MYCOPLASMA GENITALIUM

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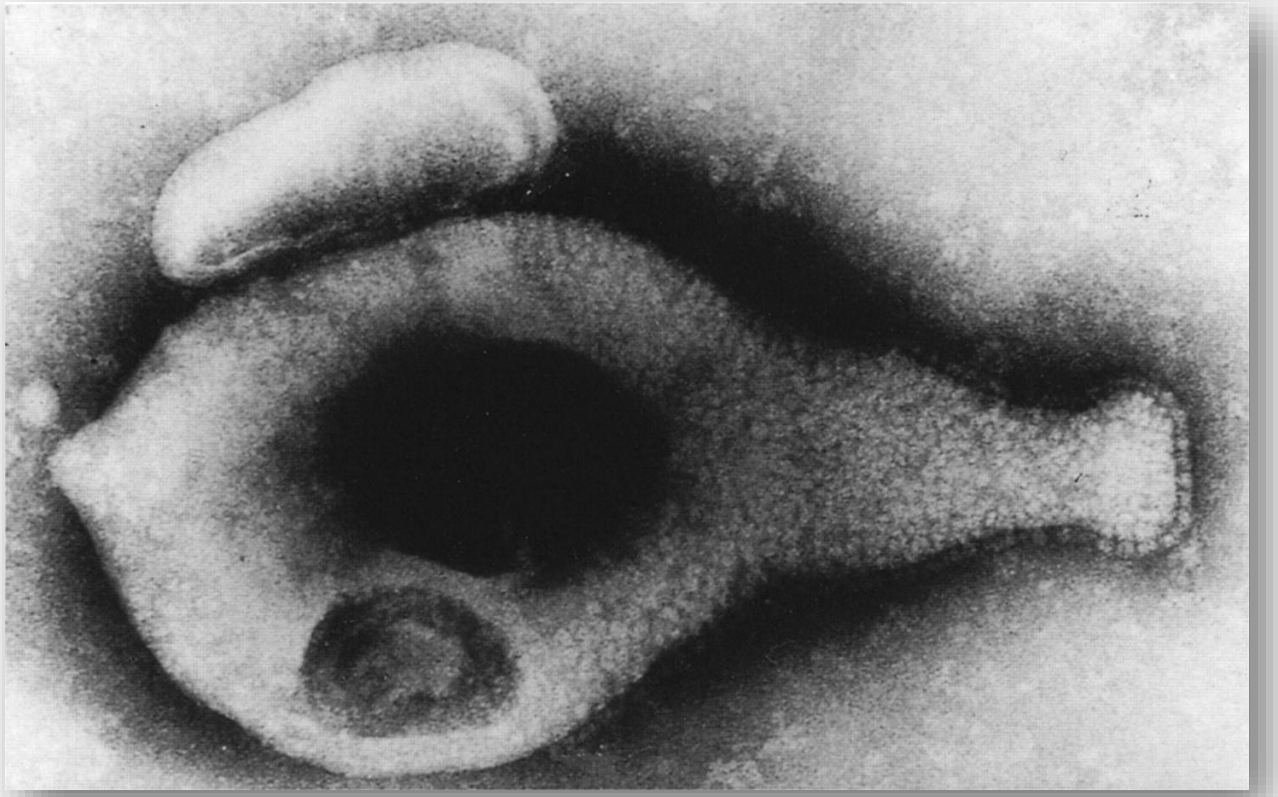
WHY ARE WE TALKING ABOUT M.GEN IN 2018?

- First isolated in 1981 from man with NGU
- Is *M.gen* an STI?
- Is *M.gen* associated with genital syndromes and reproductive system sequelae?
- So what? Syndromic management guidelines will cure most cases…right?

MICROBIOLOGY

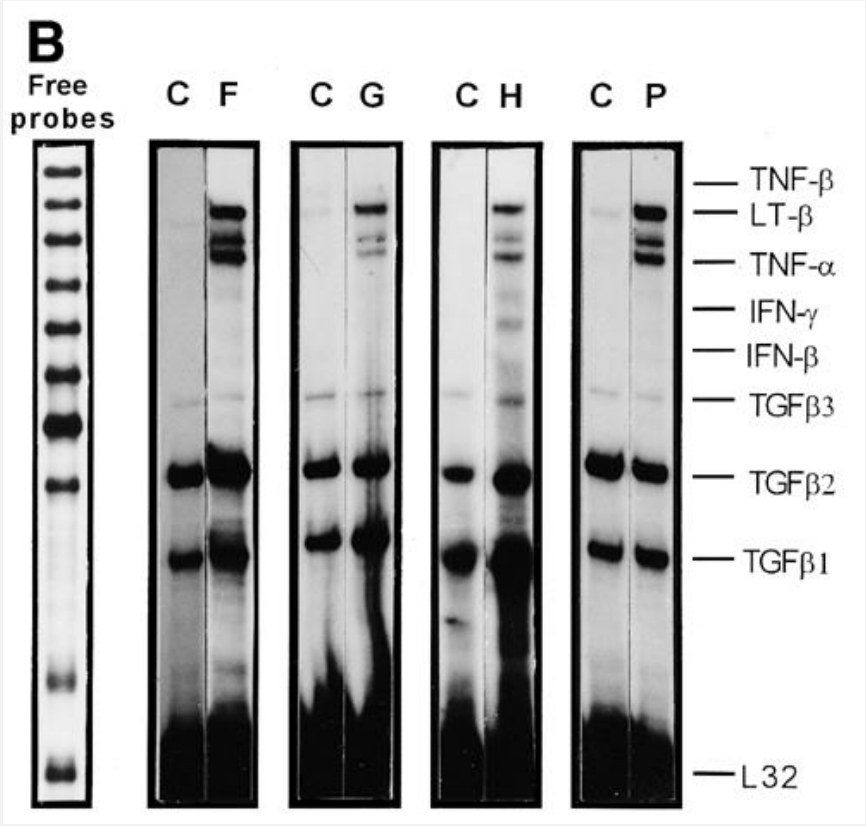
- Member of *Mycoplasmataceae* family
- Genome: 580 kilobases in size
- Smallest known self-replicating bacterium
- Fastidious and may require one to two months to grow
- “Fried egg" colonies



Transmission electron micrograph. The characteristic flask shape and the terminal truncated portion with extracellular projections are clearly seen (after Tully, 1983)

PATHOGENESIS

- *M. genitalium* has been isolated from multiple tissue sites, but disease appears to be limited to the urogenital tract
- Inoculation studies in male and female non-human primates demonstrate its pathogenicity
- A specialized terminal tip-like structure allows *M. genitalium* to attach to, adhere to the surface of, and enter epithelial cells
- *M. genitalium* evades the host immune response through modulation of the immune system, including suppression and stimulation of lymphocytes and upregulation of cytokine expression
- Infection may persist for months or years



Changes in cytokine expression in *Mycoplasma* infected immortalised cells (Zhang, 2006)

EPIDEMIOLOGY

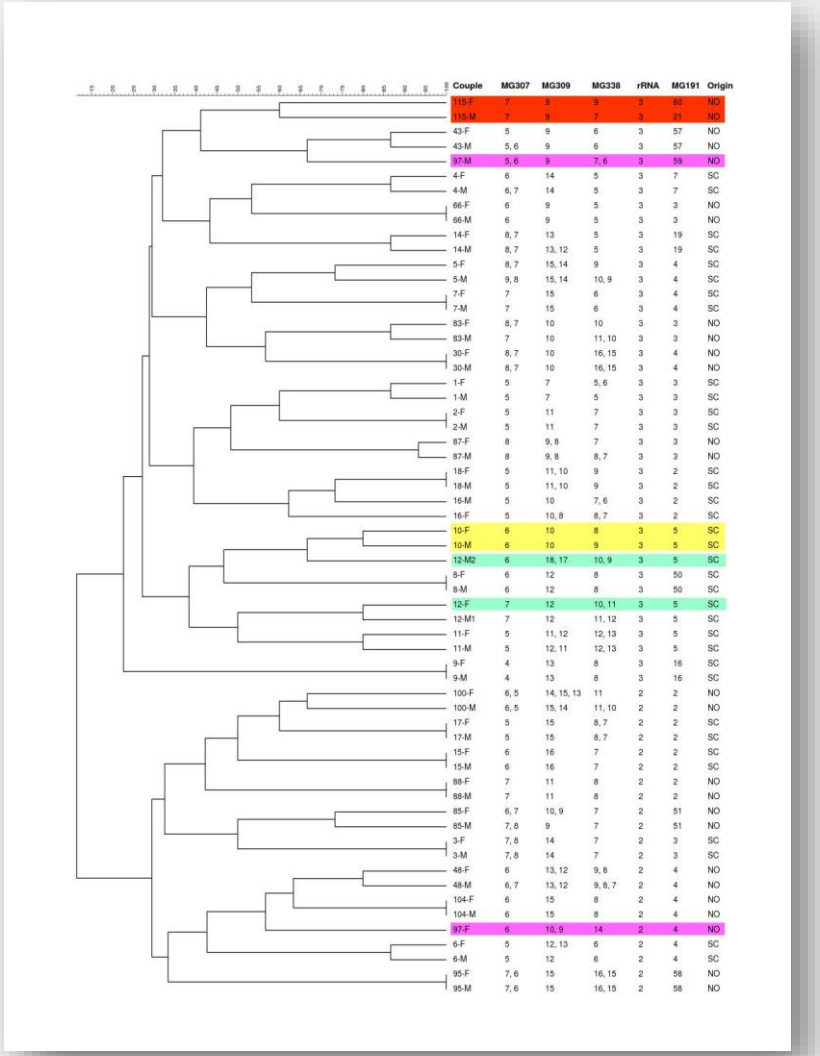
- In US, prevalence c. 1% among young adults in the general population
- Globally, prevalence estimates: 1-4% percent among men and 1-6.4% percent among women (e.g. 1.2% in men and 1.3% in women aged 16–44 in NATSAL, 2010 (n= 4507) [Sonnenburg, 2015])
- Higher *M. genitalium* prevalence rates have been reported among STI clinic attendees, ranging from 3-38% (e.g. 3% of 2441 female NCSP participants and 2172 female London STI clinic attendees in 2009 [Svenstrup, 2014])
- Risk factors:
  - Younger age (eg, <20 to 22 years old),
  - Smoking
  - Non-white ethnicity
  - Recent sexual intercourse
  - Increasing number of sexual partners

EPIDEMIOLOGY

Sexual transmissibility

Demonstrated by clinical and molecular evidence:

- Associated with sexual experience, and number of partners
- Associated with positivity in partners
- Concurrently infected partners more likely to harbour genetically linked strains (e.g. in one study of genotype profiles of 31 concurrently infected couples and 74 unrelated pairs of individuals, a concordance rate of 87.1% was observed for the couples compared with only 5.4% for the unrelated pairs of individuals [Ma, 2008])



ASSOCIATION OF *M. GENITALIUM* WITH DISEASE: MEN

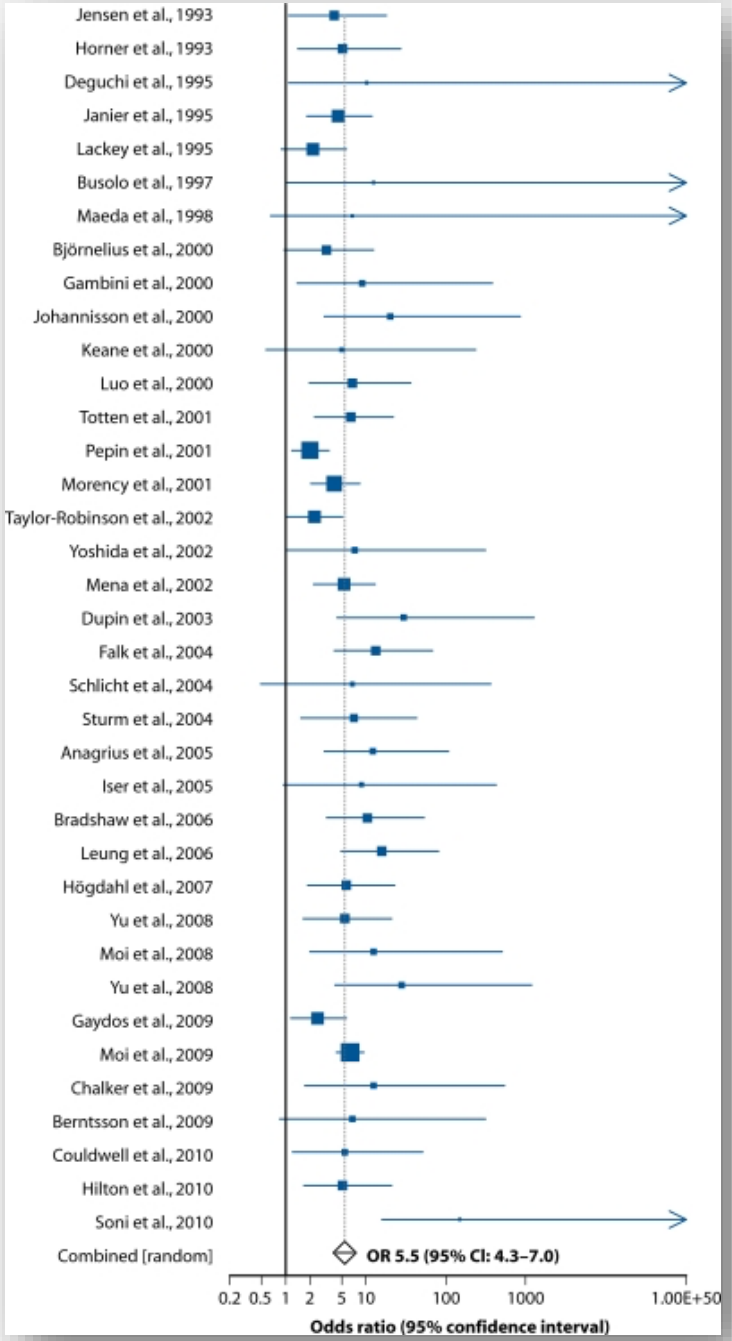
Disease	Odds ratio
Non-gonococcal urethritis (NGU)	5.5 (4.35-7.0)
Non-chlamydial NGU	7.6 (5.5-10.5)
Proctitis	No data* 12% prevalence

Taylor-Robinson D Clin Micro Rev 2011: Ong J STD 2018 on line: Bissessor M Clin M Inf 2016;22:260

CLINICAL ASSOCIATIONS: NGU

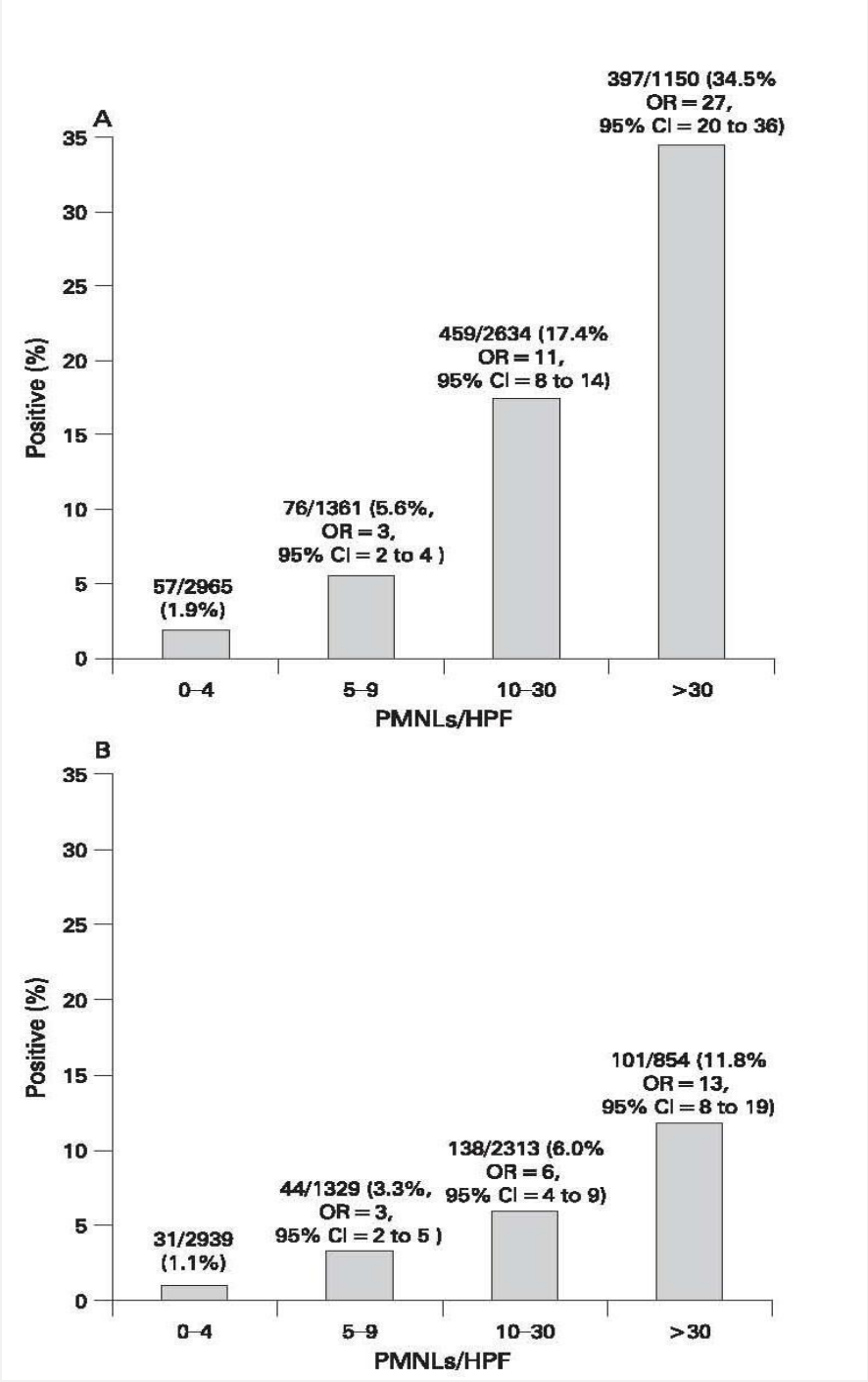
***M.gen* is unequivocally associated with NGU**

- Systematic reviews of several observational studies demonstrate OR 3-7 of detection *M.gen* in men with NGU vs. controls, with a stronger association with NCNGU
- *M.gen* detected in 15-20% men with NGU and 18-46% of men with NCNGU
- Similarly, *M.gen* detection is frequent in men with persistent or recurrent urethritis following empiric therapy



Jensen, 2011

CAN SYMPTOMS AND SIGNS PREDICT INFECTIOUS AETIOLOGY IN NGU?



Chlamydia

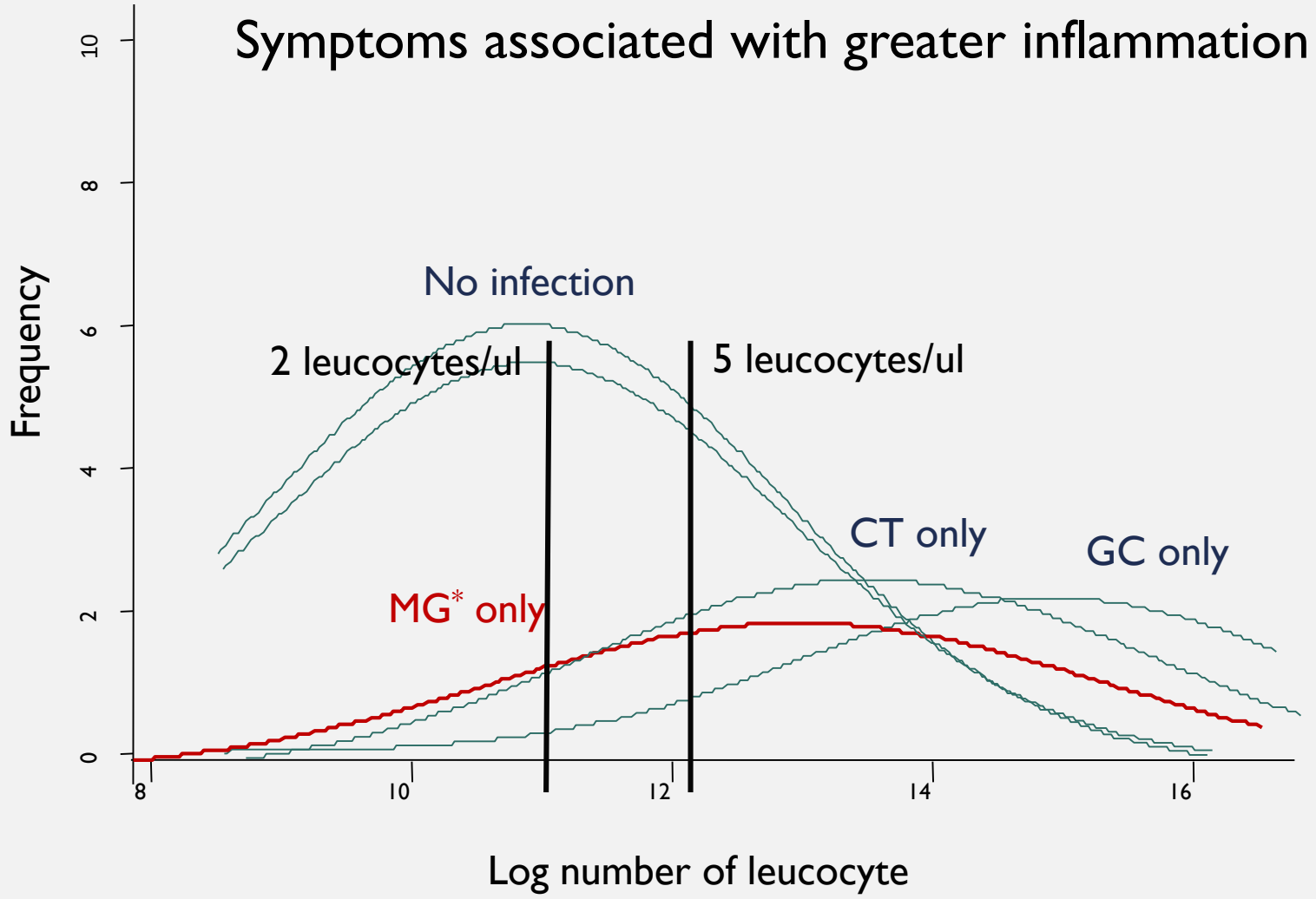
M. genitalium

Moi H et al STI 2009 & STD 2017

		Chlamydia		*M. genitalium*		*U. urealyticum*		TV	*H. influenza*		Adenovirus		HSV		Pathogen-negative	
		Sena	Ito	Sena	Ito	Sena	Ito	Sena	Sena	Ito	Sena	Ito	Sena	ito	Sena	ito
age	<31		53 (44%)		10 (45%)		7 (50%)			7(21%)		4(33%)		4(36%)		13(44%)
	≥31		57		12		7			14		8		7		16
age	comparison	younger		younger				No							older	
oral	only		20 (22%)		7 (33%)		2 (17%)			12 (63%)		5 (50%)		7 (70%)		13 (72%)
	other		71		14		10			9		5		3		5
partners 3/12	<2	29 (43%)		22 (24%)				9 (24%)							31(38%)	
	>=2	39		68				29							51	
incubation	<7		7 (16%)		3(20%)		3(33%)			8(44%)		2(22%)		5 (55%)		5 (26%)
	> 7		54		12		6			10		7		4		14
dysuria	Yes	57 (44%)	71(64%)	40(44%)	16(73%)		11 (79%)	22 (56%)		12 (57%)		12 (100%)		11(100%)	31(38%)	19(65%)
	No	72	39	50	6		3	17		9		0		0	51	10
irritation			82 (75%)		12 (55%)		6 (43%)			15 (71%)		3(25%)		3(27%)		19(65%)
			28		10		8			6		9		8		10
Discharge	Yes	82 (64%)	56(51%)	59 (66%)	9(41%)		4(29%)	23(59%)		14(66%)		1(8%)		0(0%)	44(54%)	10(34%)
	No	47	54	31	13		10	16		7		11		11	38	19
meatitis/	yes		43(39%)		9(41%)		6(43%)			3(14%)		11(92%)		7(64%)		15(52%)
balanitis	no		67		13		8			18		1		4		14
Discharge	Scanty	61(53%)	36(32%)	53(57%)	8(36%)		6(43%)	20(51%)		8(62%)		7(58%)		6 (55%)	53(74%)	19(65%)
	intermeiate	63	72	33	14		8	16		13		5		5	18	9
	profuse	5	6	4	0		0	3		0		0		0	1	1
Discharge	serous		60(55%)		14(64%)		9(64%)			10(48%)		11(92%)		11(100%)		15(52%)
	mucoid		41		6		5			6		1		0		4
	mucopurulent		9(8%)		2(9%)		0			5(24%)		0		0		10(34%)
WCC /ul	median		199		185		83.4			44.8		125		8.9		29.7
PMNs/hpf	5-15	64(60%)			51(64%)			18(54%)							63(81%)	
	>15	43			28			15							14	

Sena A JID 2012 & Ito S IJU 2016

WIGGINS R ET AL IJSA 2006



* hypothetical

CAN SYMPTOMS AND SIGNS PREDICT INFECTIOUS AETIOLOGY IN NGU?

- No
  - Too much overlap
- Pathogen-negative detection
  - Associated less inflammation

IS *M. GENITALIUM* ASSOCIATED WITH EPIDIDYMO-ORCHITIS?

- Ito S. Int J Urol 2012; 19:234–8
- 56 cases <40 years old
  - *Chlamydia trachomatis* 50% (23)
  - *M. genitalium* 8% (4)

M. GENITALIUM AND PROCTITIS

- Bissessor M. CMI 2016
- 154 MSM with proctitis
  - 12% detection rate of *M. genitalium*
  - Similar to
    - Chlamydia 19.5%
    - Gonorrhoea 25%
    - Herpes 13%
  - Higher load compared to asymptomatic infection
- Need case control studies

M. GENITALIUM AND OTHER CONDITIONS

- Sexually acquired reactive arthritis (rare)
  - *M. genitalium* has been identified in reactive joints
- Balano-posthitis
  - Horner et al 2012 – association
  - Ito et al 2016 – no association

WHAT IS THE RISK OF ASYMPTOMATIC MEN DEVELOPING URETHRITIS?

4286 cases NCNGU in GUM clinics were aged 16-44yrs
1.2% of the male population aged 16-44yrs are *M.gen* +ve (n=125 282)

Proportion of NCNGU due to M. genitalium	No of M. genitalium treated NCNGU cases	Proportion M. genitalium which is symptomatic in men (duration infection 1 year)	Proportion M. genitalium which is symptomatic in men (duration of infection 2 years)	No of M. genitalium treated NCNGU cases adjusted for 50% diagnosed in community	Proportion M. genitalium which is symptomatic in men (duration of infection 1 year)	Proportion M. genitalium which is symptomatic in men (duration of c infection 2 years)
0.1	4286	0.03	0.06	8572	0.06	0.12
0.15	6429	0.05	0.09	12859	0.09	0.17
0.2	8572	0.06	0.12	17145	0.12	0.21

Horner, personal communication

M.GEN IS DEFINITELY A PROBLEM IN MEN

- First isolated in 1981 from man with NGU
- Demonstrated associations with NGU
- Associations with proctitis, epididymo-orchitis and SARS less clear

- What is the burden of disease in women?

THERE IS A PAUCITY OF EVIDENCE IN WOMEN

- Far fewer studies have been conducted in women
- Data documenting morbidity are inconsistent
- Associations with reproductive system disease syndromes are of lower magnitude
- However, because women are more likely to suffer greatest sequelae of genital tract infections, decisions to invest in control programmes are usually based on the strength of the evidence in women

HOW COMMON IS M.GEN IN WOMEN?

Study population Country, number, year	Prevalence in % (95% CI)	Sample size	Age range or mean, years
Women in the general population			
Denmark 1, 2007[24]	2.30 (1.32, 3.24)	921	21-23
Great Britain 4, 2015[25]	1.30 (0.90, 1.90)	2632	16-44
USA 2, 2007[20]	0.80 (0.42, 1.57)	1714	18-27
Subtotal (I-squared = 73.4%)	1.39 (0.81, 2.38)		

Recent meta-analysis of 63 studies [Baumann, 2018]

Summary prevalence in women in HDI countries: 1.39%
In pregnant women: 0.9%
Female community-based CSWs: 15.9%

CLINICAL SYNDROMES: ASSOCIATION WITH M.GEN INFECTION

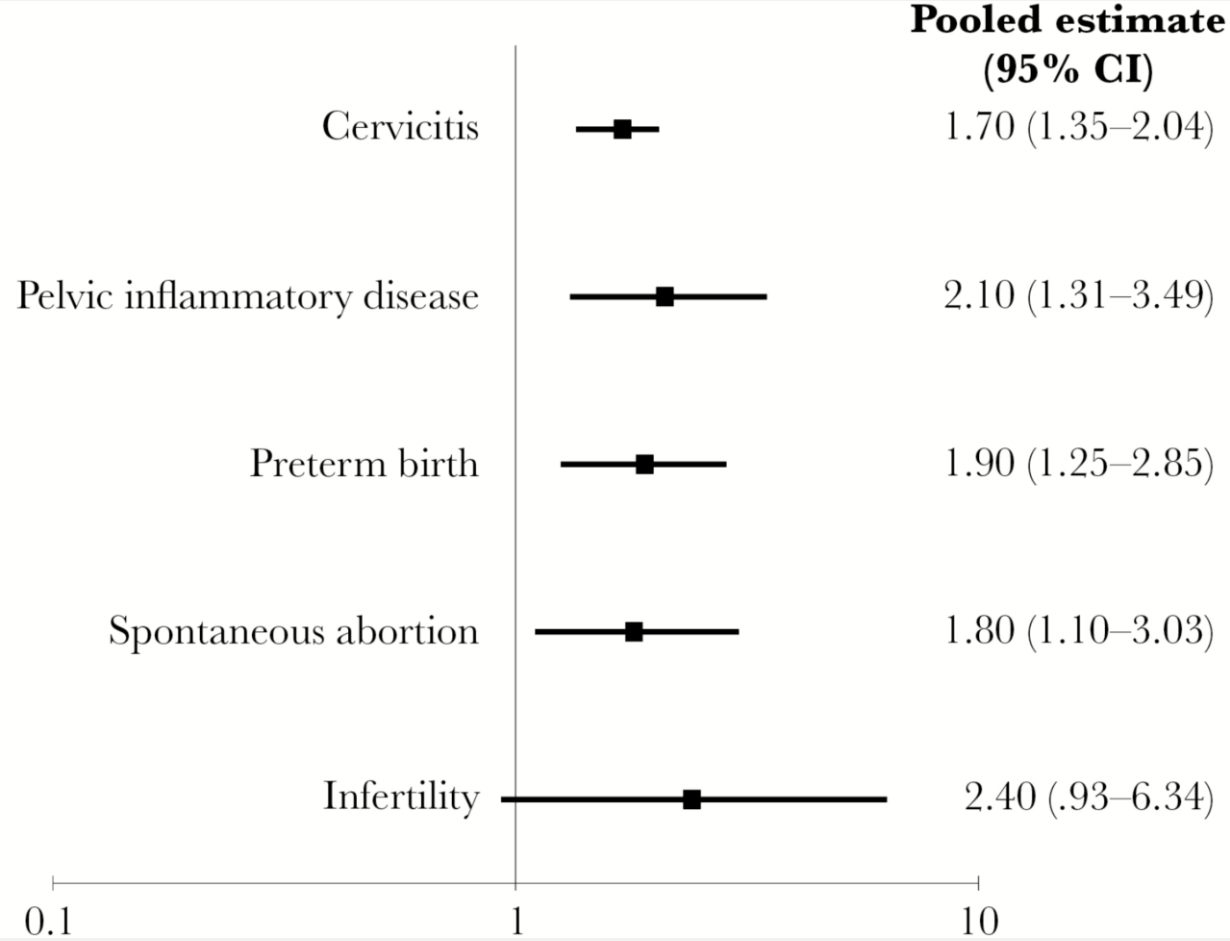
Reproductive tract syndromes and sequelae

Meta-analysis [Lis, 2015]

1980 – present

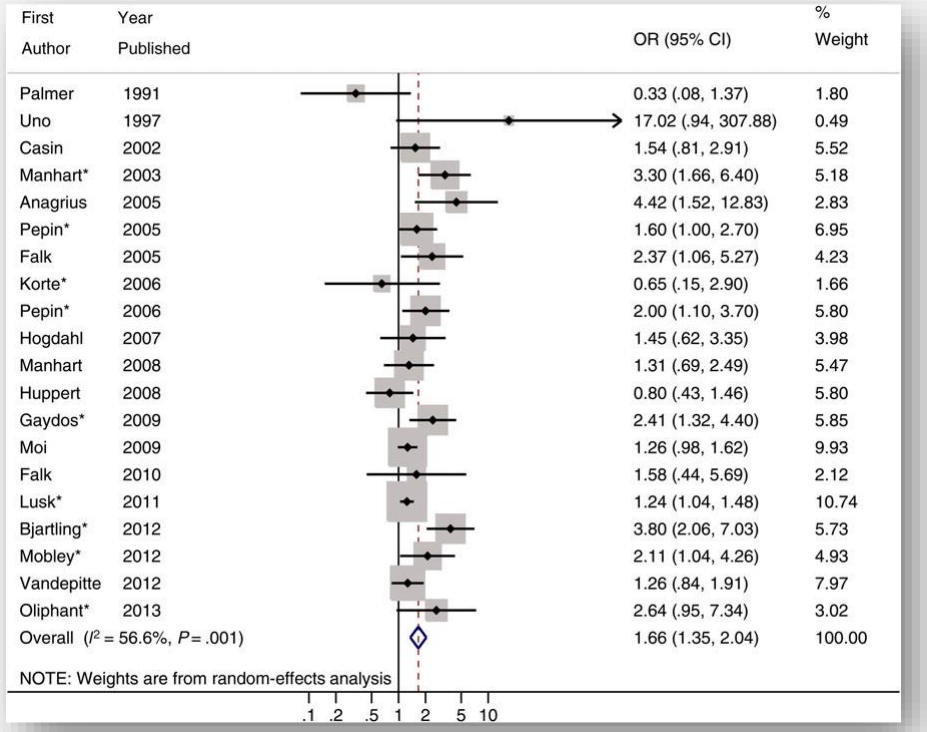
Cross-sectional, cohort or case-control design

Cervicitis n = 311
PID n = 292
Adverse pregnancy outcomes n = 174
Infertility n = 203



CERVICITIS

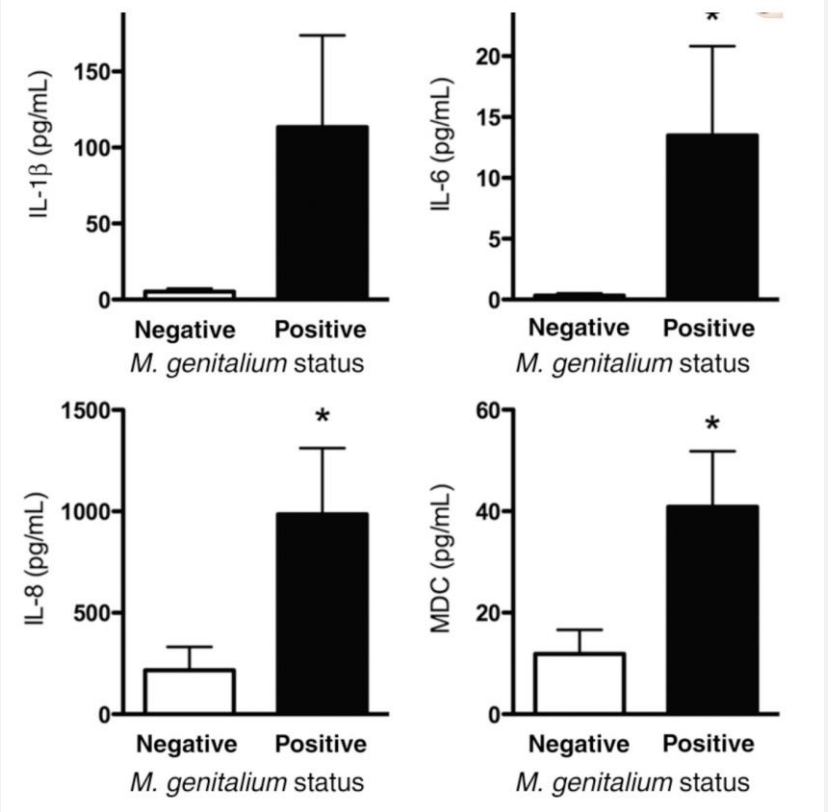
- Cervicitis often considered the female counterpart to urethritis
- Signs and symptoms in studies are variable plus heterogeneity of diagnostic definition across studies (e.g. visible mucopurulent discharge, microscopic criteria (PMNLs > X; PMNLs > epithelial cells) post-coital bleeding)
- Majority of studies demonstrate an increase in risk, but effect size is small

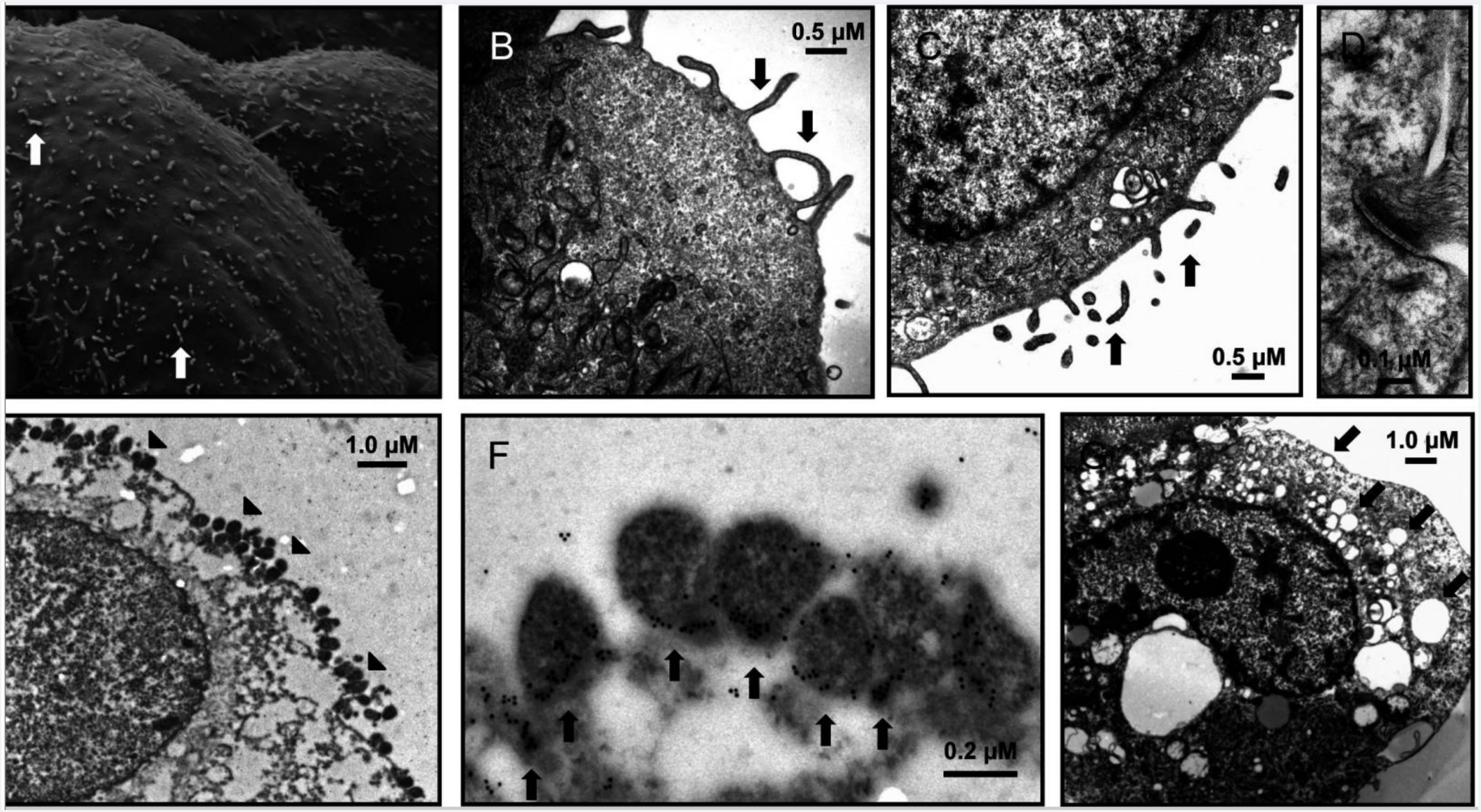


Cervicitis
Pooled OR **1.70** [95%CI 1.35-2.04]

CERVICITIS

- Nearly all studies are cross-sectional
- A longitudinal study demonstrating induction of cervicitis following infection would imply causality
- In vitro and animal studies:
  - *M. gen* can cause prolonged infection in marmoset lower genital tract with a prolonged inflammatory response [Taylor-Robinson, 1982]
  - Acute *M. gen* infection of endocervical cells causes destruction of micro-villi and increased secretory function [McGowin, 2013]
  - In vitro infection of endocervical cells causes increase in pro-inflammatory cytokine and chemokine response [McGowin, 2012]
  - Pro-inflammatory cytokine profiles seen in women with chronic *M. gen* infection [Dehon, 2016 – see right]





Pre-inoculation: abundant microvilli on the apical cell surface (*A–C*; arrows)
E: attached *M. gen* marked with arrowheads
F; arrows: Confirmation of pleomorphic *M. gen* at apical cell surface (gold particles concentrated on the tip organelle)
*G: i*ncreased secretory activity (arrows) and absence of abundant microvilli 48 hours post-inoculation

McGowin, JID, 2013

CERVICITIS

- Most infections in the lower genital tract are asymptomatic
- Thus, further epidemiological studies are unlikely to be helpful
- Better to focus on pathophysiology of cervicitis and risk factors for inflammation and disease progression
- Are any lower genital tract symptoms discriminatory?

ASSOCIATION OF M.GEN WITH SYMPTOMS: WOMEN

Disease	Author	Number	MG positive	MG negative	P value
Post coital bleeding*	Bjartling	679	22%	12%	0.008
Post coital bleeding*	Sonnenberg	2215	14.4%	2.7%	0.014
Abnormal Vaginal discharge*	Bjartling		32%	24%	0.12
Abnormal Vaginal discharge*	Sonnenberg		0.9%	4.0%	0.06
Abdominal pain	Bjartling		59%	65%	0.28
Abdominal pain	Sonnenberg		10.8%	6.3%	0.39
Dysuria*	Bjartling		9.3%	8.3%	0.76
Dysuria*	Sonnenberg		7.8%	7.8%	0.90

* Associated with chlamydia

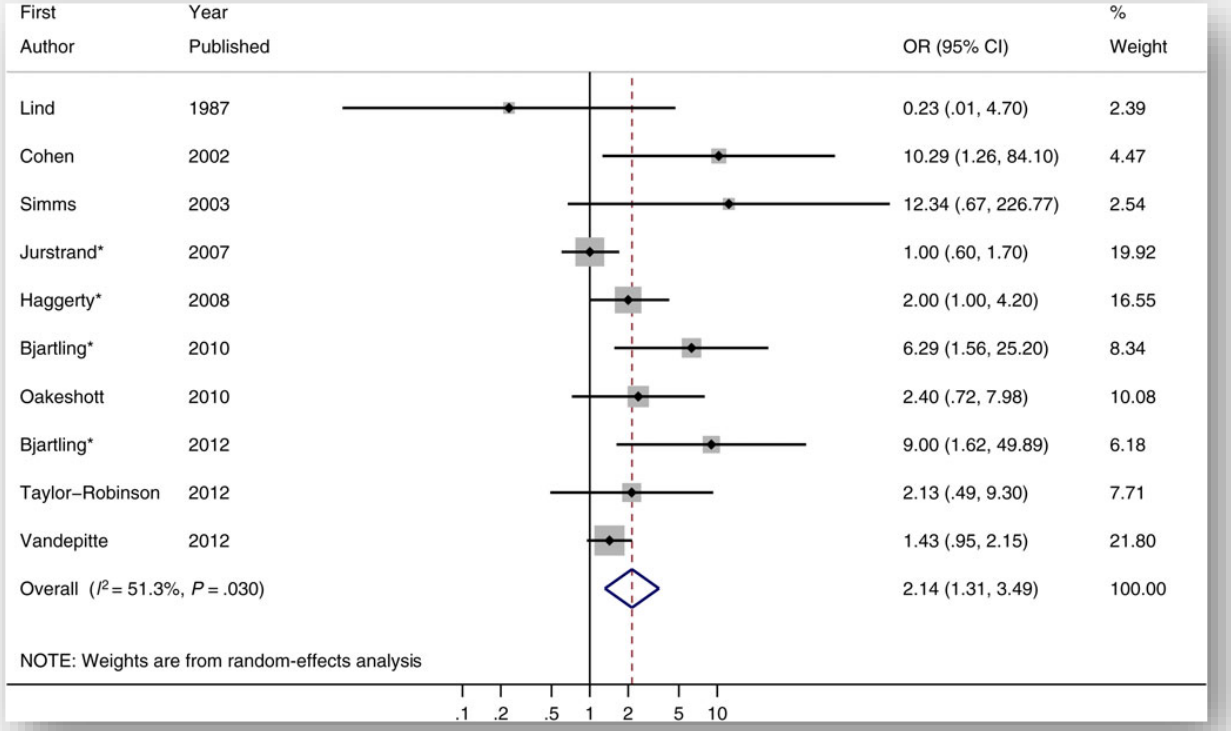
Bjartling K. 2012;206:476: Sonnenberg P. IJE 2015;44:1982

VAGINITIS

- No associations with vaginitis have been demonstrated
- No data support an association with organisms causing vaginitis (e.g. *Trichomonas vaginalis* or *C. albicans*)
- The relationship with BV is inconsistent
- One recent longitudinal study has shown that susceptibility to *M.gen* may be enhanced in the presence of BV [Lokken, 2017]

PELVIC INFLAMMATORY DISEASE

- Early studies using serology yielded conflicting results
- NAAT-based studies have demonstrated presence of *M.gen* in the upper genital tract of women with PID
  - 7/58 (12%) women with histologically defined endometritis and pelvic pain in a Kenyan STD clinic had *M.gen* detected (c.f. none in women without endometritis) [Cohen, 2002]
  - *M.gen* detected on cervical swabs in 6/45 (13%) women with clinical PID in UK GUM clinics, compared to none of the controls [Sims, 2003]



PID

Pooled OR **2.14** [95%CI 1.31-3.49]

UK PID STUDY [DEAN, 2016]

- RCT to investigate short-course azithromycin in the treatment of PID
- N = 313
- Microbiology:
  - *Chlamydia trachomatis* 27 (9.6%)
  - *Neisseria gonorrhoeae* 1 (0.4%)
  - ***Mycoplasma genitalium*** **28 (9.7%)**

  - 10 patients co-infected with *C. trachomatis* & *M. genitalium*
  - 1 patient co-infected with *N. gonorrhoeae* & *M. genitalium*

SUBCLINICAL PID

- Many women with tubal-factor infertility (TFI) lack a clinical history of PID
- An association between *M.gen* seropositivity and TFI has been observed, independent of past chlamydial infection [Svenstrup, 2008]
- Subclinical PID (endometritis) was identified in 22/43 (51%) of women with *M.gen* infection in an STD clinic (c.f. 47% in those with *C. trachomatis*)
- When controlling for chlamydia, gonorrhea, and bacterial vaginosis, cervical *M. genitalium* infection was independently associated with subclinical PID (adjusted odds ratio aOR = 2.4; 95% confidence interval [CI] = 1.2–4.6) [Wiesenfeld, 2009]
- Women with subclinical PID are at risk of infertility, but the role of *M.gen* is yet to be determined [Wiesenfeld, 2012]

WHICH WOMEN DEVELOP PID?

- Screening and treatment of women with chlamydia has resulted in a fall in the incidence of PID [Scholes, 1996]
- A similar observation with *M.gen* would verify its role in upper genital tract infection
- Secondary analysis of a prospective UK chlamydia screening trial tested stored samples for *M.gen* [Oakeshott, 2010]
  - Women with *M.gen* had a two-fold greater incidence of PID over 12 months (3.9% vs. 1.7%; relative risk = 2.35; 95% CI = 0.74–7.46)
  - Prevalence of *M.gen* in the cohort: 3.3%
  - Relatively low-risk population, and diagnosis relied on patient report of symptoms during follow-up
  - Seven (26%; 95% CI, 9%–43%) of 27 women with *M. gen* infection at baseline remained positive after 12–21 months; genotyping results suggest that these were persistent infections
- Swedish cohort of 2079 women undergoing TOP [Bjartling, 2010]
  - 2.5% had *M.gen*
  - 5/46 (10%) developed PID (OR: 6.29, 95% CI 1.56–25.2)

RISK OF ASYMPTOMATIC WOMEN DEVELOPING PID

10.3 million women, with 0.25% PID GP 26, 000 plus 14,000 GUM aged 16-44yrs = 40,000
134000 (1.3%) of the female population aged 16-44yrs are *Mgen* +ve

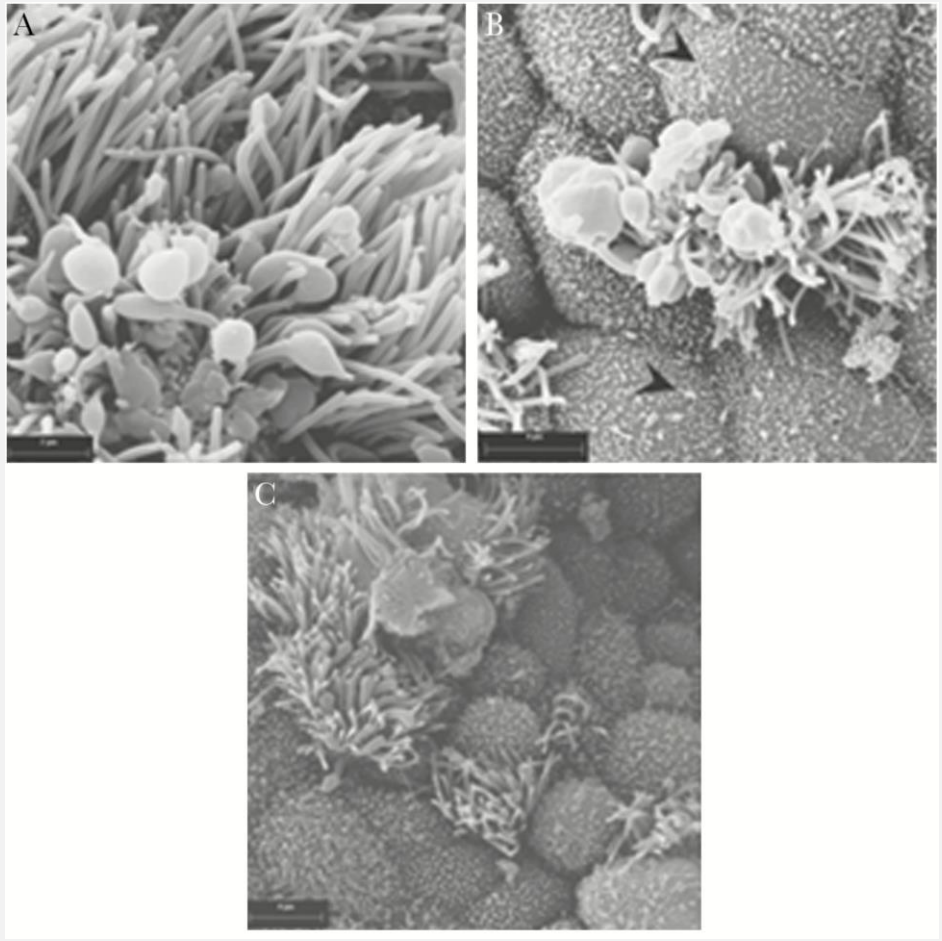
Proportion of PID due to M. genitalium	No of cases M. genitalium PID	Proportion M. genitalium which results in PID (duration of asymptomatic infection 1 year)	Proportion M. genitalium which results in PID (duration of asymptomatic infection 2 year)
0.05	2000	0.015	0.03
0.1	4000	0.03	0.06
0.15	6000	0.045	0.09

Prevalence = incidence x duration

Horner, personal communication

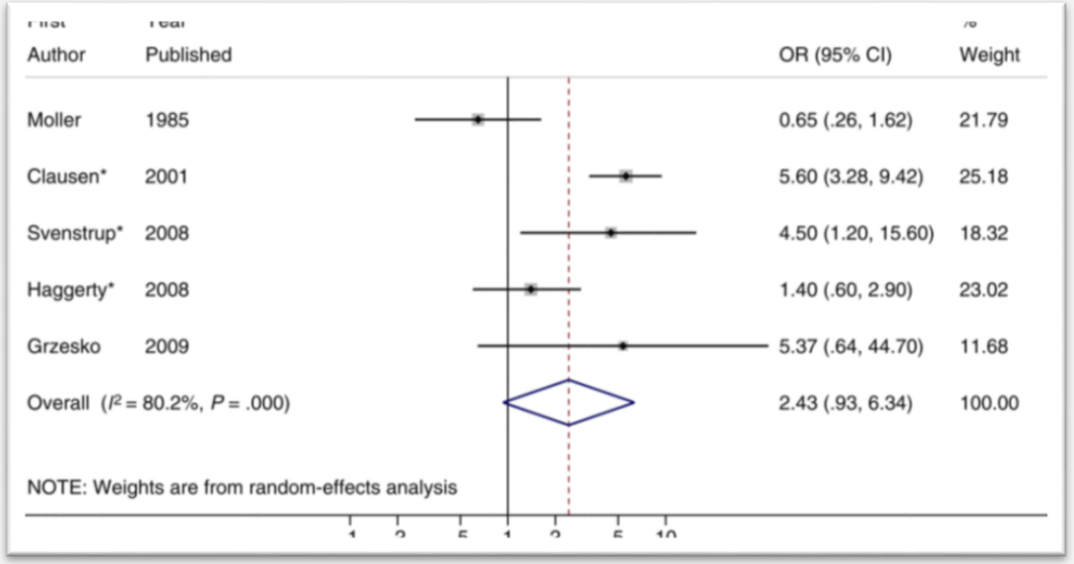
M.GEN AND TUBAL FACTOR INFERTILITY

- Microscopic evidence of ciliary damage has been demonstrated in fallopian tube explants [Baczynska, 2007]
- *A:* swelled cilia may have many shapes and swelling can appear at different places of the cilia
- *B*: Cilia fall apart, many are shortened, and the number of cilia per cell is reduced. Structures similar to *M. genitalium* cells can be seen in the preparation (arrows)
- *C:* Affected ciliated cells with two dead cells that are still partly attaching to the human fallopian tube tissue



M.GEN AND TUBAL FACTOR INFERTILITY

- Epidemiological studies yield conflicting data
- Studies are heterogenous, and aetiology of TFI/subfertility is multi-factorial
- Lis, 2015: OR 2.43 (increasing to 3.3 when restricted to studies accounting for chlamydial and gonococcal infection)



Female infertility
Pooled OR 2.43 [95% CI 0.93-6.34]

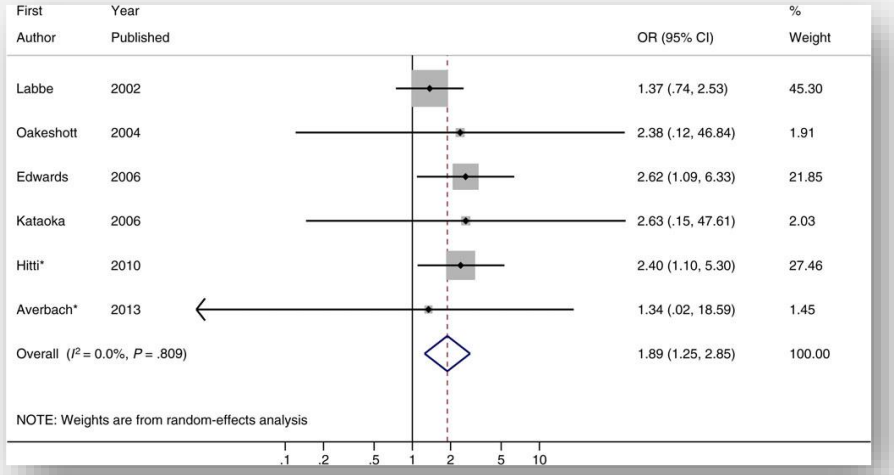
M.GEN AND ECTOPIC PREGNANCY

- Tubal damage as exemplified may imply *M.gen* also associated with ectopic pregnancy
- Paucity of literature
  - Serum specimens from 82 Swedish women with ectopic pregnancies and 246 controls screened for rubella were tested for the presence of *M. genitalium* antibodies: aOR = 1.0; [95% CI = 0.5–2.0] [Jurstrand, 2007]
  - NAAT testing for *M. gen* was performed on tubal tissue from 84 hospitalized Saudi women with ectopic pregnancies undergoing salpingectomy and 51 women undergoing hysterectomy or tubal ligation, detection of *M. gen* was significantly associated with ectopic pregnancy, even after adjustment for other pathogens (aOR = 2.3; 95% CI = 1.1–8.6) [Ashshi, 2015]

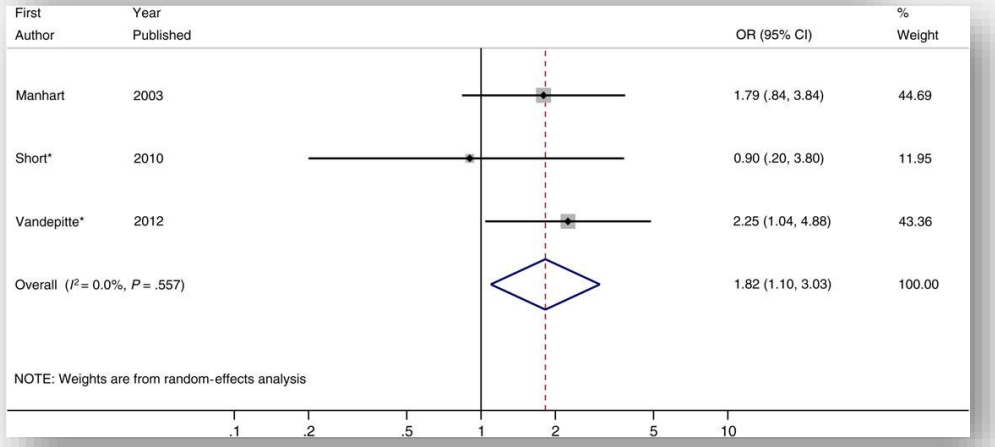
- Further work is required to understand the role of *M.gen* in TFI and ectopic pregnancy, requiring a better understanding of pathophysiology, and better mechanisms to detect past infection

M.GEN AND PREGNANCY OUTCOMES

- Data for adverse pregnancy outcomes are limited by heterogeneity of studies, and small numbers
- The OR for pre-term delivery is <syphilis and gonorrhoea but >TV and BV
- Does this warrant intervention?
- Longitudinal studies in high-risk populations are necessary



Pre-term birth
Pooled OR 1.89
[95% CI 1.25–2.85]



Spontaneous abortion
Pooled OR 1.82
[95% CI 1.10–3.03]

CASE 1

27 year-old British male

Presents with four week history of dysuria and cloudy PU discharge

Saw GP at onset of symptoms and Rx azithromycin 1g stat with apparent improvement in symptoms. Symptoms recurred after one week

Sexual Hx:

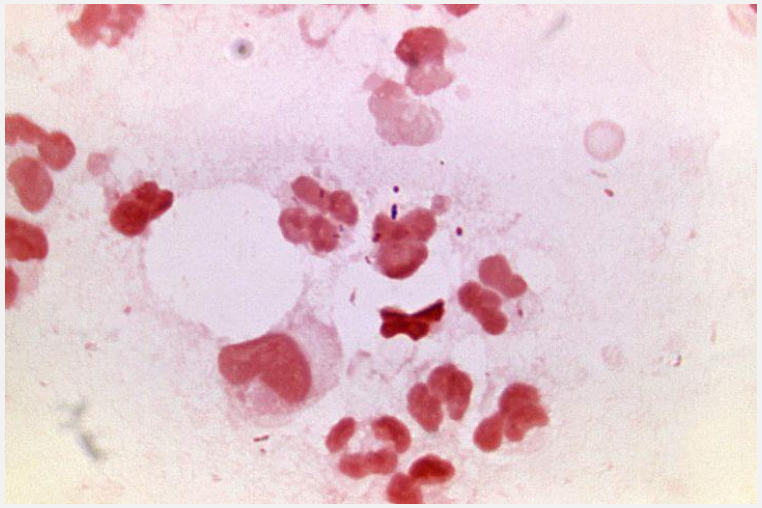
(1) 2/7 with RFP (UK; 3/12) – UPVI

(2) 3/12 with ex-RFP (UK; 1yr) – UPVI

O/E:

White discharge evident at urethral meatus

Urethral smear: 10-15 PMNLs/HPF



CASE 1

What would you test for?

How would you treat this man?

INVESTIGATION

- MG not visible under light microscopy
- Culture very slow

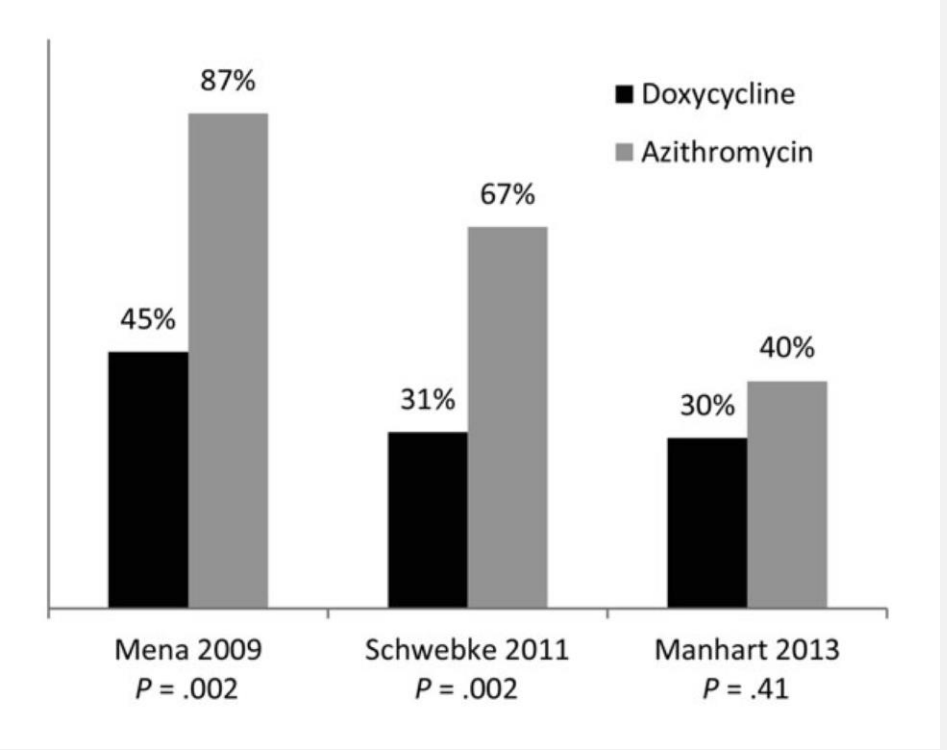
- In-house PCRs (CE-marked but not FDA licensed)
- Multiplexes:
  - FastTrack Diagnostics
  - Seegene, Bio-rad, Pathofinder
  - Aptima

- Sample – FVU, vaginal swab, rectal swab

TREATMENT

- No cell wall: penicillins and cephalosporins ineffective
- Tetracyclines: 30-40% effective
- Macrolides: azithromycin (staggered dose superior to 1g stat)
- Fourth generation quinolones effective (moxifloxacin)

Patient given azithromycin 500mg stat then 250mg OD for 4/7



RCTs of doxycycline vs. azithromycin 1g stat in men with MG urethritis (Martin, CID 2015)

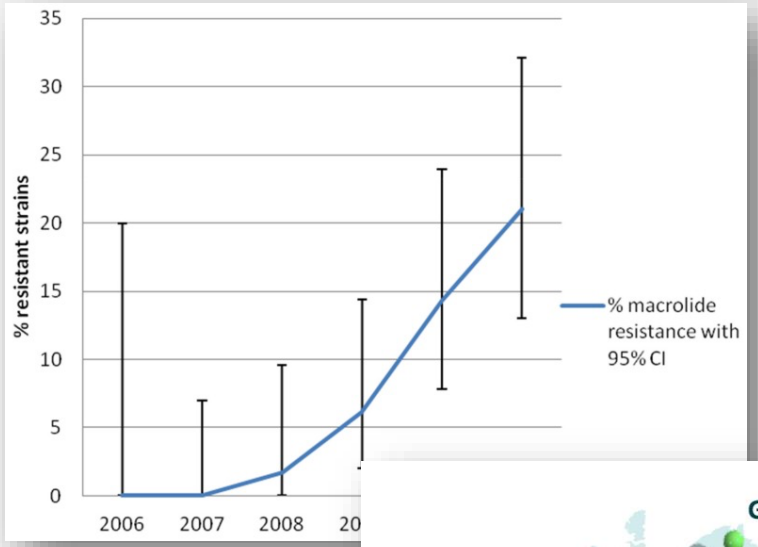
CASE 1

- Presents 2/52 later
- Symptoms no better – ongoing white PU discharge
- No sex since last seen
- MG *POSITIVE* on initial visit
- Urethral slide: 10-20 PMNLs/HPF

What next?

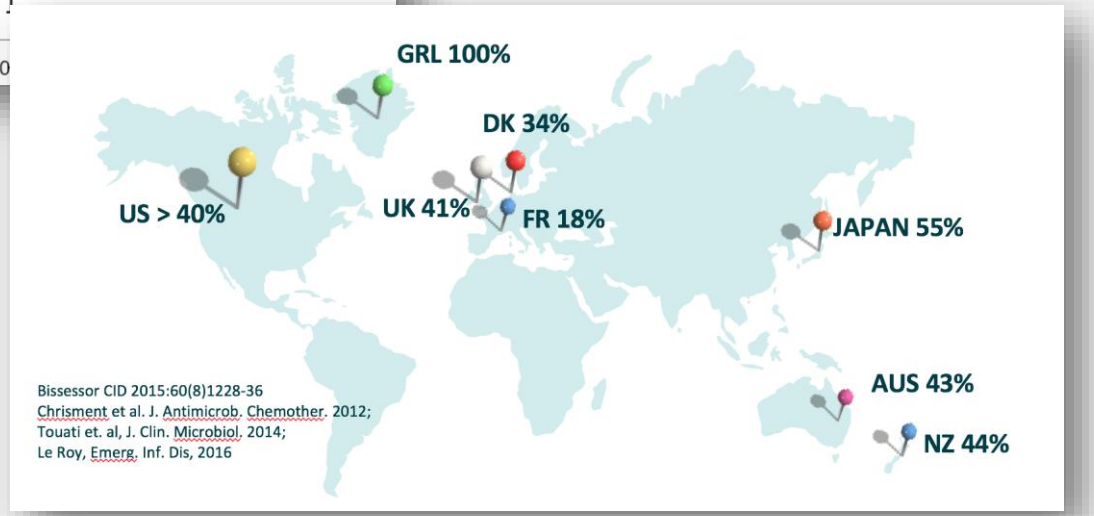
MACROLIDE RESISTANCE

- Efficacy of azithromycin 1g is declining
- Meta-analysis of cure in 21 studies (Hocking, 2015):
  - Pre-2009: 85.3%
  - Post-2009: 67.0%



Anagrius, 2013

- Clinical macrolide resistance has been demonstrated to be climbing in a number of retrospective and prospective observational and interventional studies



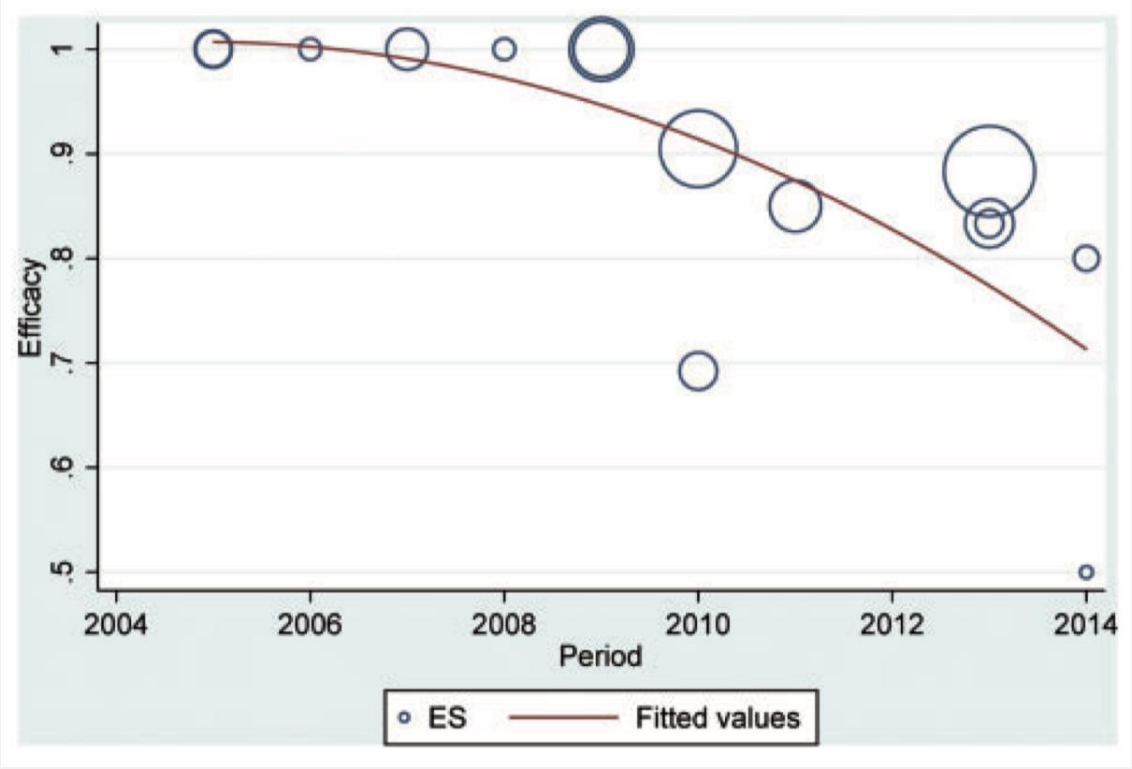
MACROLIDE RESISTANCE

- Macrolide resistance-mediating mutations (MRMMs) prevalent in pre-treatment and treatment experienced patients
- MRMMs at 2058/59 locus on 23s ribosome
- Bissessor et al (2015):
  - 155 participants treated with 1g azithromycin with TOC at 14 and 28 days: **61% cured**
  - Pretreatment MRMM detected in 36% and strongly associated with treatment failure [adjusted OR 47.0]
  - All 11 participants who had MRMM detected in **post**-treatment samples had failed azithromycin
  - Organism load predicts likelihood of failure and induction of resistance

- Evidence of induction of resistance by stat (>12%) and staggered dose regimens (>5%)
- Efficacy of staggered dose regimens is also falling
- New evidence that pre-treatment with doxycycline improves outcomes (Read, JID 2018)
- **Staggered dose regimens will not reliably work in patients with MRMMs**
- **Detection assays should incorporate AMR testing**

MOXIFLOXACIN

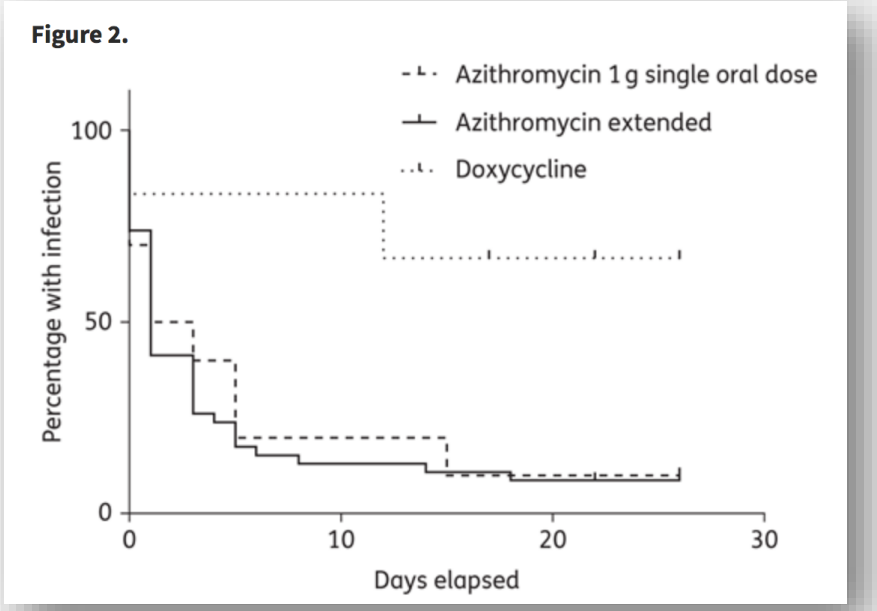
- Initially highly effective, with cure rates >100%
- Well tolerated drug (drug-induced hepatitis very rare)
- Recent meta-analysis suggests declining efficacy
  - Pre-2010, pooled cure rate 100% (95% CI, 99%–100%)
  - Post-2010, pooled cure rate 89% (95% CI, 82%–94%)
- Reduced efficacy driven by fluoroquinolone-associated resistance mutations in *gyrA* and *parC*
- Drivers unclear, as resistance occurs in both high and low-use settings



Li, Int J STD & AIDS, 2016

CASE 1

- Patient treated with moxifloxacin 400mg OD for 14 days
- Test of cure negative at 21 days
- RFP treated with same regimen
- All's well that ends well!
- But if not, what's next?
  - pristinamycin (streptogramin antimicrobial used for VRE and MRSA; limited availability and high cost)



Time to negative TOC, Falk, 2015

DRAFT BASHH GUIDELINES

- Consultation closed 1st September

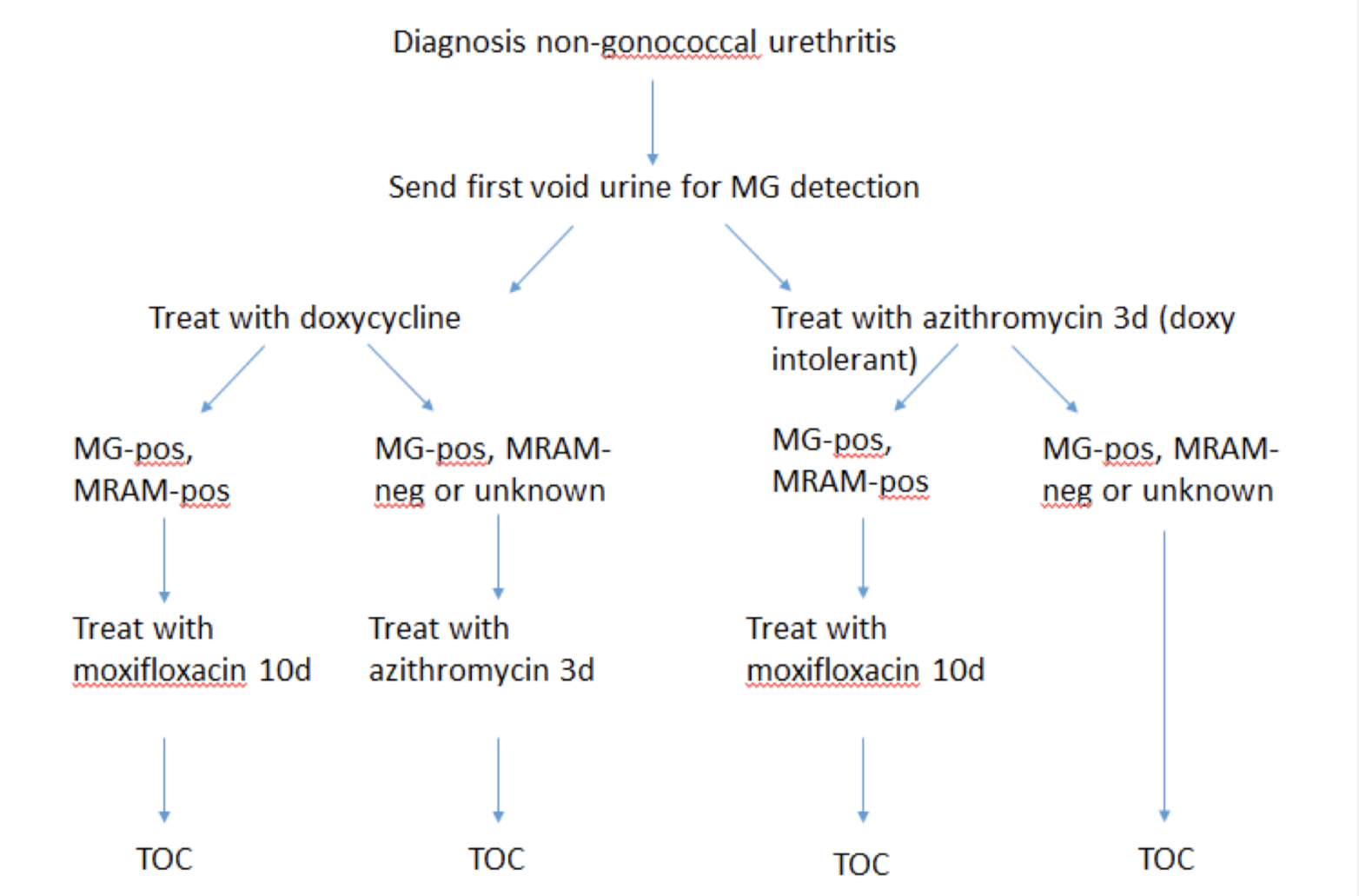
SUMMARY OF BASHH GUIDELINE RECOMMENDATIONS: TESTING

DOMAIN	GRADE
Testing based on signs and symptoms	
Male with NGU Pelvic inflammatory disease MPC Post-coital bleeding	1B 1B 2B 2B
Testing based on risk factors	
Current partner of someone with *M.gen*	1D
Specimen collection	
Self-taken or clinician-taken vulvo-vaginal swab	1C

SUMMARY OF BASHH GUIDELINE RECOMMENDATIONS: TREATMENT

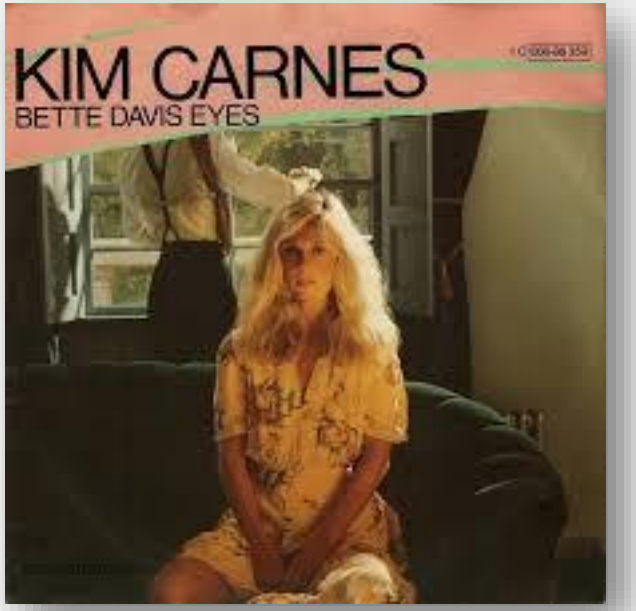
DOMAIN	GRADE
Uncomplicated infection	
Doxycycline 100mg BD for 7 days followed by azithromycin 1g stat then 500mg BD for 2 days	1D
Moxifloxacin 400mg OD for 10 days	1B
Complicated infection	
Moxifloxacin 400mg OD for 10 days	1D
TOC	
All patients should attend for a TOC five weeks (and no sooner than three weeks) after the start of treatment	1D

SUGGESTED MANAGEMENT FLOW CHART IN MEN



LEARNING POINTS

- *M.gen* is a definite cause of STI syndromes, and complications
- It has been neglected and beleaguered since 1981 by a paucity of diagnostic tests and contention about his pathogenicity
- Emergent antimicrobial resistance has pulled it into the spotlight
- Routine testing is warranted in all patients with urethritis and PID
- When testing not available, antimicrobial stewardship is key (viz BASHH NGU Guidelines update)
- What about co-infection when treating other syndromes/STIs?
- AMR detection should be incorporated into routine tests



Betty Davis Eyes, Carnes, 1981

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