ESGMI ESCMID STUDY GROUP FOR MYCOPLASMA INFECTIONS
European Society of Clinical Microbiology and Infectious Diseases

IOM The International Organization for Mycoplasmology
Dedicated to the study of the Mollicutes

Mycoplasma genitalium: epidemiology, diagnostics and antimicrobial resistance

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INRA SCIENCE & IMPACT

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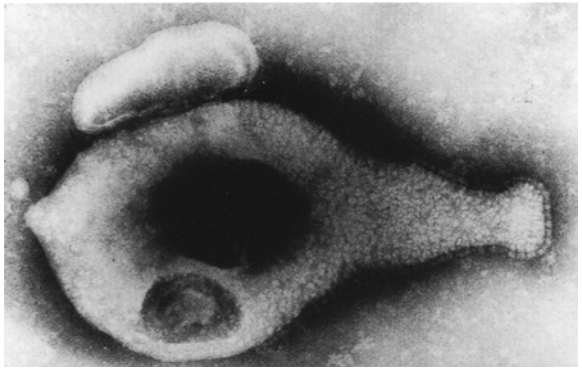
Déclaration d'intérêts de 2012 à 2015

Investigateur principal d'une recherche ou d'une étude clinique :

- **Roche Diagnostics,**
- **Diagenode,**
- **Hologic,**
- **SpeeDx**

Mycoplasma genitalium

- **1980: *Mycoplasma genitalium* isolated from 2 of 13 men with nongonoccocal uretritis (NGU)**
  - **Mollicutes class: no cell wall**
  - **Very slow growth (>50 days)**
  - **Very few isolates available**
  - **Animal model in chimpanzees**



Tully, Int J Syst Bacteriol 1983

- **1990's: development of PCR assays, allowed study of disease association**

- **1995: smallest genome known (580 kbp, ≈ 480 genes)**
  - **The 2nd bacterial genome fully sequenced (Himmelreich, 1995)**
  - **Minimal requirements of life, concept of minimal cell**

M. genitalium: prevalence and incidence

- **Prevalence**
  - **Community-based populations 1–3%**
    Carriage may be asymptomatic
  - **STI testing centers populations (high risk) 4 – 38%**
- **Incidence**
  - **University women: 0.9 per 100 WY**
  - **Kenyan female sex workers: 23 per 100 WY**

Anderson Sex Transm Infect 2007; Baczynska Syst Biol Reprod Med 2008; Cohen Sex Transm Dis 2007; Jensen J Eur Acad Dermatol 2013; Manhart Am J Public Health 2007; Clin Infect Dis 2011; Oakeshott Clin Infect Dis 2010; Peuchant Diagn Microbiol Infect Dis 2015; Svenstrup BMJ Open 2014; Walker Clin Infect Dis 2013

M. genitalium: disease association

Men	Women
NGU	Urethritis
Balanoposthitis	Cervicitis
Epididymitis Prostatitis	Endometritis, Salpingitis (PID)
Proctitis (MSM)	Adverse pregnancy outcomes
	Female infertility
	Increased HIV transmission

Association between *M. genitalium* and male NGU (1)

- **Responsible for 15-20% NGU (pooled OR 5.5),**
  20-25% NCNGU,
  30% persistent-recurrent U

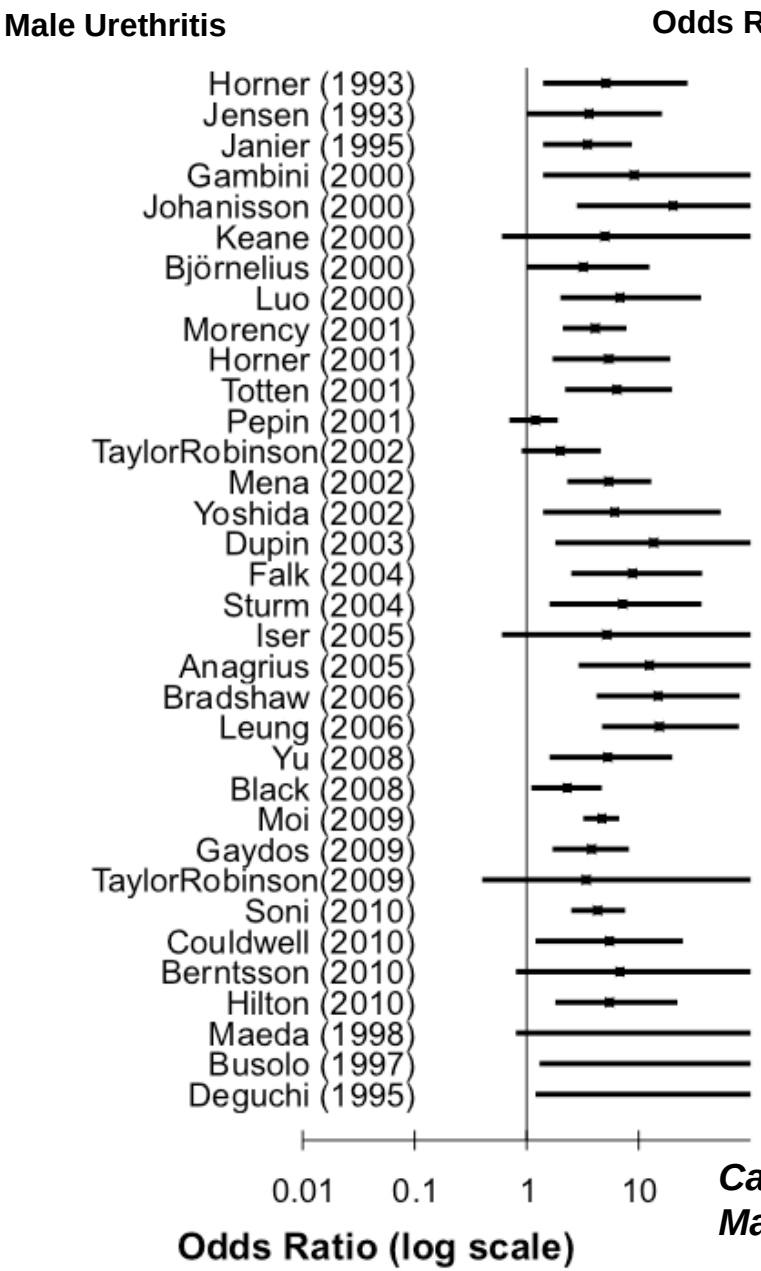
- **2nd cause of NGU after *Chlamydia trachomatis***

- **Coinfection with *C. trachomatis* not incommon**

- **2015 Updated CDC STD guidelines, 2016 European guideline on NGU, 2016 European guideline on *M. genitalium* infection:**
  role of Mg in urethritis and treatment-related implications

Taylor-Robinson, Jensen Clin Microbiol Rev 2011; Manhart Clin Infect Dis 2011; Mena J Infect Dis 2012; www.cdc.gov/std/tg2015; http://www.iusti.org/regions/europe/pdf/2016/2016EuropeanMycoplasmaGuidelines.pdf http://www.iusti.org/regions/europe/pdf/2016/2016EuropeanNGUGuideline.pdf (Int J STD AIDS 2016, Eurosurveillance 2016)

Association between *M. genitalium* and male NGU (2)



Cazanave et al, Med Mal Infect 2012
Manhart et al, Clin Infect Dis 2011

Association between *M. genitalium* and female reproductive tract disease (1)

- **Fewer studies than in men, small sample sizes**
- **Commonly asymptomatic**
- **Mg detected in 10-30% clinical cervicitis, 2-22% PID**
- **Similar to *C. trachomatis:*** **Mg can cause PID (proportion of cases unknown), but less frequently than with *C. trachomatis***
- **Adverse pregnancy outcomes and female infertility:** **more research needed**
- **2015 Updated CDC STD guidelines, 2016 European guideline on *M. genitalium* infection:** **role of Mg in cervicitis and treatment-related implications**

Taylor-Robinson and Jensen, Clin Microbiol Rev 2011; Manhart et al, Clin Infect Dis 2011; Mc Gowin et al, PLoS Pathog 2011, Lis et al, Clin Infect Dis 2015; www.cdc.gov/std/tg2015 http://www.iusti.org/regions/europe/pdf/2016/2016EuropeanMycoplasmaGuidelines.pdf

Association between *M. genitalium* and female disease (2)

Metanalysis 1980-2014

(Lis et al, Clin Infect Dis 2015, 61:418, PMID: 26042815)

- ***M. genitalium* infection significantly associated with approximately 2-fold increased risk of:**
  - **Cervicitis (20 included studies): pooled OR, 1.66**
  - **PID (10 studies): pooled OR, 2.14**
  - **Pre-term birth (6 studies): pooled OR 1.89**
  - **Spontaneous abortion (3 studies): pooled OR 1.82**

- **Elevated risk of female infertility**
  - **5 included studies, risk about 2.5-fold**
  - **Only statistically significant in subanalyses**

Diagnosis of *M. genitalium* infections (1)

- **Only a direct diagnosis, no serology kit commercialized**
- **Culture extremely fastidious (very few strains isolated worldwide, coculture with Vero cells)**
- **By nucleic acid amplification tests:**

  - A lot of in-house PCRs, real-time PCR ++, TMA

  - MgpA adhesin gene, 16S rRNA

  - Monoplex and multiplex tests commercialized, some CE-marked, no FDA-approved (RUO tests)

  - Load very low even in symptomatic infections

  - Some specimens better than others:
  FVU> urethral swabs in men
  vaginal swabs >cervix>FVU in women

Hamasuna J Clin Microbiol 2007; Jensen J Clin Microbiol 2004; Le Roy J Microbiol Methods 2012, 2014; Lee J Infect Chemother 2012; Lillis J Clin Microbiol 2011; Wroblewsky J Clin Microbiol 2006

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

Manufacturer	Kit	Technique	Pathogens targeted
Diagenode	S-DIAMGTV	qPCR	*M. genitalium, Trichomonas vaginalis*
Fast-track Diagnostics	Several kits	qPCR	*M. genitalium* and several STI pathogens and urogenital mycoplasmas
Hologic	*Mycoplasma genitalium* Aptima assay	TMA	*M. genitalium*
Roche/TIB MolBiol	LightMix *Mycoplasma genitalium*	qPCR	*M. genitalium*
Sacace	Several kits	qPCR	*M. genitalium* alone or multiplexed with several STI pathogens and/or urogenital mycoplasmas
Seegene	Several kits	qPCR	*M. genitalium* and several STI pathogens and urogenital mycoplasmas

Diagnosis of *M. genitalium* infections (2)

- **Barriers for Mg testing:**

  - No reimbursement

  - Lack of validated commercial assays

  - Low throughput in commercial available assays

  - Test diagn. performance varies significantly between labs

⇒ Need for quality assessment

⇒ Diagnostic activity is predicted to increase

Diagnosis of *M. genitalium* infections (3)

- **Indications for Mg testing:**

Symptoms

Risk factors

- **Symptoms in a regular sexual partner**
- **Persons with high-risk sexual behavior (<40 yo, >3 new sexual contacts, >5 life-time partners)**
- **Sexual contact of persons with STI or PID, Mg-infected persons**
- **Before termination of pregnancy or other procedures, that break the cervical barrier**
- **Regular testing of MSM including anal sampling (role of Mg in increased HIV transmission risk)**

http://www.iusti.org/regions/europe/pdf/2016/2016EuropeanMycoplasmaGuidelines.pdf

M. genitalium and antibiotics

- **Intrinsic resistance related to β-lactams +++, fosfomycin, glycopeptides and rifampicin**

- **Active antibiotics *in vitro***

  - Macrolides, lincosamides, streptogramins, ketolides (MLSK), tetracyclines, fluoroquinolones

  - Early *in vitro Mg* studies:
  highly S to macrolides (azithromycin, AZM),
  reduced S to tetracyclines and older fluoroquinolones (CIP, OFX)

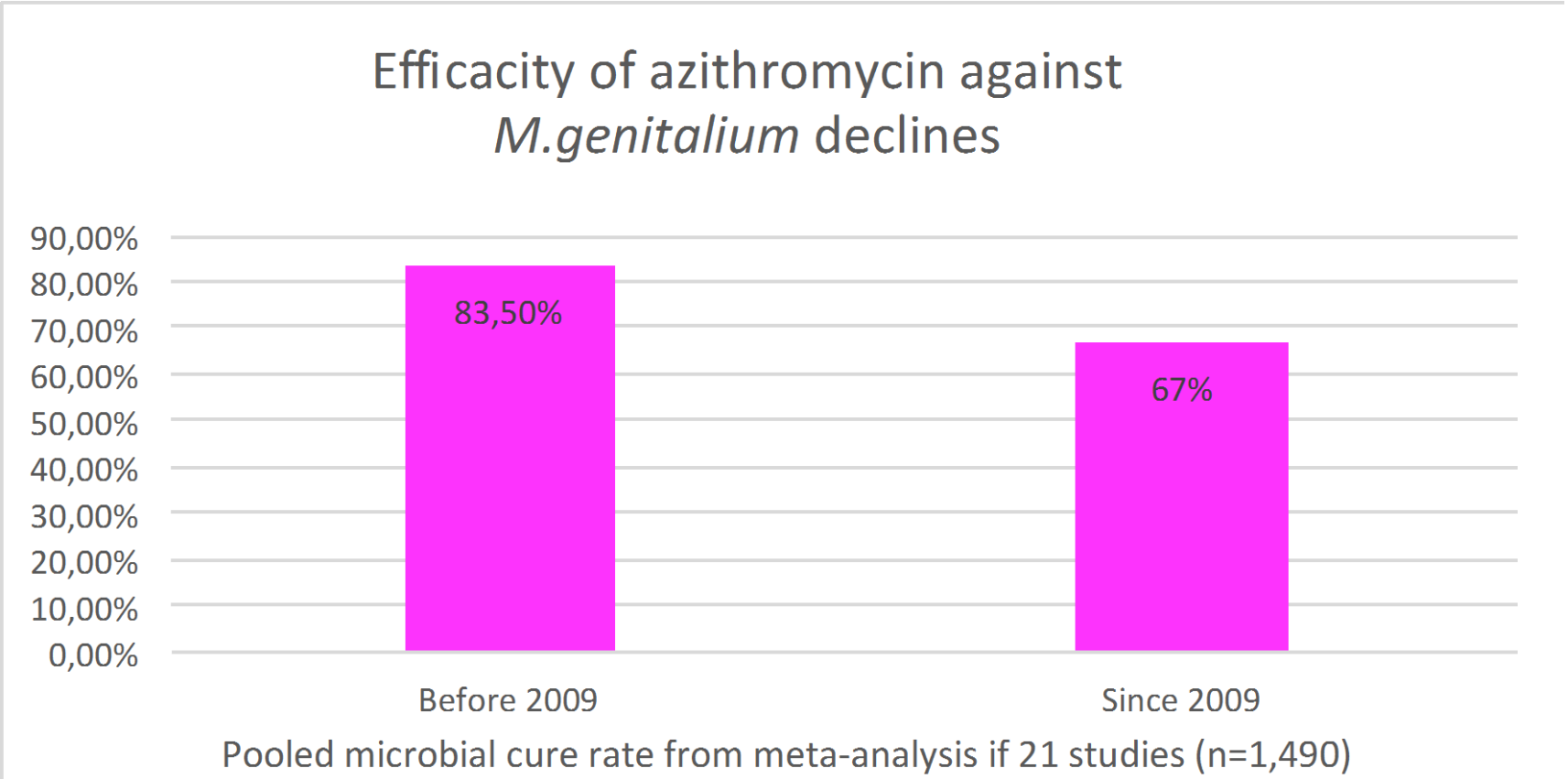
- **No antimicrobial susceptibility testing done in routine**

- **Acquired resistance**
  - Genetic support: chromosomal mutations ++
  - Target modification

Bébéar Clin Infect Microbiol 2008; Bébéar in Antibiogram, ASM Press 2010; Hamasuna Antimicrob Agents Chemother 2005; Renaudin Antimicrob Agents Chemother 1992; **Pereyre in Antimicrobial Therapy and Vaccines,www.antimicrobe.org, 2015**

M. genitalium treatment studies

- **Most male NGU studies**
- **Tetracyclines not useful, effective in only 30-40% (no acquired R)**
  AZM 1g single dose = 1st line treatment



Source: Lau A, et al. Clin infect Dis. 2015;doi:10.1093/cid/civ644.PMID: 26240201

M. genitalium treatment studies

- **Most male NGU studies**
- **Tetracyclines not useful, effective in only 30-40% (no acquired resistance)**
  AZM 1g single dose = 1st line treatment
- **Metanalysis on the efficacy of AZM for Mg treatment**
  (Lau Clin Infect Dis 2015)

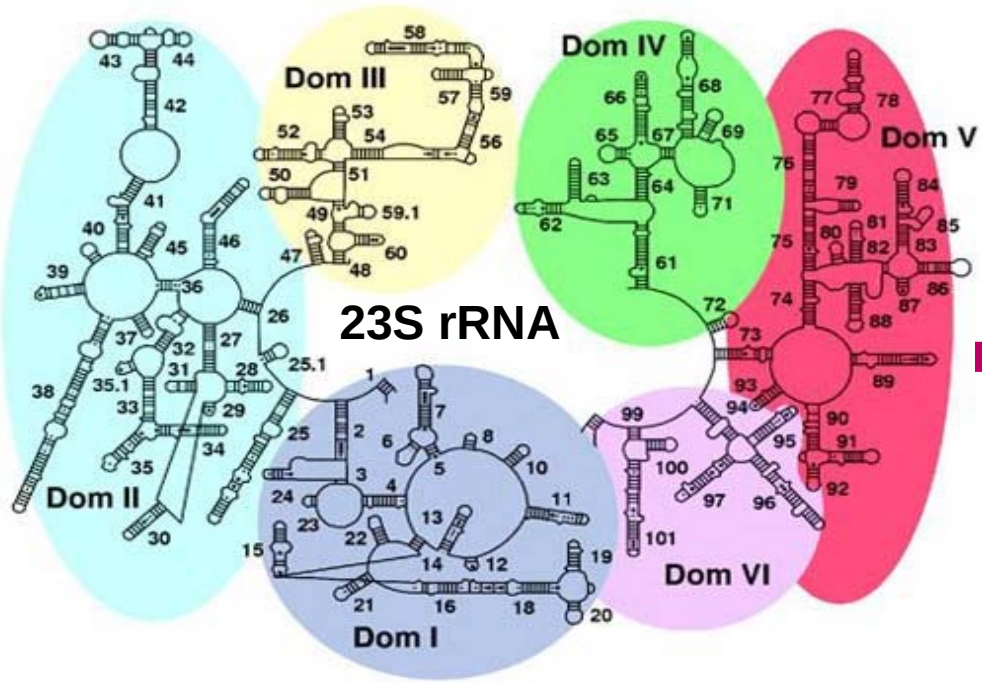
⇒ **Declining treatment efficacy of AZM 1g for the treatment of urogenital *M. genitalium***

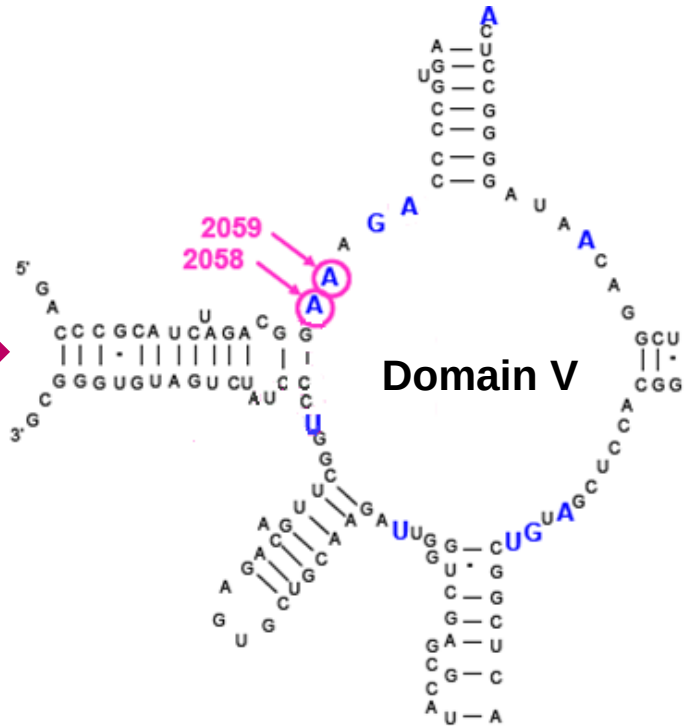
⇒ **Why? Increasing prevalence of macrolide resistance due to widespread use of AZM 1g single dose**

Björnelius Sex Transm Infect 2008; Manhart Clin Infect Dis 2013; Mena Clin Infect Dis 2009; Schwebke CID 2011, Sena J Infect Dis 2012; Stamm Sex Transm Infect 2007; Lau Clin Infect Dis 2015; Horner Clin Infect Dis 2015; Jensen BMC Infect Dis 2015

Macrolide resistance in *M. genitalium* (1)

- **Mutations in domain V of 23S rRNA**
  - **A2058G/C, A2059G (*E. coli* numbering)**
  - **AZM 1g single dose**
    - ➔ **Selection of resistant mutants during AZM treatment**
    - ➔ **Therapeutic failure if patient infected with a mutated strain**





Macrolide resistance in *M. genitalium* (2)

- **Extended 1.5 g AZM (500 mg d1, 250 mg d2-4) 85-95% effective and associated with lower risk of inducing AZM R**

  Anagrius PLoS One 2013, Bjornelius Sex Transm Infect 2008

- **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

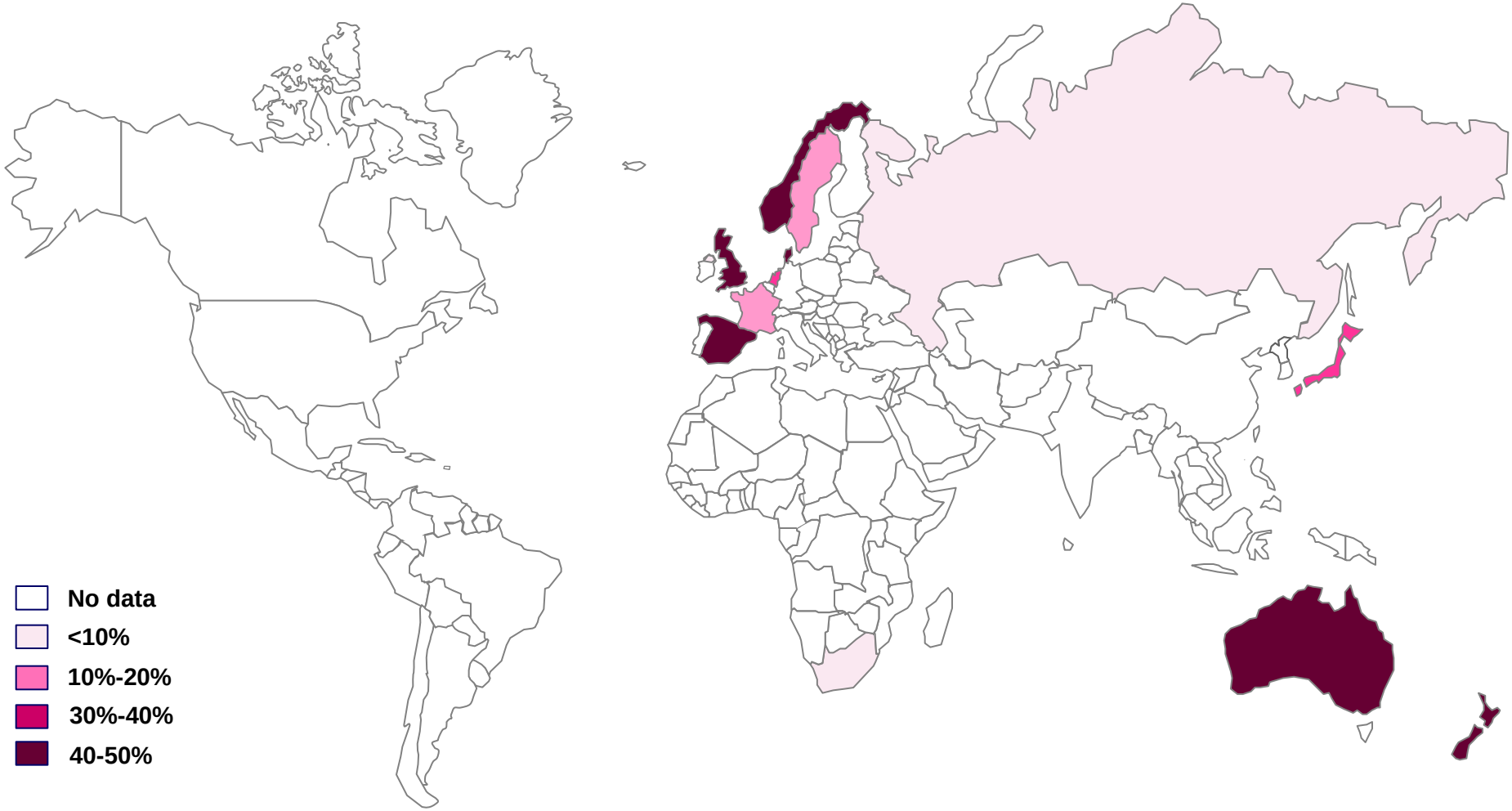
- **Macrolide resistance since 2005 in Australia, New-Zealand, Japan, Scandinavia, The Netherlands, France, Spain, Russia, South Africa**

  France: 17.2% of specimens (2013-2014)

  Denmark, UK: ≈ 40%...Greenland 100%!!

Bradshaw Emerg Infect Dis 2006, Bissessor Clin Infect Dis 2015, Chrisment J Antimicrob Chemother 2012, Hay Sex Transm Dis 2015, Gesink Int J Circumpolar Health 2012, Gushin BMC Infect Dis 2015, Le Roy Emerg Infect Dis 2016, Nijhuis J Antimicrob Chemother 2015, Pond Clin Infect Dis 2014, Shimada Emerg Infect Dis 2011, Touati J Clin Microbiol 2014, Salado-Rasmussen Clin Infect Dis 2014, Yew J Clin Microbiol 2011

Frequency of macrolide resistance in *M. genitalium*



Fluoroquinolone resistance in *M. genitalium*

- **Moxifloxacin 400 mg for 7-10 d in case of AZM failure but…**

www.cdc.gov/std/tg2015;
http://www.iusti.org/regions/europe/pdf/2016/2016EuropeanMycoplasmaGuidelines.pdf
http://www.iusti.org/regions/europe/pdf/2016/2016EuropeanNGUGuideline.pdf ***(Int J STD AIDS, Eurosurveillance 2016)***

- **Emerging MXF resistance with few MXF-R isolates and failures described in Australia, Japan, Scandinavia and Europe:**

- Australia and Japan: prevalence ranges from 10-47% between 2006 and 2014
- France: prevalence of 7% in 2013-2014
- UK: prevalence of 4.5% in 2011

Bissessor Clin Infect Dis 2015, Bradshaw PLoS One 2008; Deguchi, Emerg Infect Dis 2015;Jernberg Sex Transm Infect 2008, Jensen BMC Infect Dis 2015; Kikuchi J Antimicrob Chemother 2014; Le Roy Emerg Infect Dis 2016; Pond Clin Infect Dis 2014; Shimada Int J Antimicrob agents 2010; Tagg J Clin Microbiol 2013

- **Mutations in the bacterial target genes of fluoroquinolones**

- Most frequent mutations in *parC*

- A few mutations in *gyrA*

Molecular diagnosis of *M. genitalium* antibiotic resistance

- **Molecular detection of macrolide resistance**
  - **Sanger sequencing, SNP detection using pyrosequencing, qPCR (FRET or TaqMan probes)**
  - **In-house and commercial assays (Speedx)**
  - **Simultaneous detection of Mg and macrolide resistance directly from specimens -> treatment to be adjusted**
- **Molecular detection of fluoroquinolone resistance**
  - **amplification and sequencing of the gene targets (*parC*+++)**

Jensen Clin Infect Dis 2014; Twin PLoS one 2012; Manhart Clin Infect Dis 2014; LE Roy Emerg Infect Dis 2016; Pond Clin Infect Dis 2014, Salado-Rasmussen Clin Infect Dis 2014; Shimada, Int J antimicrob Agents 210; Touati J Clin Microbiol 2014

Conclusion

- ***M. genitalium*, a STI pathogen, has emerged!!**
  An accepted cause of male NGU and female cervicitis,
  Significant association with PID
- **Diagnostic activity is predicted to increase (commercially available NAAT tests):** **testing Mg on symptomatic patients and patients with high STI risk behavior**
- **Increasing prevalence of macrolide resistance ⇒ Decreasing efficacy of AZM monotherapy**
- **2nd line treatment with MXF …under pressure**

⇒ Need to detect Mg and macrolide resistance in the same time (implementation of molecular diagnostic tests): change from syndromic to <u>etiologic treatment</u>

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

Horner Clin Infect Dis 2015; Jensen BMC Infect Dis 2015; Manhart Clin Infect Dis 2014
http://www.iusti.org/regions/europe/pdf/2016/2016EuropeanMycoplasmaGuidelines.pdf

Acknowledgments

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