Mycoplasma genitalium increasingly resistant?

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Epidemiology of STI worldwide

WHO 2020: 374 millions new cases of 4 curable STIs, adults 15-49 yo

- ***Chlamydia*** 129 millions
- ***N. gonorrhoeae*** 82 millions
- **Syphilis** 7.1 millions
- ***Trichomonas vaginalis*** 156 millions
- More than one million new STIs per day

- Another STI : ***Mycoplasma genitalium***, increased cases

☞ Improve their diagnostic: **new molecular tools**

☞ **Antimicrobial resistance:**
Priority STIs- *N. gonorrhoeae* and ***M. genitalium***

Global progress report on HIV, viral hepatitis and sexually transmitted infections, 2021. WHO, 2021

Highlights of the CDC's 2019 antibiotic resistance threat report

#IA

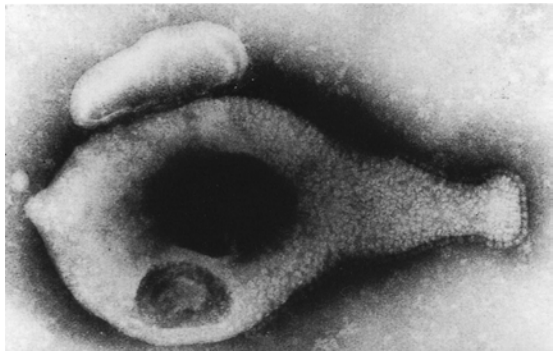
U.S. Department of Health and Human Services
Centers for Disease Control and Prevention

☞**4 categories of 21 AMR bacteria and fungi based on level of concern on human health:**

- **Urgent threats: 5 microbes including drug-resistant *N. gonorrhoeae***
- **Serious threats**
- **Concerning threats**
- **Watch list: 3 microbes including drug-resistant *M. genitalium***

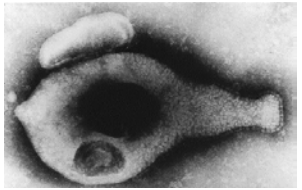
https://www.cdc.gov/drugresistance/pdf/threats-report/2019-ar-threats-report-508.pdf

Mycoplasma genitalium



Tully, Int J Syst Bacteriol 1983

Mycoplasma genitalium



STI pathogen

Prevalence

General population: 1-4% (France ?)

Key populations:5-35%

(MSM, PLWH, PrEPers)

Clinical diagnostic

- Culture
- **Molecular diagnostic**
  - Guidelines
  - symptomatic and partners

Pathologies	*M. genitalium*
Infections génitales masculines	
UNG	+
Epididymites, prostatites	±
Infections gynécologiques	
Vaginose bactérienne	±
Cervicites	+
Endométrites	+
Salpingites	+
Infections au cours de la grossesse	
Chorioamniotites	?
Fièvre, endométrites post-partum	-
Avortement spontané	±
Prématurité	±
Retard de croissance intra-utérin	?
Atteintes néonatales	
Hypotrophie	?
Infections respiratoires, neurologiques, bactériémies, abcès	?
Dysplasie broncho-pulmonaire	?
Infections extra-génitales	
Arthrites septiques	±
Arthrites réactionnelles	±

Wood, Infect Dis Clin N Am 2023, Waites and Bébéar, MCM 13th ed, 2023; Waites *et al*. J Clin Microbiol, 2023; Bébéar and Pereyre, REMIC 2022

Quand rechercher *Mycoplasma genitalium* ? HAS juillet 2022

- **Symptômes**
  - Urétrite chez l'homme, aigue, persistante, récurrente
  - Cervicite

  - Métrorragies ou saignements post-coïtaux
  - Dysurie sans autre étiologie chez la femme
  - Douleurs pelvienne aigue, endométrite, salpingite
  - Orchi-epididymite aigue chez l'homme <50 ans
  - Rectite après exclusion de Ct et Ng

- **Pas de screening chez les patients asymptomatiques**
  - Infections asymptomatiques >70%
  - Clairance spontanée
  - Limiter l'utilisation d'azithromycine et donc la résistance aux macrolides

- **Sauf : contact sexuels avec**
  - Une personne en cours de traitement d'une infection à *M. genitalium* (Reco Eur 2021)
  - Chez partenaires sexuels des patients infectés par *M. genitalium* (HAS)

➢ **Avis favorable HAS en 2022 : détection MG et résistance aux macrolides (MRM)**
➢ **Inscription nomenclature et remboursement à venir**
➢ **Recommandations françaises à venir (CNS-ANRS-HAS)**

Avis n° 2022.0046/AC/SEAP du 21 juillet 2002, Diagnostic biologique des mycoplasmes urogénitaux dans les infections génitales basses

M. genitalium and antimicrobials

- **Intrinsic resistance in the Mollicutes class**
  - **No cell wall: resistance to β-lactams, glycopeptides, fosfomycin**
  - **Mutation in the *rpoB* gene: resistance to rifampicin**

- **Potent antibiotics**
  - **Macrolides and related antibiotics: azithromycin (1st line), pristinamycin (3rd line)**
  - **Tetracyclines: doxycycline (3rd line : active in only 30-40% of cases), minocycline**
  - **Fluoroquinolones: moxifloxacin only ! (2nd line)**

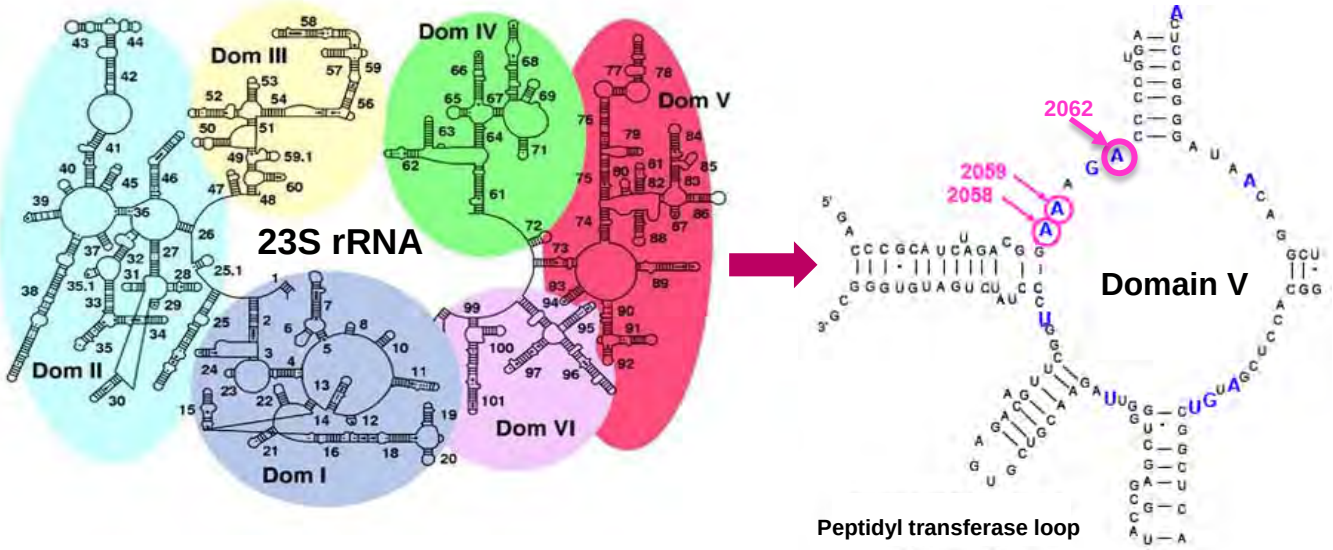
- **Acquired antimicrobial resistance (AMR)**
  - **Target modification: chromosomal mutations +++**
    - ✓ High rate of mutations (as in all *Mycoplasma* spp.)
  - **No mobile genetic elements carrying AMR genes**

Macrolide resistance

Acquired resistance to macrolides

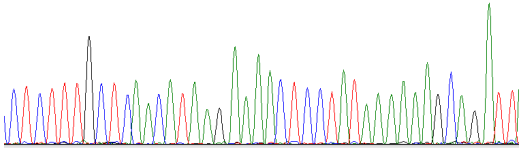
- **Point mutations in the peptidyl transferase loop of domain V of 23S rRNA**
  - **Single operon encoding 16S and 23S rRNA**
  - **A2058G**/C/T**,** **A2059G**/C/T, A2062G/T **(*E. coli* numbering)**

- **High-level resistance to macrolides**



M. genitalium: detection of macrolide resistance-associated mutations

- **Amplification and sequencing of 23S rRNA**
  - **Time-consuming, not adapted to routine**



- **Published in-house NAATs**
  - **Several real-time PCR methods**

- **CE-IVD commercial kits**
  - **Multiplex real-time PCR: detection of Mg and 4-5 mutations in 23S rRNA**

SpeeDx diagendde diagnostics Patho Finder ELITechGroup Cepheid Seegene

  - **Recommended by HAS July 21, 2022 for each *M. genitalium*-pos. specimen to implement resistance-guided therapy (RGT)**

CNR des IST bactériennes
Bordeaux

HAS HAUTE AUTORITÉ DE SANTÉ

Le Roy, J. Clin. Microbiol. 2017; Le Roy, J. Clin. Microbiol. 2020, Le Roy, J. Clin. Microbiol. 2021, Bébéar, CPMH 5th ed, ASM Press 2023

M. genitalium macrolide and fluoroquinolone resistance: worldwide epidemiology

THE LANCET
Infectious Diseases

Volume 20, Issue 11, November 2020, Pages 1302-1314

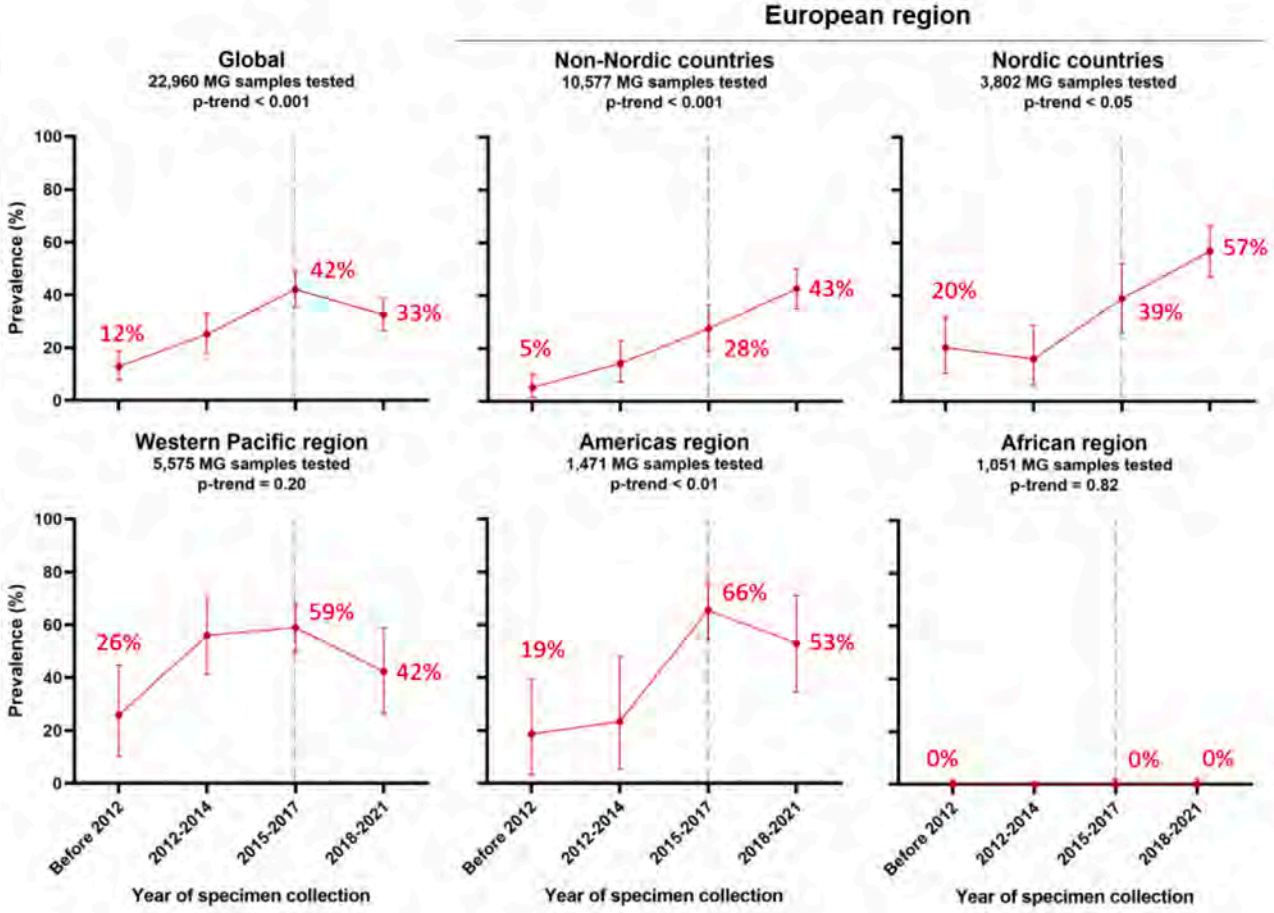
Articles

Prevalence of mutations associated with resistance to macrolides and fluoroquinolones in *Mycoplasma genitalium*: a systematic review and meta-analysis

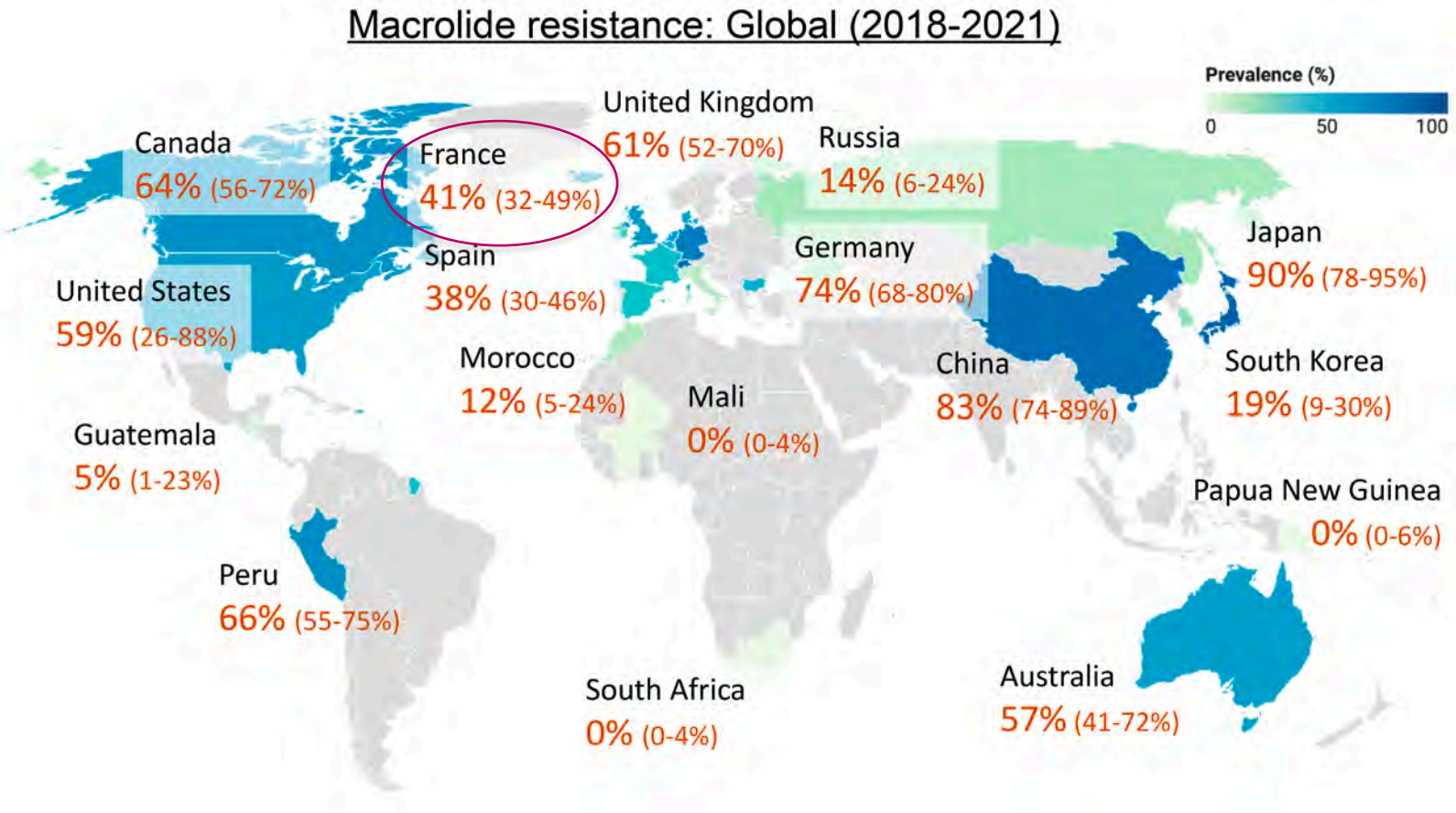
Dorothy A Machalek PhD [a b c], Yusha Tao MPH [e f g], Hannah Shilling GradDip [a i], Prof Jørgen S Jensen MD [j], Magnus Unemo PhD [k], Gerald Murray PhD [a d i], Eric P F Chow PhD [e f], Prof Nicola Low MD [l], Prof Suzanne M Garland MD [a d i], Lenka A Vodstrcil PhD [e f], Prof Christopher K Fairley PhD [e f], Prof Jane S Hocking PhD [c], Lei Zhang PhD [e f g h m †], Catriona S Bradshaw PhD [c e f †]

Macrolide resistance

41 countries



Courtesy Chua T.-P., Evolving AMR in *M. genitalium*: an updated global systematic review and meta-analysis, STI and HIV world congress, Chicago, USA, 2023



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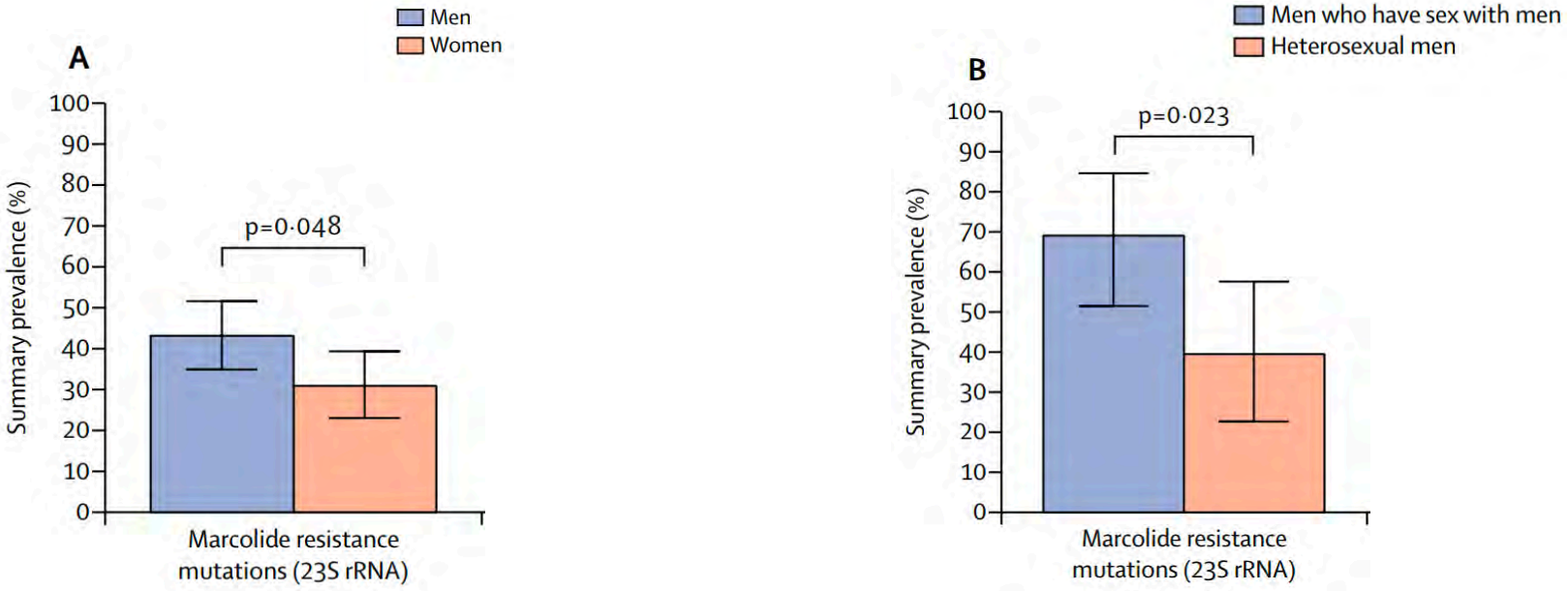
Macrolide resistance in *M. genitalium* according to gender and sexual orientation

Meta-analysis : 59 studies from 21 countries



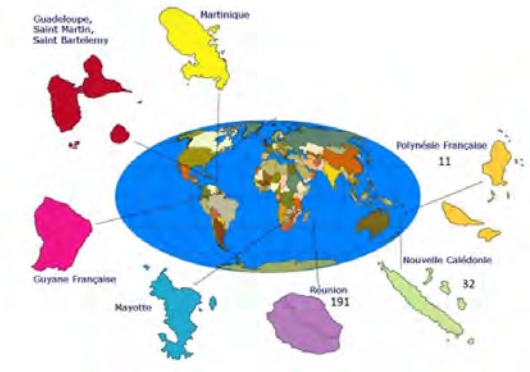
Machalek et al. Lancet Infect Dis 2020

Macrolide resistance in *M. genitalium* in France

CNR des IST bactériennes

Enquêtes MG-MET, MG-DROM

- **Each year, in a one-month prospective study, 37 diagnostic laboratories in metropolitan France in 2022**
- **Each year, in a three-month prospective study, 4 diagnostic laboratories in French overseas in 2022**

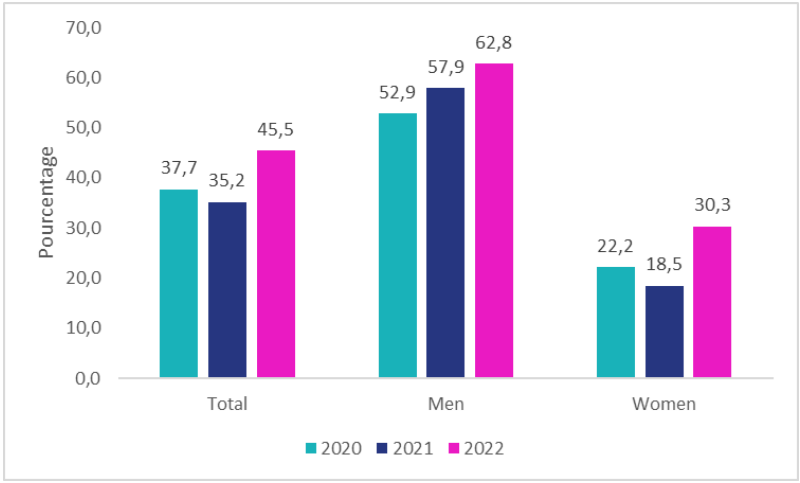


Macrolide resistance in *M. genitalium* in France

CNR des IST bactériennes **Enquêtes MG-MET, MG-DROM**

- **Metropolitan France, 2022**
  - **Macrolide resistance: 38.6%**
  - **Stable between 2020 and 2022**
  - **51.5% in men *vs* 24.9% in women (p<0.001)**
  - **70.8% in MSM *vs* 33.2% MSW (p<0.001)**
  - **Tendency to increase in women (p =0.053)**

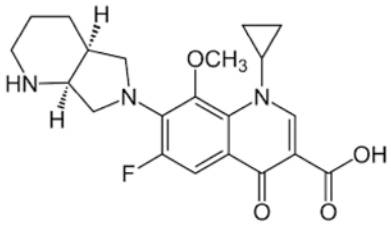
- **French Overseas, 2022**
  - **Macrolide resistance: 18.4%**
  - **Lower resistance than in metropolitan France**
  - **28.9% in men *vs* 12.5% in women (p<0.05)**
  - **64.3% in MSM *vs* 35.3% MSW (p>0,05)**



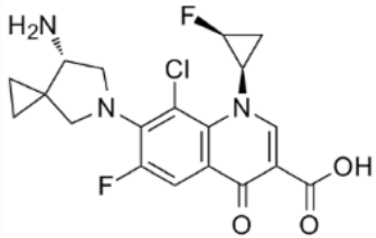
In 14 labs that participated in the 3 years of study (metropolitan France)

Pereyre et al. Sex Transm Inf 2022; rapport CNR IST 2023, www.cnr-ist.fr

Fluoroquinolone resistance



Moxifloxacin



Sitafloxacin

M. genitalium and fluoroquinolones

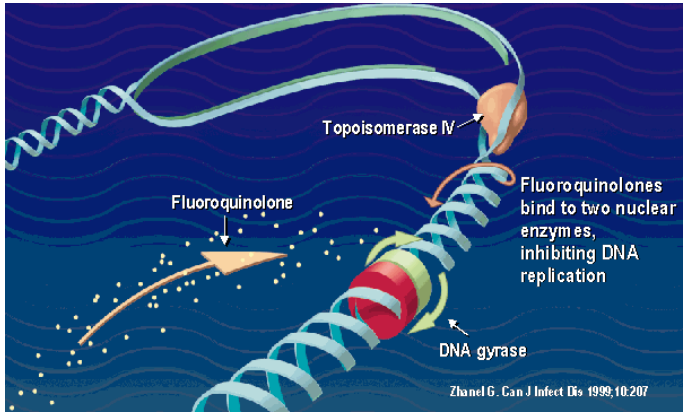
- **Only moxifloxacin has low MICs (mg/l)**
  - **+ Sitafloxacin (registered in Japan)**

Antibiotics	*M. genitalium*	*M. hominis*	*Ureaplasma* spp.
Fluoroquinolones			
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	0.125		

Deguchi, J Infect Chemother 2014; Waites and Bébéar, MCM 13th ed, ASM Press, 2023

Fluoroquinolone resistance in *M. genitalium*

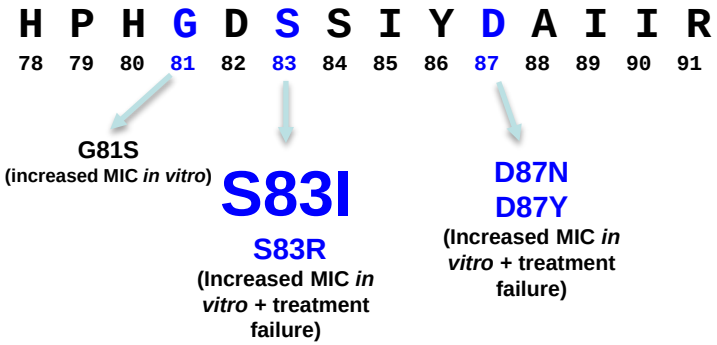
- **Mutations in the bacterial target genes of fluoroquinolones**
  - ***parC*** **gene (topoisomerase IV), most frequently mutated**
  - ***gyrA*** **gene (DNA gyrase), rare mutations**



- **Many mutations have not been evaluated by *in vitro* MIC determination**

Mutations associated with moxifloxacin resistance or treatment failure in *M. genitalium*

- **In the *parC* gene (topoisomerase IV)**



ParC Amino acid change	Treatment failure (381 moxifloxacin treatments)	
S83I	60%	(44/75)
S83R	50%	(3/6)
S83C	0	(0/2)
S83N	0	(0/3)
D87Y	25%	(1/4)
D87N	12%	(2/17)
D87G	0	(0/1)
Not S83I	4%	(11/306)
Wild type	2%	(6/273)

Murray, Antimicrob Agents Chemother 2022

- **In the *gyrA* gene (DNA gyrase)**
  - **M95I, D99N (corresponding to S83 and D87 in ParC)**
  - **~5% of fluoroquinolone-resistant strains only, usually associated with *parC* mutations (S83I)**
  - **More frequent therapeutic failure when *parC*+ *gyrA***
  - **Not correlated alone with *M. genitalium* MIC increase or treatment failure to date**

Deguchi J Antimicrob Chemother 2001; Yamaguchi, Antimicrob Agents Chemother 2013; Couldwell Int J STD AIDS 2013; Murray Emerg Infect Dis 2017, J Infect Dis 2020, Clin Infect Dis 2023; Hamasuna Plos One 2018; Hamasuna, J Infect Chemother 2021; Murray, STI and HIV World Congress, Chicago, 2023

M. genitalium: detection of fluoroquinolone resistance-associated mutations

- **Amplification and sequencing of the *parC* gene (+/- *gyrA*)**
  - **Time-consuming, not adapted to routine**

- **Commercial kits**

TIB MOLBIOL NYtor Seegene

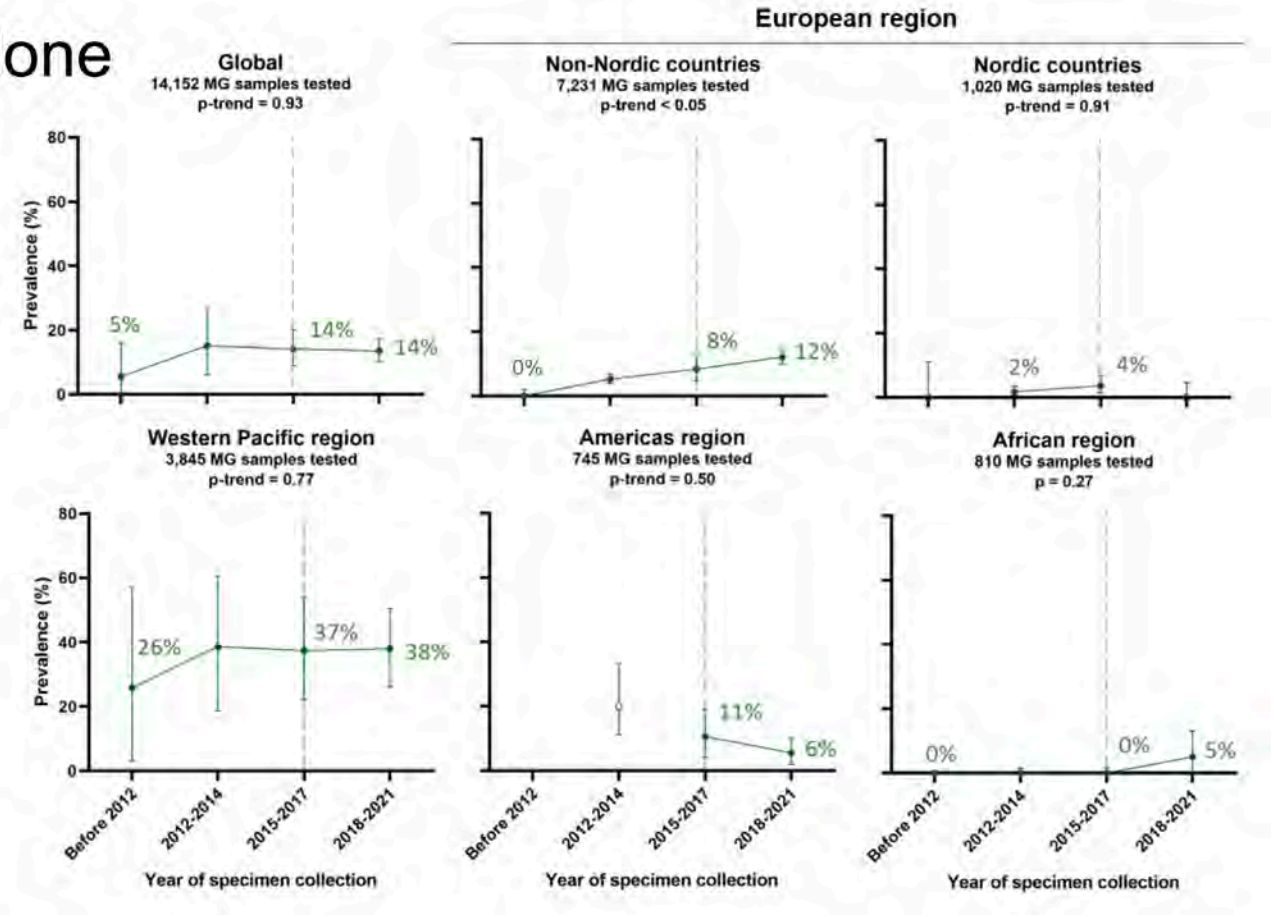
Gardette et al. J. Clin. Microbiol. 2022

- **When to look for?**
  - **Macrolide-resistant MG or macrolide treatment failure**
  - **Moxifloxacin therapeutic failure**
  - **Complicated infections**

CNR des IST bactériennes

Fluoroquinolone resistance

35 countries



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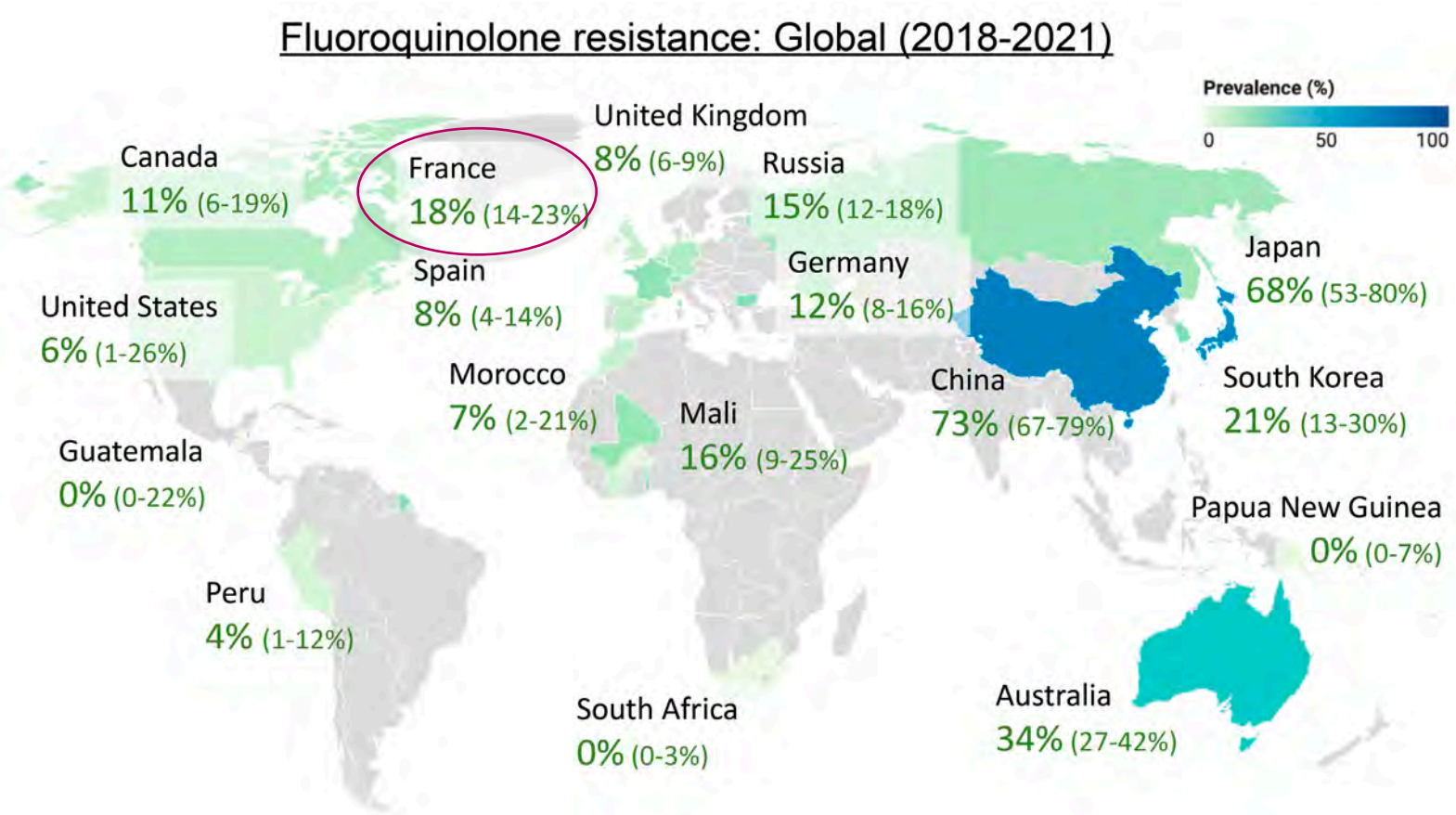

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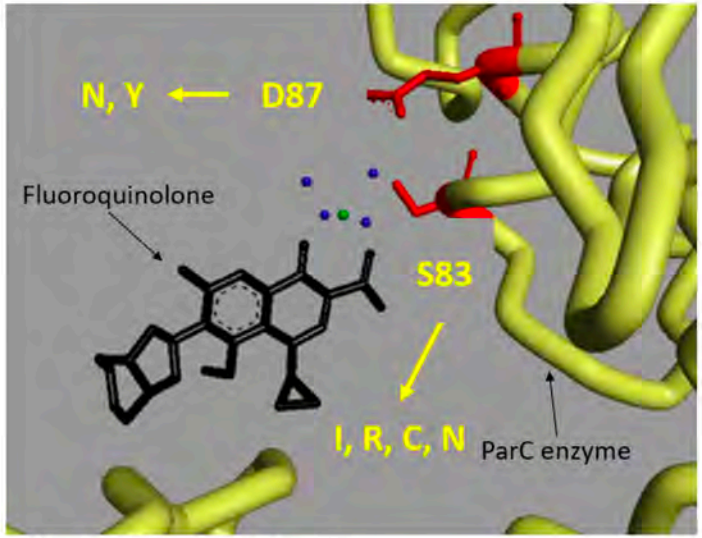
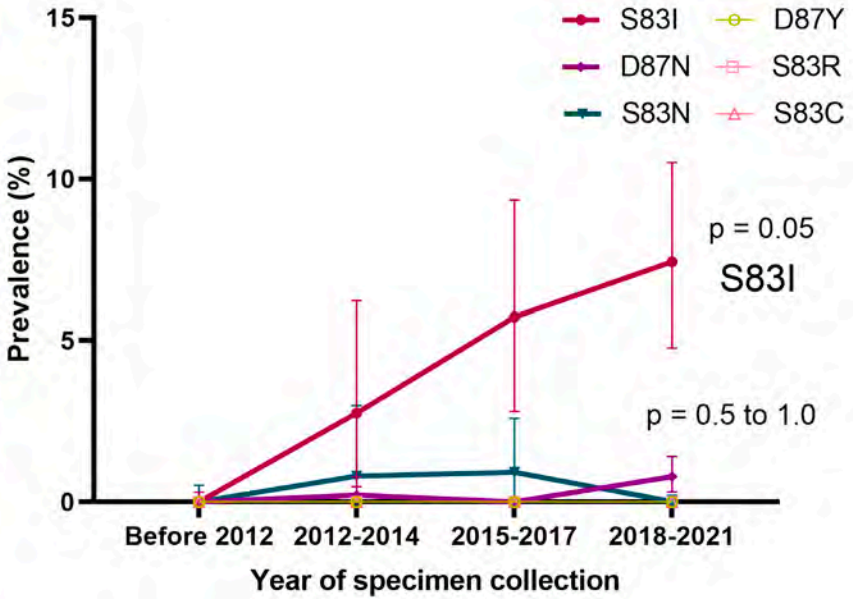
Fluoroquinolone resistance: ParC amino acid changes



Aldred *et al.* (2014) *Biochemistry.*



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Fluoroquinolone resistance in *M. genitalium* in France

CNR des IST bactériennes **Enquêtes MG-MET, MG-DROM**

One-month prospective collection of *M. genitalium*-positive specimens each year (37 labs in 2022)

- **Metropolitan France, 2022**
  - **Fluoroquinolone resistance: 17.1%**
  - **Stable between 2020 and 2022**
  - **25.8% in men *vs* 10.8% in women (p<0.001)**
  - **39.3% in MSM *vs* 22.5% MSW (p>0,05)**

- **French Overseas, 2022**
  - **Fluoroquinolone resistance: 4.8%**
  - **Lower resistance than in metropolitan France**
  - **7.6% in men vs 3.3% in women (p>0.05)**
  - **25.0% in MSM *vs* 0% MSW**

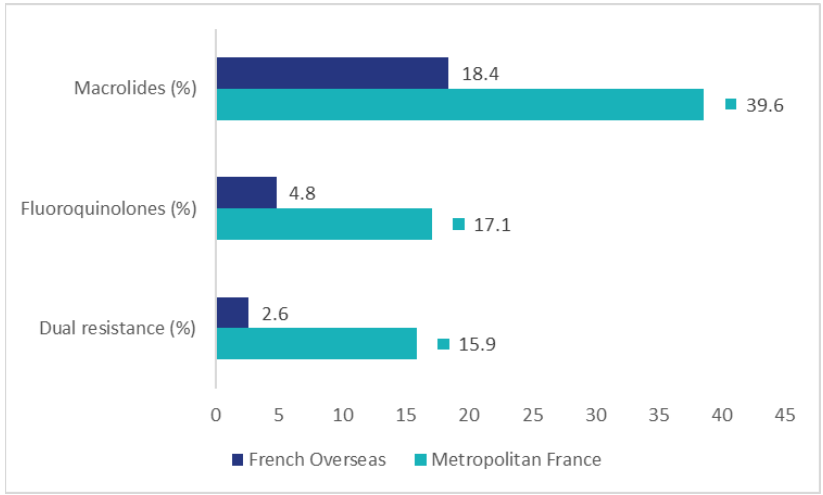
In 14 labs that participated in the 3 years of study (metropolitan France)

Pereyre et al. Sex Transm Inf 2022; rapport CNR IST 2023, www.cnr-ist.fr

Dual resistance in *M. genitalium* in France

CNR des IST bactériennes

Enquêtes MG-MET et MG-DROM 2022



Rapport CNR IST 2023, www.cnr-ist.fr

Mycoplasma genitalium

DOI: 10.1111/jdv.17972 JEADV

GUIDELINE

2021 European guideline on the management of *Mycoplasma genitalium* infections

	Recommended antibiotic	Resistance mechanism	% of cure
1st line	**Azithromycin 1.5 g, 5 d**	23S rRNA mutations	52%
2nd line	**Moxifloxacin** 400 mg/7d	*parC*/*gyrA* mutations	91%
3rd line	**Pristinamycin** 4 g/10d	23S rRNA mutations	75%
	Minocycline 200 mg/14d	~~*tet*(M)~~	67%
	Doxycycline 200 mg/14d	16S rRNA mutations? Efflux?	59%

AMS

- ~~Culture~~
- **Molecular biology**

Sequential RGT using assays to detect MRMG

Wood *et al. J. Clin. Microbiol. 2021;* Sutormin *et al.* Acta naturae 2021; Hamasuna *et al.* J Infect Chemother, 2022; Deguchi *et al*. J Antimicrob Chemother, 2001; Couldwell *et al*. *Int J STD AIDS* 2013; Murray *et al. Emerg Infect Dis* 2017; Murray *et al.* J Infect Dis, 2020; Murray *et al.* Clin Infect Dis, 2023; Jensen, JEADV, 2022; Machalek *et al*. Lancet Infect Dis, 2020; Doyle *et al*. Open Forum Infect Dis, 2020; Read *et al*. Emerg Infect Dis, 2018; Palich *et al.* Sex Trans Dis, 2021; Gossé *et al*. BMC Infect Dis, 2021; Chua *et al*. Antimicrob Agents Chemother, *2022, Clarke et al.,* Open Forum Infect Dis, 2023

Sequential RGT of uncomplicated MG infections

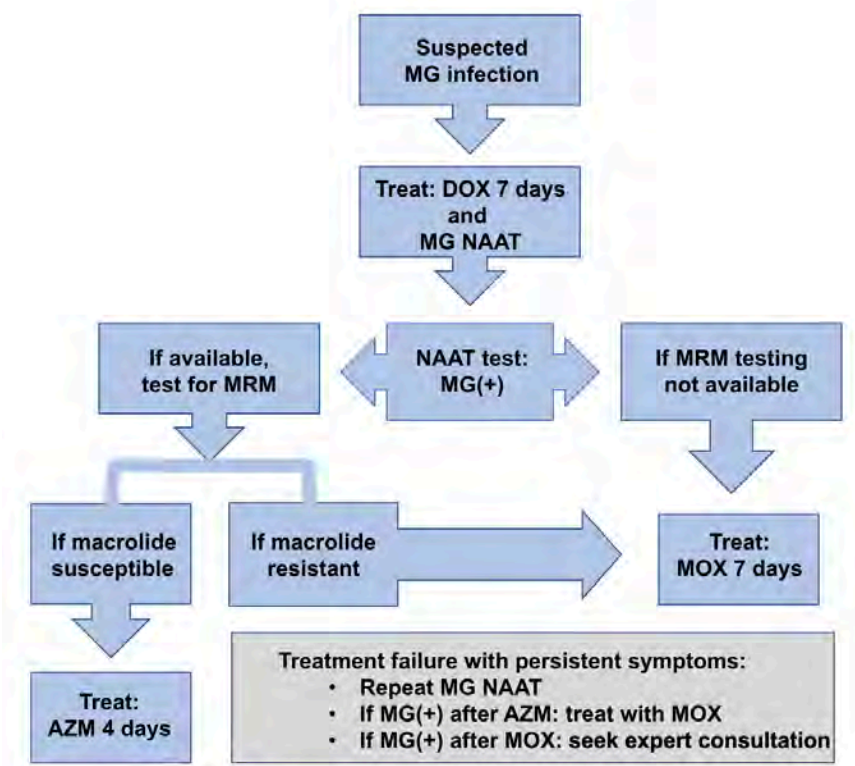


Fig. 1. Resistance-guided treatment of uncomplicated *Mycoplasma genitalium* infections. AZM, azithromycin (1 g initial dose, then 3 days, 500 mg qd); DOX, doxycycline (7 days, 100 mg bid); MOX, moxifloxacin (7 days, 400 mg qd).

Wood, Infect Clin Dis N Am 2023

Test of cure?

NAAT testing recommendation	Indication in guideline		
	United States[c]	United Kingdom[d]	Europe[e]
Asymptomatic persons	No	No	Not specified
Test of cure following treatment of persons who test positive	Only if resistance testing is not available and moxifloxacin cannot be used	Yes, no earlier than 3 wks	Consider, no earlier than 3 wks
Sex partners of symptomatic persons who test positive	Yes	Yes	Yes
Resistance testing on all persons who test positive	Yes, if available[a]	Yes (macrolide)	Yes (macrolide)[b]

[c]Workowski, MMWR Recommend Rep 2021; [d]Soni, Int J STD AIDS 2019; [e]Jensen, JEADV 2022; Waites, J Clin Microbiol 2023

- **Is a TOC needed in case of clinical cure?**
- **Updated French guidelines to be released (CNS-ANRS-HAS)**

Conclusion

- **AMR is impacting on the efficacy of all available antimicrobials for *M. genitalium***
  - **High prevalence of macrolide resistance**
  - **Moxifloxacin and dual resistance (MSM +++)**
  - **A few other antimicrobials available for 3rd line treatment**

- **Increasing understanding of molecular markers of resistance to a number of key antimicrobials**

- **Untreatable *M. genitalium* infections**

Acknowledgments

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Cochin hospital,
National Reference Center for bacterial STI

Nicolas Dupin

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https://www.cnr-ist.fr/