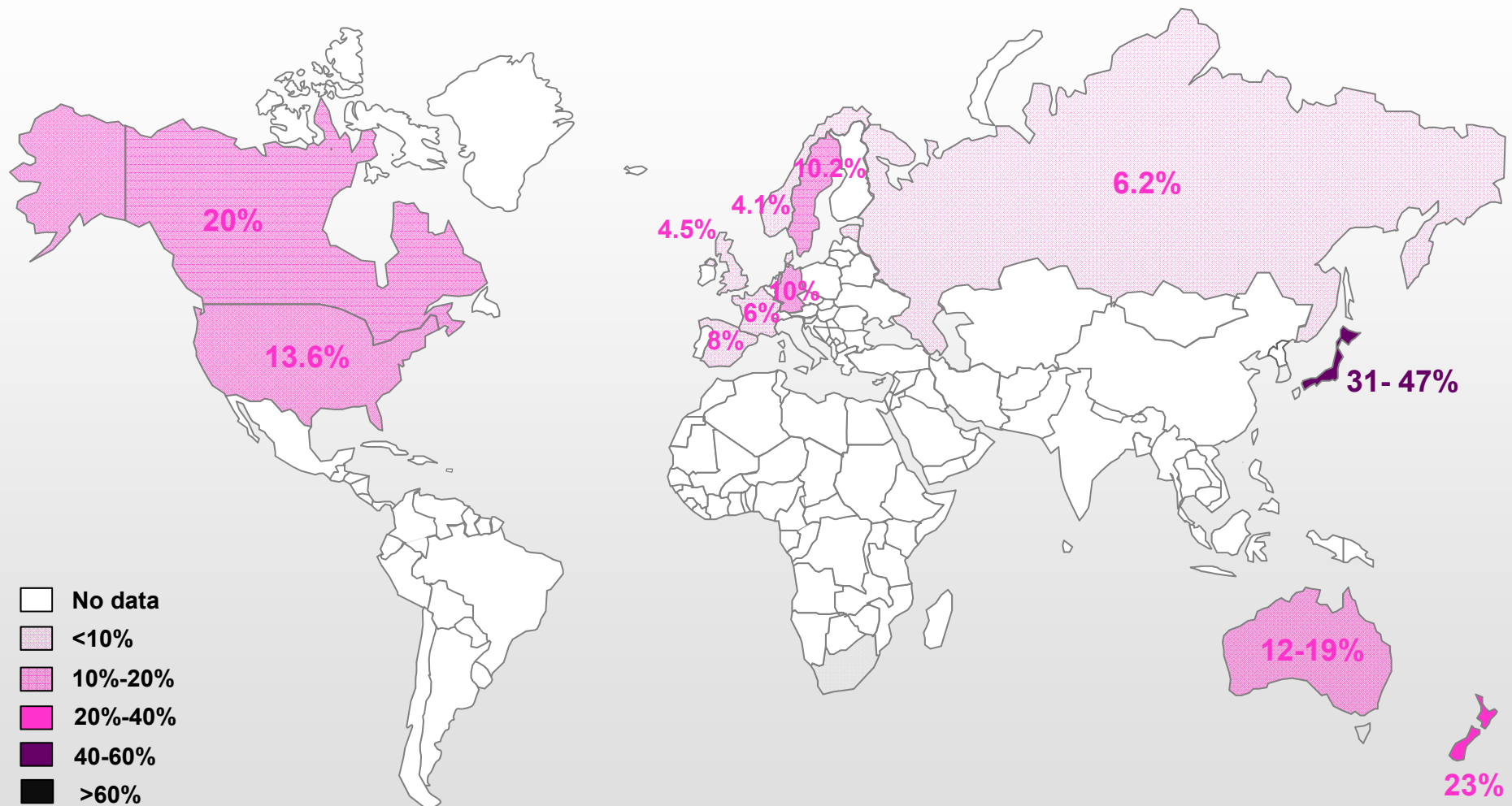
# Fluoroquinolone resistance in *M. genitalium*

- **Mutations in the bacterial target genes of fluoroquinolones**
  - **Most frequent mutations in *parC* (Topoisomerase IV)**  
Primarily Ser83 and Asp87
  - **A few mutations in *gyrA* (DNA gyrase)**



- **Molecular detection only: amplification and sequencing of target genes**

# Prevalence of fluoroquinolone resistance-associated mutations

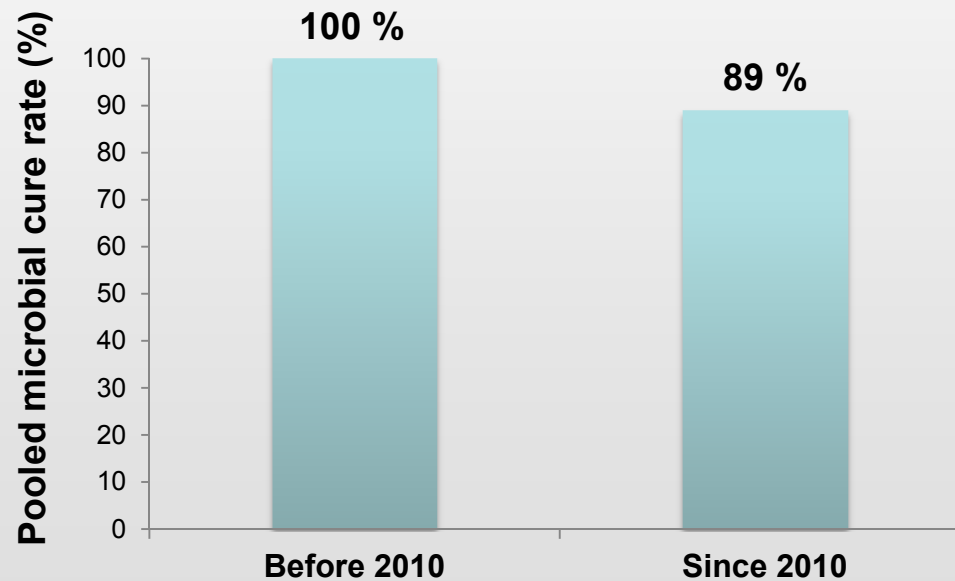


*Bissessor Clin Infect Dis 2015; Deguchi, Clin Infect Dis 2016; Dumke, DMID 2016; Kikuchi J Antimicrob Chemother 2014; Le Roy Emerg Infect Dis 2016; Pond Clin Infect Dis 2014; Shipitsina PLoS one 2017; Couldwell Int J STD and AIDS 2013; Gesink Can family Physian 2016; Tagg J Clin Microbiol 2013; Murray Emerg Infec Dis 2017; Barbera Sex Transm infect 2017; Anderson, J Clin Microbiol 2017, Unemo, Clin Microbiol Infect 2017.*



# *M. genitalium* fluoroquinolone treatment studies

- **Meta-analysis on the efficacy of moxifloxacin for *M. genitalium* treatment** (*Yi et al Int J STD AIDS 2017*)
  - **17 studies, 252 patients**



# 2016 European guideline on *Mycoplasma genitalium* infections

Jørgen Skov Jensen<sup>\*1</sup>, Marco Cusini<sup>2</sup>, Mikhail Gomberg<sup>3</sup>

<sup>1</sup>Microbiology and Infection Control, Statens Serum Institut, Copenhagen, Denmark.

<sup>2</sup>Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

<sup>3</sup>Chief Researcher, Moscow Scientific and Practical Centre of Dermatovenereology and Cosmetology.



JEADV 2016 DOI 10.1111/jdv.13849

- **Uncomplicated *M. genitalium* infection:**
  - In the absence of macrolide resistance-associated mutations
    - Azithromycin 500 mg (day 1), then 250 mg (days 2-5)**
    - Josamycin 500 mg 3 times daily - 10 days- IV
  - Macrolide-resistant *M. genitalium* infection
  - Second line treatment for persistent *M. genitalium* infection
    - Moxifloxacin 400 mg/d - 7 to 10 days**



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<sup>3</sup>Chief Researcher, Moscow Scientific and Practical Centre of Dermatovenereology and Cosmetology.



JEADV 2016 DOI 10.1111/jdv.13849

- **Third-line treatment for persistent MG infection after AZM and MXF**
  - Doxycycline 100 mg x2 daily for 14 days**
  - Pristinamycin 1 g x4 daily for 10 days**
- **Complicated MG infection (PID, epididymitis)**
  - Moxifloxacin: 400 mg - 14 days**
- **Test of cure:** no earlier than 3 weeks after the start of antibiotic treatment



## 2016 European guideline on *Mycoplasma genitalium* infections

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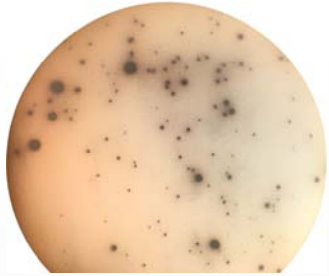
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- All *M. genitalium*-positive test should be followed up with an assay capable of **detecting macrolide resistance-associated mutations**
- The extended azithromycin ttt after failure with 1g single dose will **NOT** eradicate *M. genitalium*



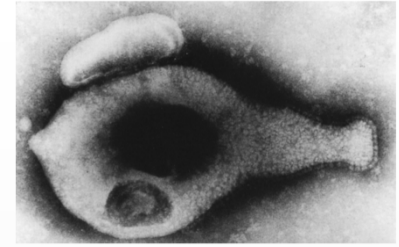
## Take-home message



### *Ureaplasma* spp. and *M.hominis* are NOT STI agents

- **Commensal of the urogenital tract**
  - **Quantitative culture to interpret their pathogenic role**
    - *Ureaplasma* in men, *M. hominis* in women
    - Adverse pregnancy outcomes and neonates : both
  - **PCR : specimens from sterile sites**
- **Antibiotic susceptibility testing**
  - **Commercialized kits, CLSI breakpoints**
- **Prevalence of resistance in France, 2010-2015**
  - **Tetracycline: 7.5% (U) to 15% (Mh)**
  - **Fluoroquinolones: 1% (U), 3% (Mh)**
  - **Macrolide : very rare**

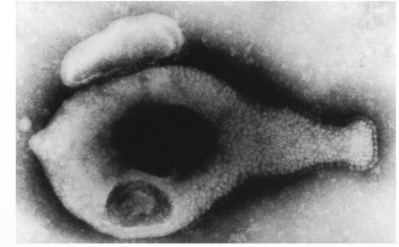
## Take-home message



### *M. genitalium*, a STI pathogen, has emerged!

- Male NGU, female cervicitis and PID
- Pauci-symptomatic infections
- Highest prevalence in high-risk sexual behavior patients +++
- Diagnostic : NAAT assays
  - Activity is predicted to increase (commercially available NAAT assays)
- Prevalence of resistance in France, 2015-2016
  - Tetracycline: no resistance but 70% treatment failure
  - Azithromycin: 18%
  - Moxifloxacin : 6%

## Take-home message



- **1<sup>st</sup>-line treatment for uncomplicated *M. genitalium* infection**
  - Azithromycin 1.5 g on 5 days in absence of macrolide resistance
- **Moxifloxacin 400 mg/j 7-10 days in the other cases**
  - Under pressure
- **Always test of cure after 3-5 weeks**

⇒ **Superbug? New XDR bacteria?**

Might become **untreatable** in certain circumstances

Need for trials of combinations of registered drugs and new antimicrobial compounds

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## Bordeaux and its surroundings

