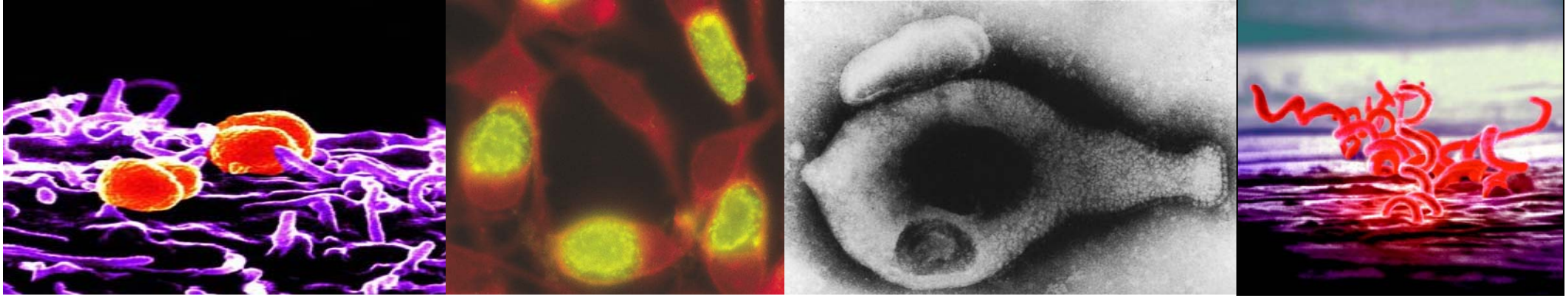


Microbiologic diagnostics of STIs

Magnus Unemo

WHO CC for Gonorrhoea and other STIs

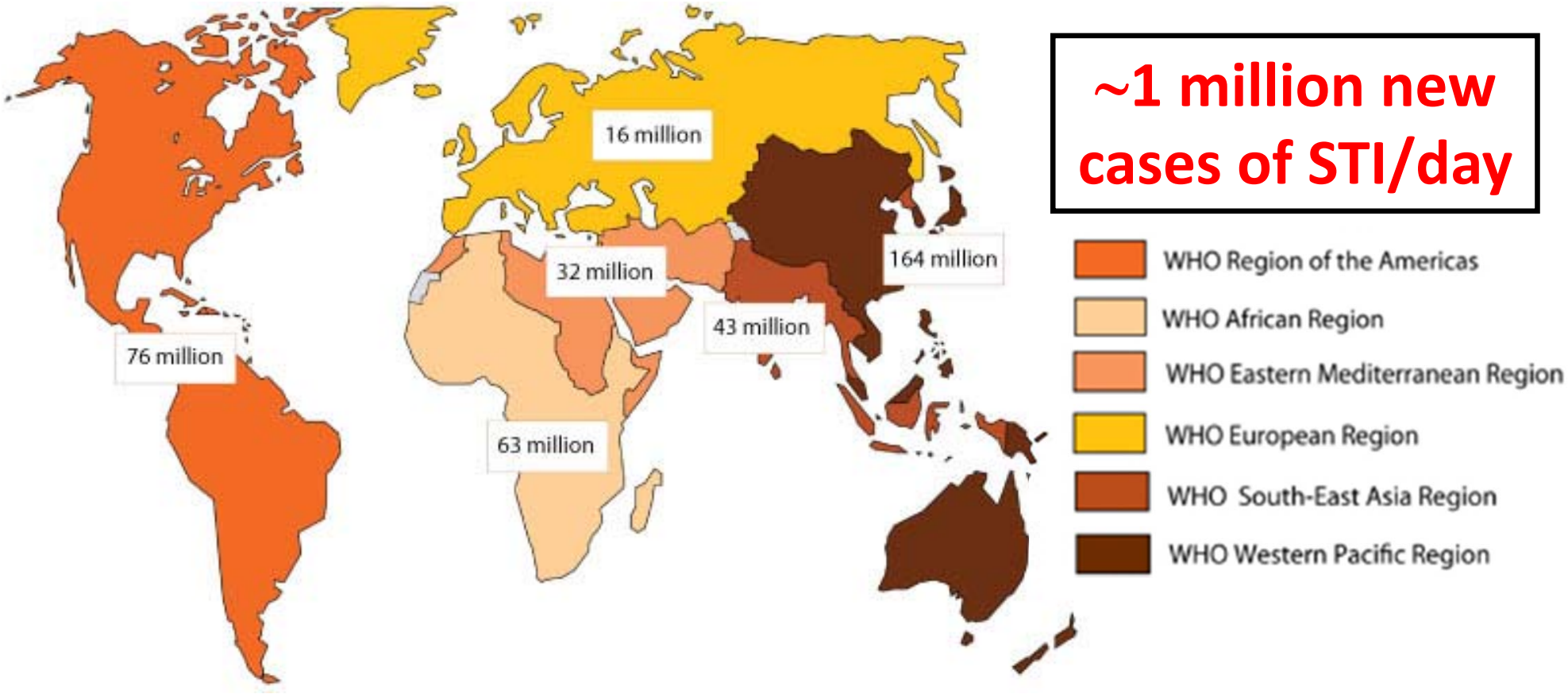
Swedish Reference Laboratory for Pathogenic Neisseria

Department of Laboratory Medicine, Microbiology

WHO Collaborating Centre for Gonorrhoea and other Sexually Transmitted Infections

Örebro University Hospital, Sweden

WHO estimates: 357 million new cases of four curable STIs in adults in 2012



Chlamydia (~131 million), Gonorrhoea (~78 million), Syphilis (~6 million), Trichomoniasis (~142 million)

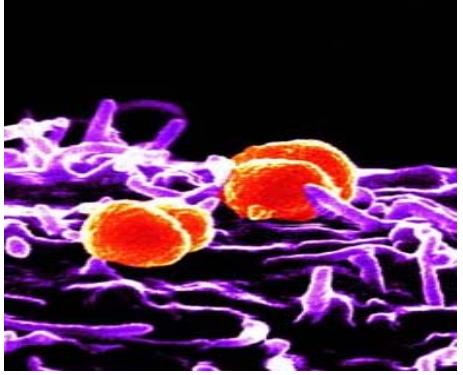
Newman, et al. PloS One. 2015

Challenges

Solved?

Unsolved?

- High incidence
- Natural course – Severe sequelae (incidence, prevalence)
- Low number of etiologically diagnosed STIs
- Suboptimal diagnostics, testing, case reporting, and treatment in many countries
- Antimicrobial resistance (AMR) in NG, *M. genitalium* (MG), *T. vaginalis*, *T. pallidum* (only azithromycin)
- Empiric first-line treatment
- High cost



Neisseria gonorrhoeae (gonococcus, GC, NG)

- Identified 1879 (Albert Neisser)
- Cultured in vitro 1882 (Leistikow and Loeffler)
- Gram-negative diplococci, aerobic but require CO_2 (4-6% ideally), and nutritional fastidiuous
- Obligate human pathogen, don`t survive in environment

Diagnosis of gonorrhoea (”Clap”)

- **Clinical**: only **suggestive for bacterial STIs**!

Laboratory diagnostics

- **Microscopy of stained smear**: **presumptive and not only test** (if not urethral specimen from symptomatic male)!

- **Culture (optimized + quality assured)**: **Old gold standard** for definitive diagnosis (”100%” specificity and **AMR testing**)!
- **Nucleic acid amplification tests (NAATs)**: **Highly sensitive and specific, but supplementary testing recommended**, i.e. at PPV≤90% and for extragenital samples!

- ~~**Antigen- or antibody-detection: Not recommended!**~~

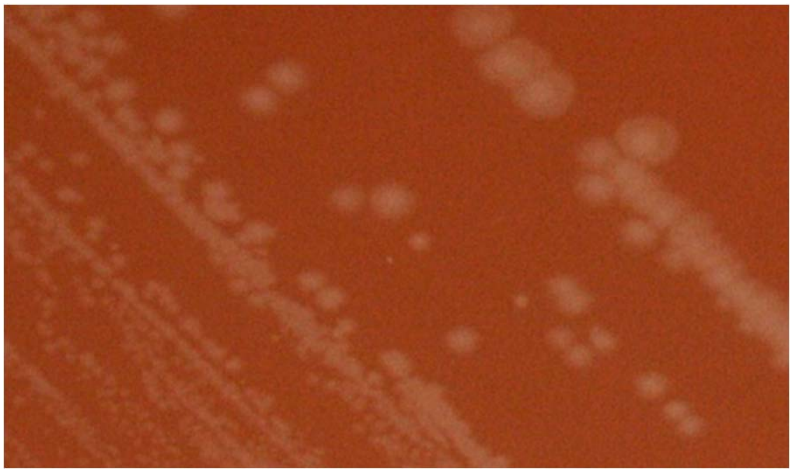
Unemo & Ison. WHO Manual. 2013; Bignell & Unemo. IJSTDA. 2013

Detection of *N. gonorrhoeae*

Presumptive

- characteristic colonies

on selective culture plates

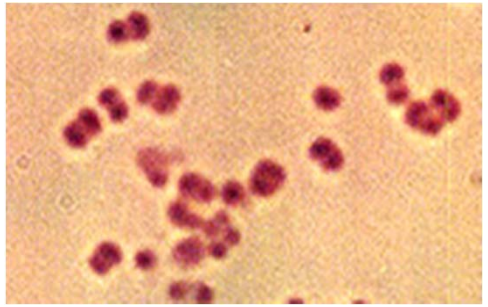


- rapid (5-30 s) positive

oxidase reaction

- typical Gram-negative diplococci

in microscopy

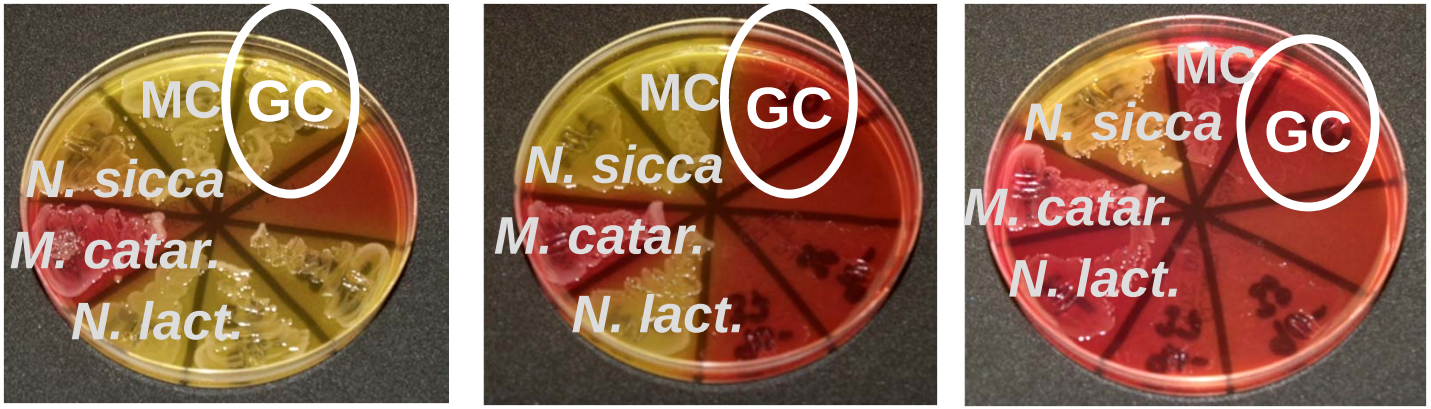


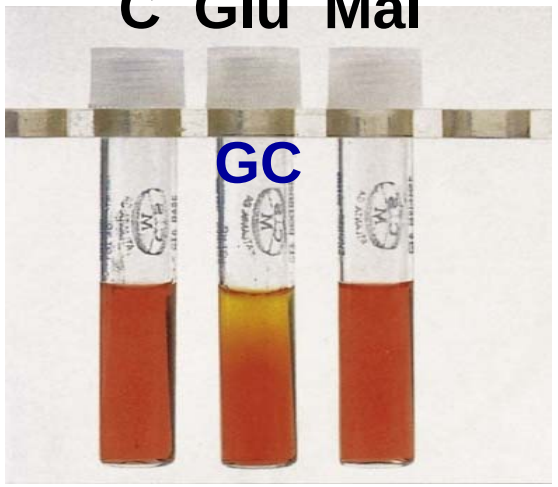
Culture of *N. gonorrhoeae*

Definitive diagnosis

- **Carbohydrate utilisation tests** (growth-dependent and/or -independent)

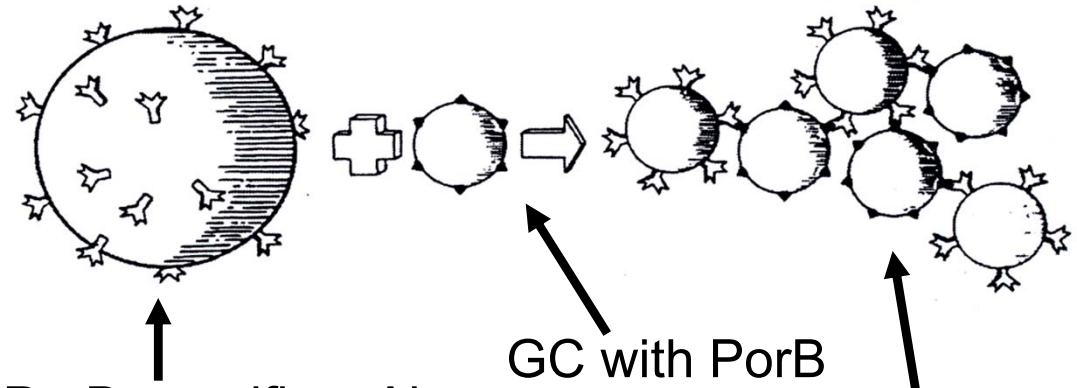
Glucose (1%) Maltose (1%) Fructose (1%)

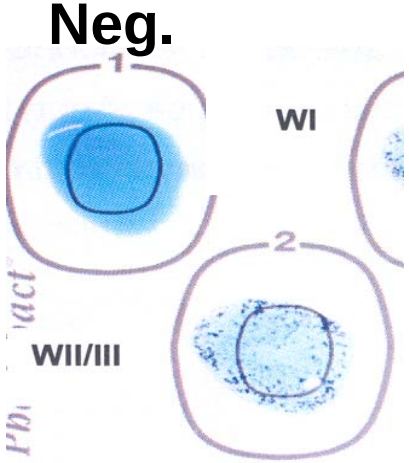




CTA medium

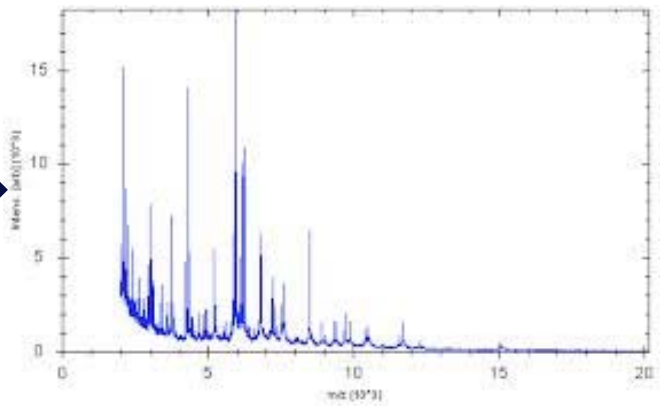
- **Phadebact Monoclonal GC Test** (coagglutination)

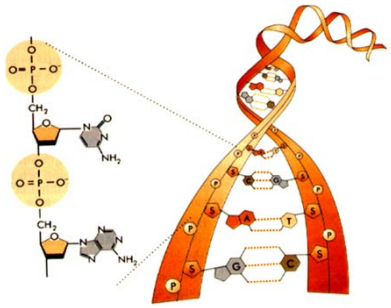




Matrix Assisted Laser Desorption Ionisation Time of Flight (MALDI-ToF)

- Mass spectrometry detects the mass-to-charge ratio (*m/z*) of proteins to produce a unique mass spectral fingerprint of each organism
- Can identify bacteria direct from the agar plate.
- Equipment costs high - cost of individual tests is low.
- Rapid and easy to perform.





NAATs: revolutionized NG diagnostics

- **Superior sensitivity**, and mostly high specificity
- Allow **automation, rapid, non-invasively collected specimens (urine and vaginal swabs) and screening of asymptomatic individuals**
- Opportunities for **simultaneous detection of several agents**, e.g. NG and CT (MG, *T. vaginalis*, HPV, (……))

Unemo & Ison. WHO Manual. 2013

Table 4.5: United States of America Food and Drug Administration (FDA)-approved NAATs for detection of *N. gonorrhoeae* (June 2012) Unemo & Ison. WHO Manual. 2013

Test	APTIMA Combo 2 (AC2)	Cobas Amplicor	Cobas 4800	Probetec ET	Probetec GC Qx	Real Time CT/NG
Manufacturer	Gen-Probe	Roche	Roche	Becton, Dickinson	Becton, Dickinson	Abbott
Target	16S rRNA	Cytosine DNA methyl-transferase gene	Direct Repeat Region 9 (DR9)	PivNg (Pilin inverting protein homologue)	Pilin (different region from Probetec ET)	Opa genes
Technology	Transcription-mediated amplification (TMA)	Polymerase chain reaction (PCR)	Real-time PCR	Strand displacement amplification (SDA)	SDA	Real-time PCR
Supplementary test available	Yes (different region of 16S rRNA)	No	No	No	No	No

Other commercially available NAATs: Siemens, GeneXpert, Seegene, Bio-Rad Dx, GeneProof, AmpliSens…

In house PCR targets: *porA* pseudogene, *opa*, *cppB*, *CMT*, *gyrA*, *gyrB*….

Table 4.5: United States of America Food and Drug Administration (FDA)-approved NAATs for detection of *N. gonorrhoeae* (June 2012) Unemo & Ison. WHO Manual. 2013

Test	APTIMA Combo 2 (AC2)	Cobas Amplicor	Cobas 4800	Probetec ET	Probetec GC Qx	Real Time CT/NG
Supplementary test available	Yes (different region of 16S rRNA)	No	No	No	No	No

- New generation NG NAATs superior sensitivity and higher specificity ⇒ increase their use!

- AMR testing not possible (culture remains essential!) and not 100% specificity!

Tabrizi, et al. JCM. 2012; Unemo & Ison. WHO Manual. 2013; Bignell & Unemo. 2012 European Guideline. IJSTDA. 2013

Other commercially available NAATs: Siemens, GeneXpert, Seegene, Bio-Rad Dx, GeneProof, AmpliSens…

In house PCR targets: *porA* pseudogene, *opa*, *cppB*, *CMT*, *gyrA*, *gyrB*….

Table 4.6: Effect of prevalence on positive predictive value (PPV) for single tests

Tests		A	B	C
Sensitivity		97.8%	96.4%	98.0%
Specificity		99.2%	97.9%	99.7%
PPV	10% prevalence	93%	84%	97%
	5% prevalence	87%	73%	95%
	1% prevalence	55%	35%	77%

Unemo & Ison. WHO Manual. 2013

Table 4.6: Effect of prevalence on positive predictive value (PPV) for single tests

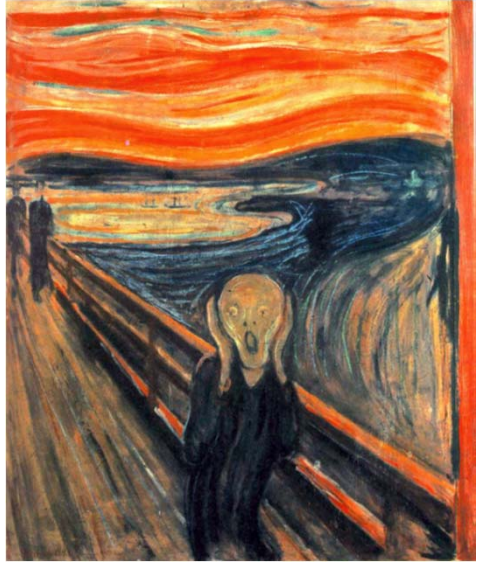
- **Positive NAAT samples recommended to be confirmed with another NAAT in Europe,** i.e. if the assay displays a PPV≤90% and for extragenital samples (Bignell & Unemo. IJSTDA. 2013)

- **Commonly results in PPV of up to 100%!**

	5% prevalence	87%	73%	95%
	1% prevalence	55%	35%	77%

Unemo & Ison. WHO Manual. 2013

The Scream (Edvard Munch, 1893)



~70 years

Current options for empiric monotherapy

~~**Penicillins**~~

~~**Tetracyclines**~~

~~**Aminoglycosides**~~

~~**Quinolones (ciprofloxacin, ofloxacin etc.)**~~

~~**Macrolides (erythromycin, azithromycin)**~~

~~**Spectinomycin (Resistance selection! Not available!)**~~

Only left ⟹ **Cephalosporins (ceftriaxone, ~~cefixime~~)**

Unemo & Nicholas. Fut Microbiol. 2012; Unemo & Shafer. Clin Microbiol Rev. 2014

Antibiotic resistance rates (%) and β-lactamase production of Swedish *Neisseria gonorrhoeae* strains 2007-2015

	2007 (n=406)	2008 (n=447)	2009 (n=384)	2010 (n=618)	2011 (n=805)	2012 (n=877)	2013 (n=967)	2014 (n=384)	2015 (n=462)
Ampicillin	30	28	44	31	24	23	18	28	22
Cefixime	<1	1	5	6	8	10	4	2	2
Ceftriaxone	0	<1	0	2	2	1	<1 (0.3)	<1 (0.3)	0
Azithromycin	7	13	6	12	11	10	13	9	10
Ciprofloxacin	70	63	75	56	55	62	53	60	53
Spectinomycin	0	0	0	0	0	0	0	0	0

Magnus Unemo, Susanne Jacobsson, Hans Fredlund. SWEDRES 2015

First NG Super Bug - H041

- **High-level resistance to ceftriaxone** (MIC=2-4 mg/L) **and most antimicrobials** (extensively-drug resistant; **XDR**)

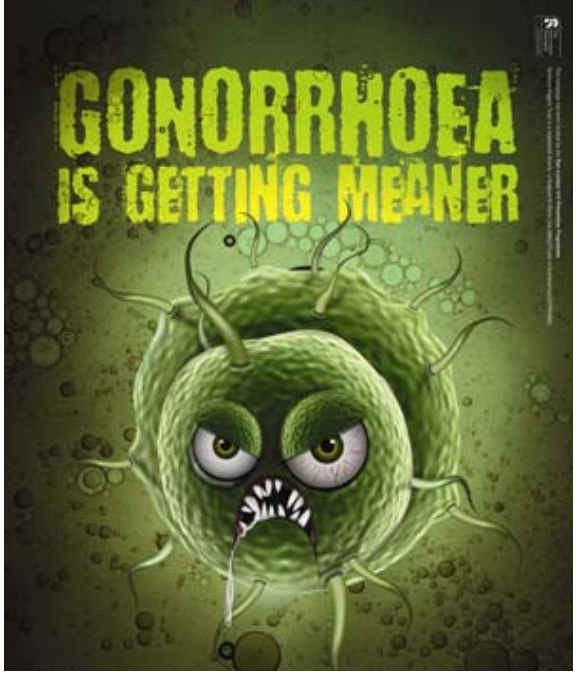
- **Treatment failure** of pharyngeal gonorrhoea (1g)

Kyoto, Japan: Female sex worker

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, July 2011, p. 3538–3545 Vol. 55, No. 7
0066-4804/11/$12.00 doi:10.1128/AAC.00325-11

Is *Neisseria gonorrhoeae* Initiating a Future Era of Untreatable Gonorrhea?: Detailed Characterization of the First Strain with High-Level Resistance to Ceftriaxone▽†

Makoto Ohnishi,[1] Daniel Golparian,[2] Ken Shimuta,[1] Takeshi Saika,[3] Shinji Hoshina,[4] Kazuhiro Iwasaku,[5] Shu-ichi Nakayama,[1] Jo Kitawaki,[5] and Magnus Unemo[2]*

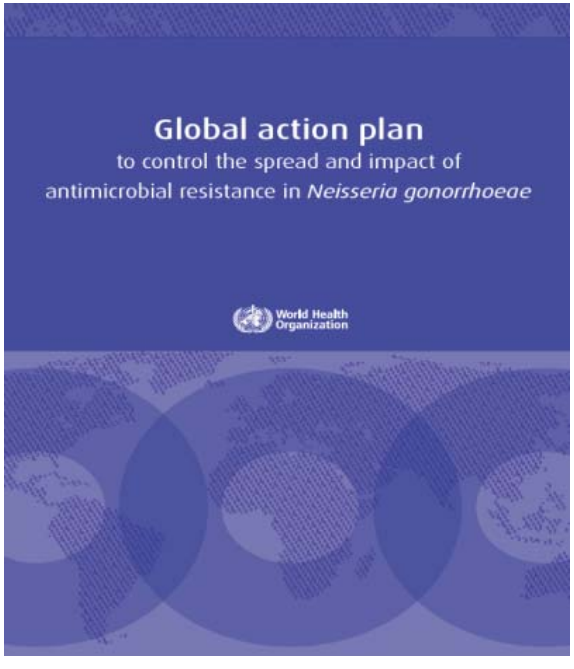


'Worse than AIDS' - sex 'superbug' discovered in Japan called disaster in waiting

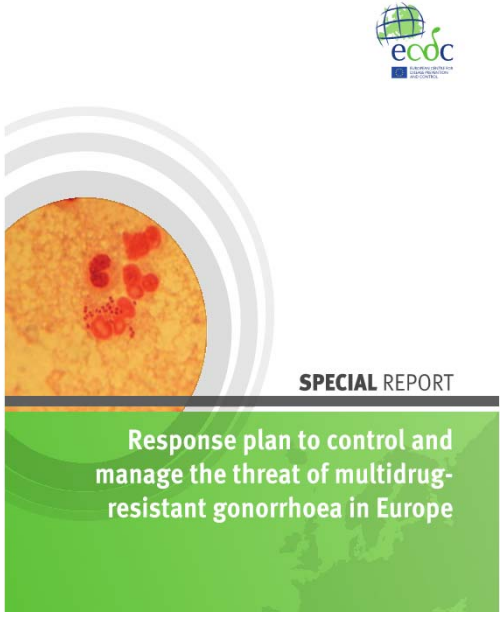
Published time: May 06, 2013 20:36
Edited time: May 08, 2013 09:41

Get sho

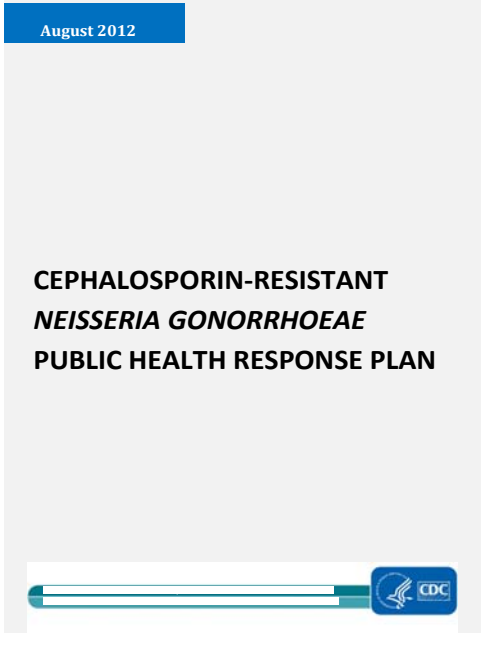
WHO 2012



ECDC for EU/EEA



CDC for USA



Future treatment of gonorrhoea?

~~1. Increased doses of ceftriaxone~~
~~Limited time period (based on the MICs of *N. gonorrhoeae* Superbugs, pharmacodynamics and resistance emergence)!~~

2. Dual antimicrobial therapy

Already introduced first-line (when susceptibility unknown)

i) Ceftriaxone 250 mg + Azithromycin 1 g (USA[1] +Canada)
ii) Ceftriaxone 500 mg + Azithromycin 1 g (UK[2] +Australia)
iii) Ceftriaxone 500 mg + Azithromycin 2 g (Europe)[3]

(Ceftriaxone 500 mg + Azithromycin 1.5 g [e.g. Germany])

- Empirical treatment eradicating also Chlamydia

[1]CDC. MMWR. 2010, 2012 and 2014
[2]Bignell & Fitzgerald. Int J STD AIDS. 2011
[3]Bignell & Unemo. Int J STD AIDS. 2013

Future treatment of gonorrhoea?

~~1. Increased doses of ceftriaxone~~

~~Limited time period (based on the MICs of *N. gonorrhoeae* Superbugs, pharmacodynamics and resistance emergence)!~~

2. Dual antimicrobial therapy

Effective for how long?

1. **Already resistance to both antimicrobials**
2. **Too expensive for wide use in less-resourced settings**
3. **First global treatment failure recently verified!**

[1]CDC. MMWR. 2010, 2012 and 2014

[2]Bignell & Fitzgerald. Int J STD AIDS. 2011

[3]Bignell & Unemo. Int J STD AIDS. 2013

FAILURE OF DUAL ANTIMICROBIAL THERAPY IN TREATMENT OF GONORRHEA

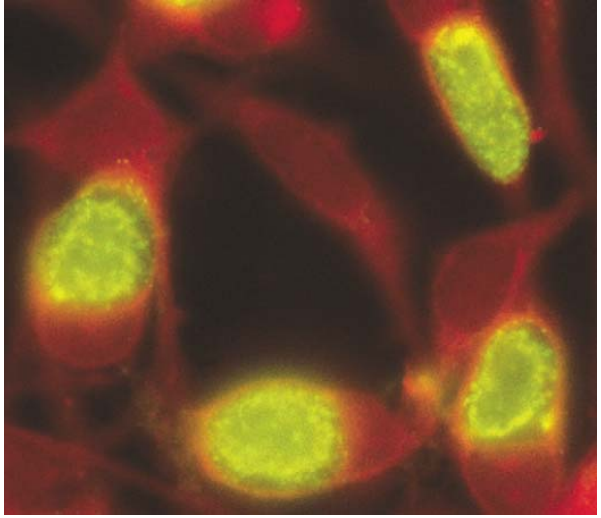
Fifer H, Golparian D, Natarajan U, Alexander S, Hughes G, Jones L, and Unemo M

New Engl J Med. 2016

- **Heterosexual male with pharyngeal gonorrhoea in UK, but infected in Japan**
- **XDR strain: Resistant to ceftriaxone, cefixime, azithromycin, penicillins, tetracyclines, and fluoroquinolones, but susceptible to spectinomycin**

Women's Soccer World Cup 2015: Official Swedish slogan

"The clap" = nickname of gonorrhoea since the 16th century



***Chlamydia trachomatis*:**

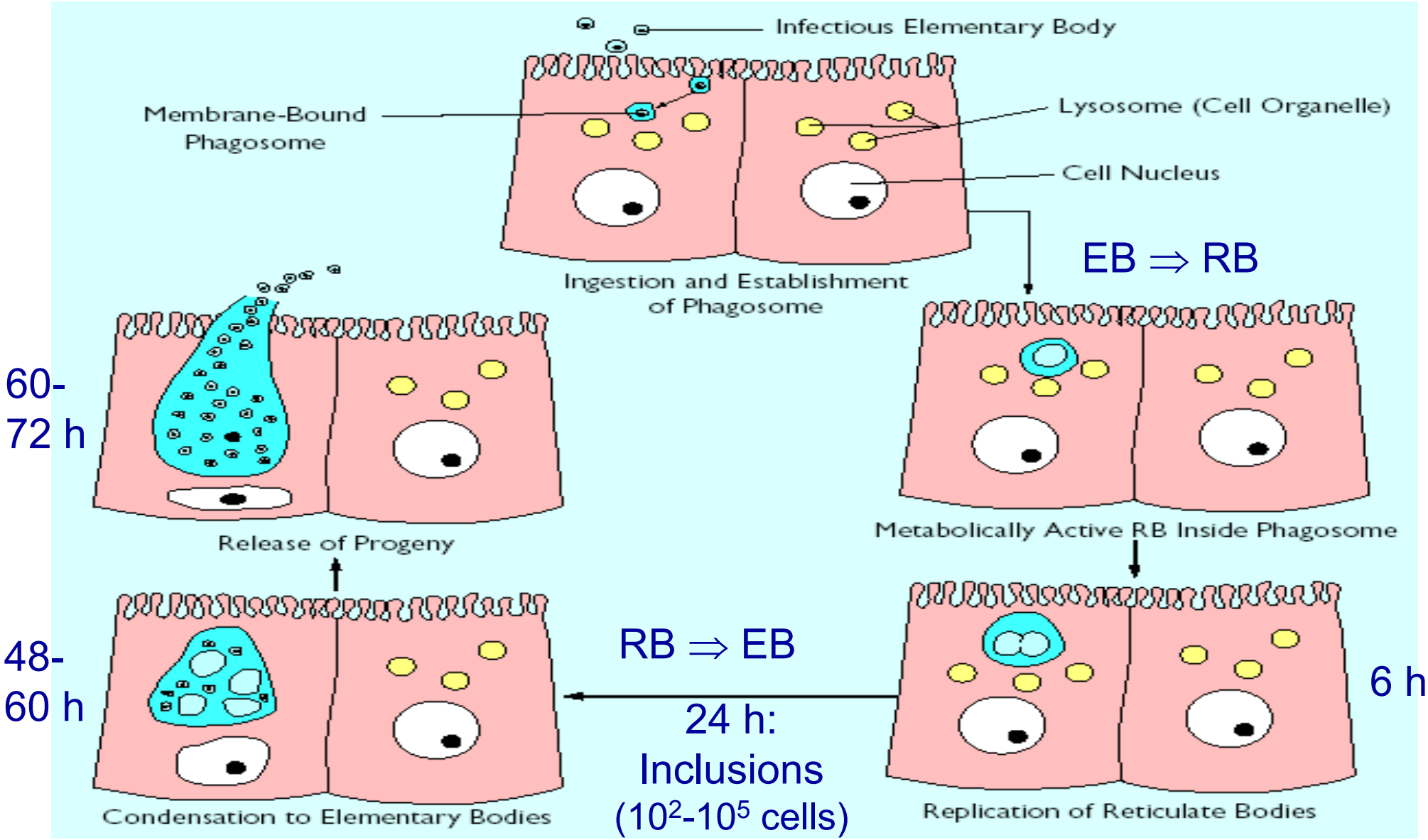
- Earliest reliable description Trachoma, in Ebers papyrus (1553-1550 BC) – isolated in yolk sac in 1950s (Tang, et al. *Chin Med J*. 1957)

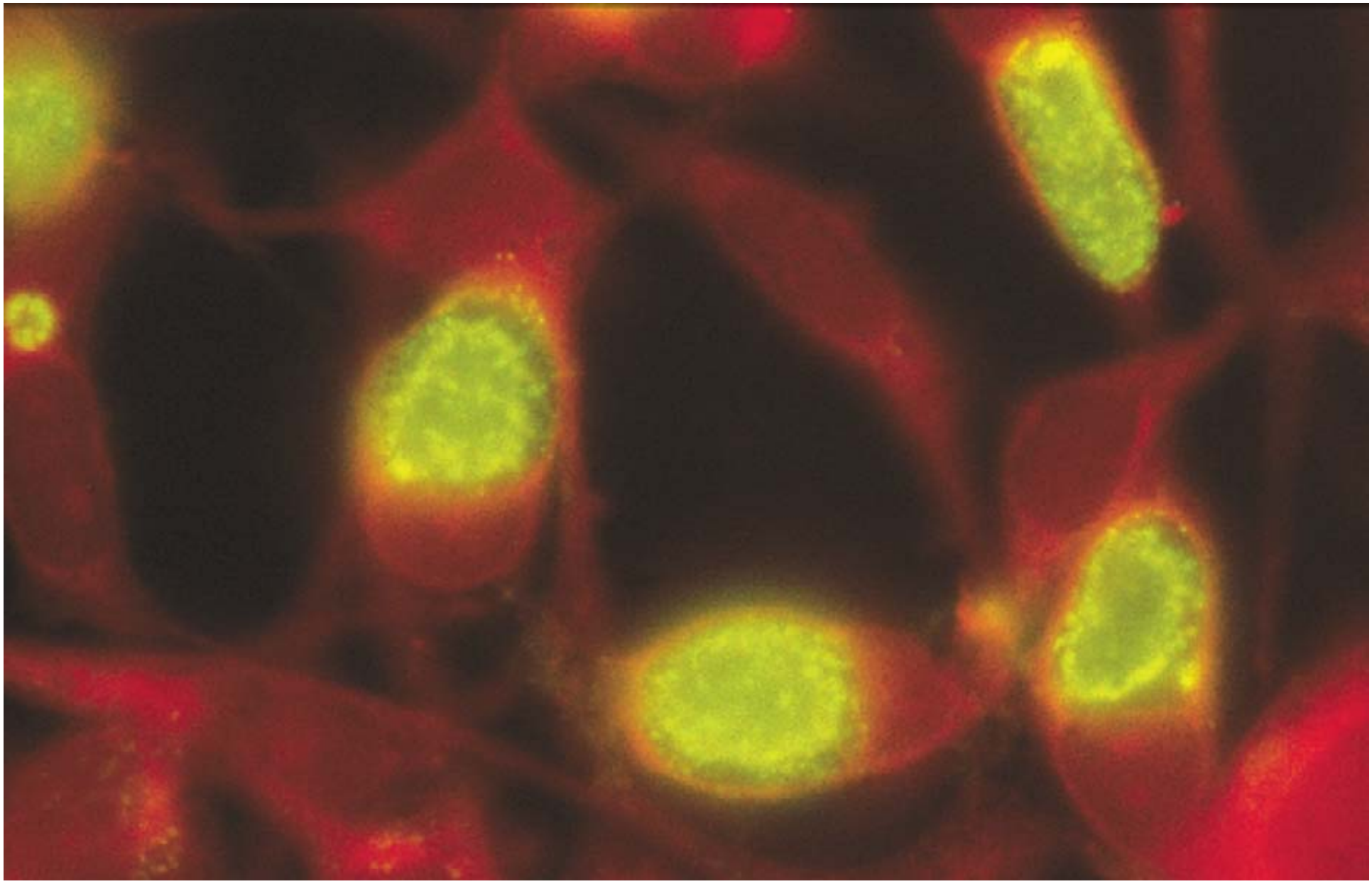
- Highly conserved small genome (~1.04 Mbp; Stephens, et al. *Science*. 1998; Jeffrey, et al. *Infect Immun*; Unemo, et al. *Microbiology*. 2010; Harris, et al. *Nature Gen*. 2011)

- Highly adapted to its parasitic lifestyle

C. trachomatis - life cycle

Obligate intracelllular human pathogenic ”parasite” (lacks ATP-synthesis!)



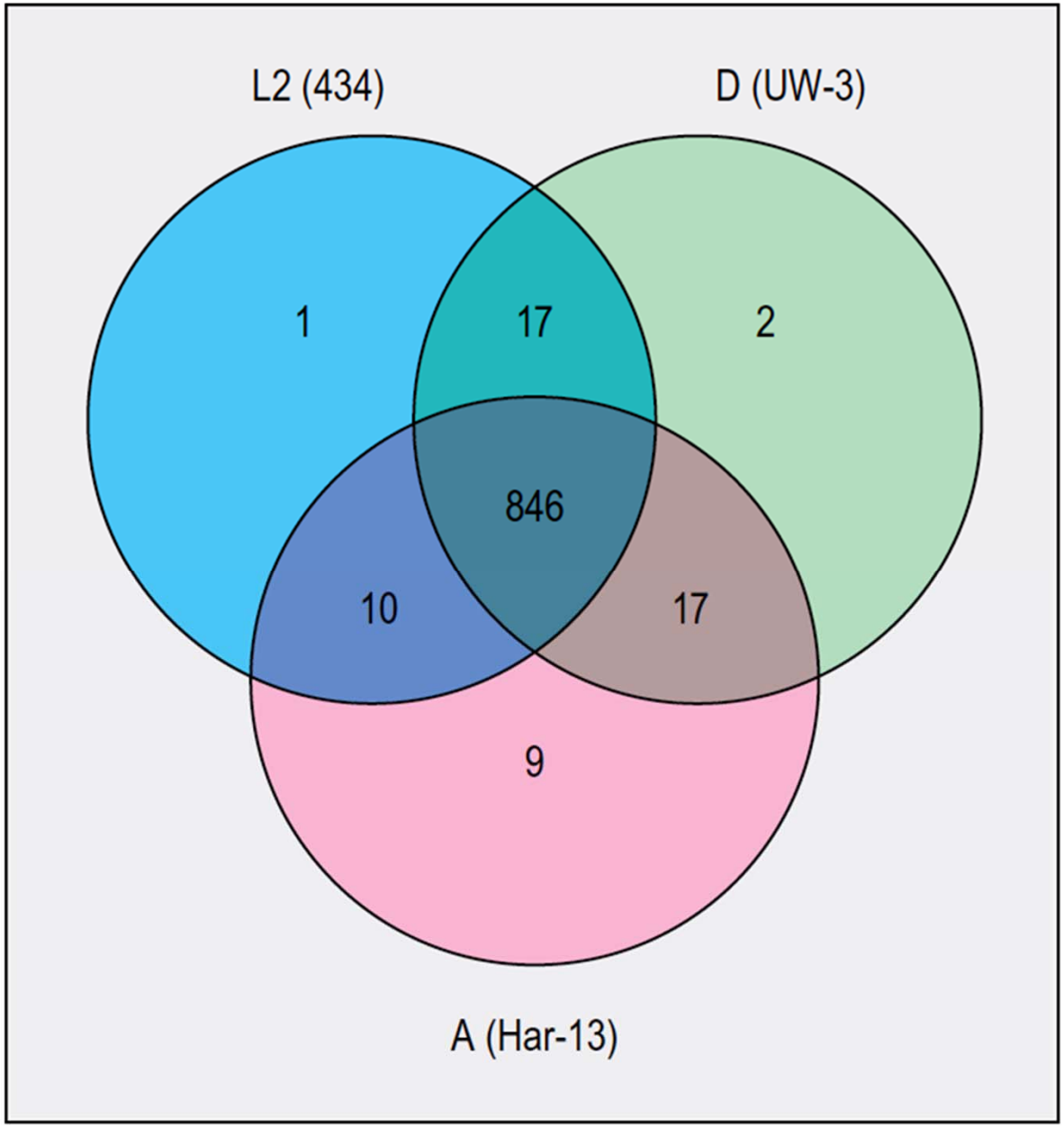


Characteristics and infections associated with different serovars (two biovars, three "pathovariants") of *C. trachomatis*

Serovar	Characteristics	Tissue tropism/ Biovar	Infection
A–C (Incl. Ba)	Non-invasive, Tryptophan synthase –	Epithelial cells/ Trachoma	Endemic blinding trachoma
D–K (Incl. Da, Ia, Ja)	Non-invasive, Tryptophan synthase +	Epithelial cells/ Trachoma	Urogenital, conjunctivitis, neonatal pneumonia
L1, L2, L3 (Incl. L2a)	Invasive, Increased growth	Lymphatic cells/ LGV	LGV

(Unemo, Papp. *Atlas of STDs and AIDS*. 2010)

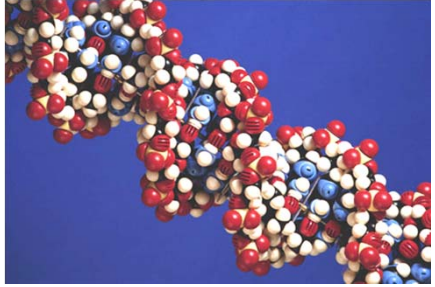
C. trachomatis core genome (three pathovariants) – 836-846 genes (>>90% of genes)?



Tissue tropism and disease presentation:
minor differences, including some gene loss/gain, pseudogenes, minor mutations, and altered levels of gene expression?

(Thomson, et al. *Genome Research*. 2008; Joseph, et al. *Biology Direct*. 2011)

Diagnosis of genital chlamydia



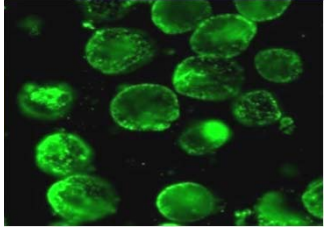
- Clinical (including microscopy): only suggestive

Laboratory diagnostics crucial!

- **Nucleic acid amplification tests (NAATs)!**
- Culture in viable cell lines – fluorescence labelled antibodies [MOMP/LPS])
- Enhanced enzyme immunoassay (EIA) tests – Antigen (Ag; MOMP/LPS) detection!
- Direct fluorescent antibody (DFA) tests?– Ag detection!
- EIA – Ag detection!
- (Rapid tests (”Point-of-care”) [MOMP/LPS]?)
- Serology: Not useful for uncomplicated infection!

Decreased sensitivity

Lanjouw et al. IJSTDA. 2015; van der Pol & Unemo. WHO Manual. 2013



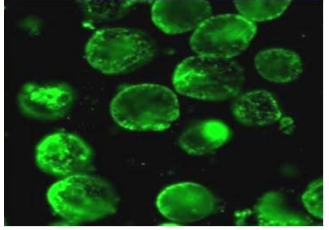
International commercial CT(/NG) NAATs

(GeneXpert, Biorad Dx)

Genetic target	Test/system	Manufacturer
Cryptic plasmid	Cobas Amplicor	Roche Diagnostics
Cryptic plasmid (+*ompA*)	Cobas TaqMan48, 4800	Roche Diagnostics
Cryptic plasmid (+plasmid)	*m2000*	Abbott Laboratories
Cryptic plasmid	BD ProbeTec/Viper	Becton Dickinson
23S rRNA (16S confirm.)	Aptima Combo 2	Gen-Probe
ompA (*omp1*) gene	artus	Qiagen
ompA gene + cryptic plasmid	artus Plus	Qiagen

VERSANT CT/GC DNA 1.0, Siemens

Unemo, Papp. Atlas of STDs and AIDS. 2010



International commercial CT(/NG) NAATs

(GeneXpert, Biorad Dx)

- **FDA approved systems: Evidently an excellent sensitivity and specificity, however, too expensive for many less-resourced settings!**

- **Confirmatory testing and pooling is NOT recommended!**

Cry			
Cryp			
23S			
	ompA gene + cryptic plasmid	artus Plus	Qiagen

VERSANT CT/GC DNA 1.0, Siemens

Unemo, Papp. Atlas of STDs and AIDS. 2010

Choice of specimen

- Men: first-void urine
- Women: (self-collected) vaginal swabs

(female urine has a suboptimal sensitivity)

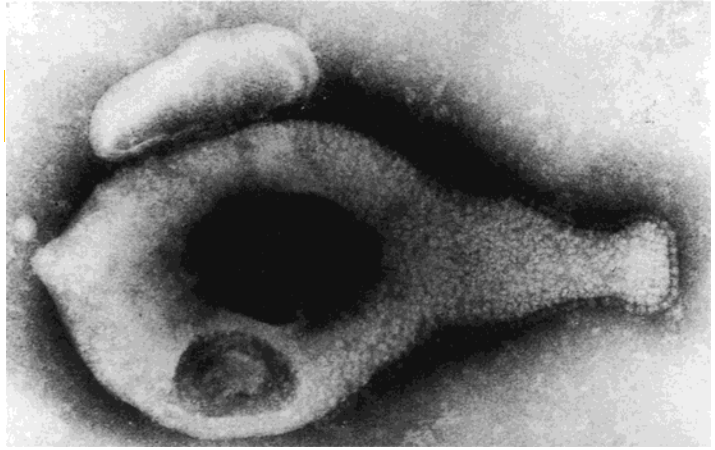
Other specimens:

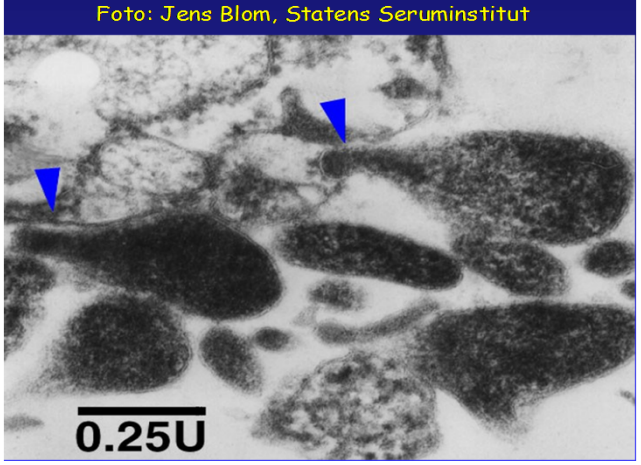
- Pharyngeal: NAAT recommended!
- Rectal: NAAT recommended; consider confirmation with another NAAT and LGV typing of positive specimens!
- Conjunctival: NAAT recommended!
- Semen: NOT recommended to test!

Lanjouw et al. IJSTDA. 2010; Unemo & Papp. Atlas of STD and AIDS. 2010; van der Pol & Unemo. WHO Manual. 2013

Lymphogranuloma venereum (LGV)

- *Chlamydia trachomatis* serovars L1, L2 or L3
- Disease symptoms include genital ulceration, inguinal lymphadenopathy and an anorectal stage
- Historically: tropical disease endemic in parts of Africa, Asia, and Latin America but rare in Europe (reports of MSM back to 1940s)
- Since 2003, outbreak/endemic? of LGV proctitis and proctocolitis in western Europe and North America in men who have sex with men (MSM)
- All commercial *C. trachomatis* NAATs detect LGV, however, none distinguish LGV from other genovars $\Rightarrow$ in house assays (PCRs)





Mycoplasma genitalium

Class Mollicutes – mycoplasmas

- Free-living small bacteria (0.3 - 0.5 µm)

- Lack the rigid cell wall found in other bacteria

Commonly found in the human urogenital tract:

- *M. genitalium*: 580 kbp
- *M. hominis*: 665 kbp
- *Ureaplasma urealyticum* (formerly *U. urealyticum*, biovar 2): 840-950 kbp
- *U. parvum* (formerly *U. urealyticum*, biovar 1): 751 kbp

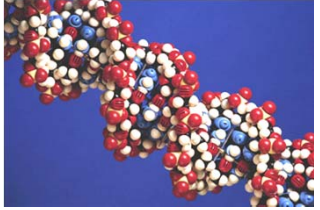
Jensen and Unemo. WHO Manual. 2013

M. genitalium only myco-/ureaplasma clearly associated with urethritis and cervicitis!

Species	Disease associations[a]						
	Urethritis	Cervicitis	Bacterial vaginosis	Endometritis and/or PID	Preterm birth	Infertility (Women)	HIV transmission
M. genitalium	++++	+++	–	+++	+/–	+	+
M. hominis	–	–	++++	+/–	+/–	–	ND
Ureaplasmas (undifferentiated)	+/–	–	+++	ND	+	+/–	ND
U. urealyticum	+	ND	ND	ND	ND	ND	ND
U. parvum	–	ND	ND	ND	ND	ND	ND

⇒ Focus on detection and treatment of *M. genitalium* infections!

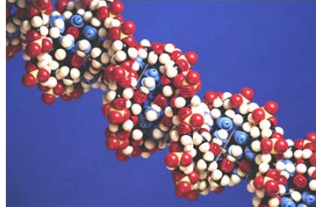
Jensen and Unemo. WHO Manual. 2013



Diagnosis of *M. genitalium* infection

- **Culture: low sensitive, extremely slow** and technically challenging (Jensen, et al. JCM. 1996)
- **No serological assays, antigen detection assays or POC tests** for appropriate diagnosis
- **NAATs only effective and practical methods!**
  - No strictly evaluated, commercially available assays – validate and quality assure in your setting!

Jensen and Unemo. WHO Manual. 2013



Diagnosis of *M. genitalium* infection

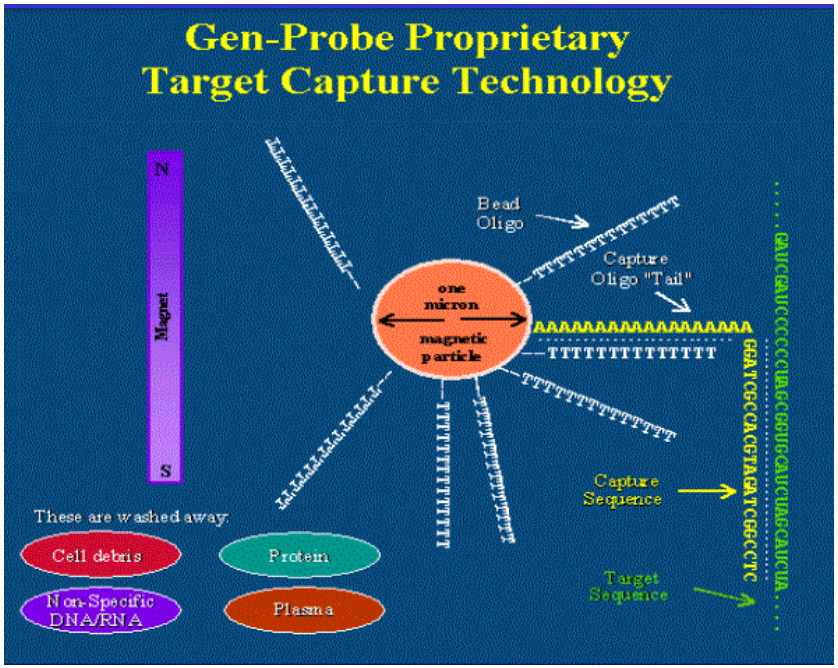
- Culture: low sensitive, extremely slow and technically

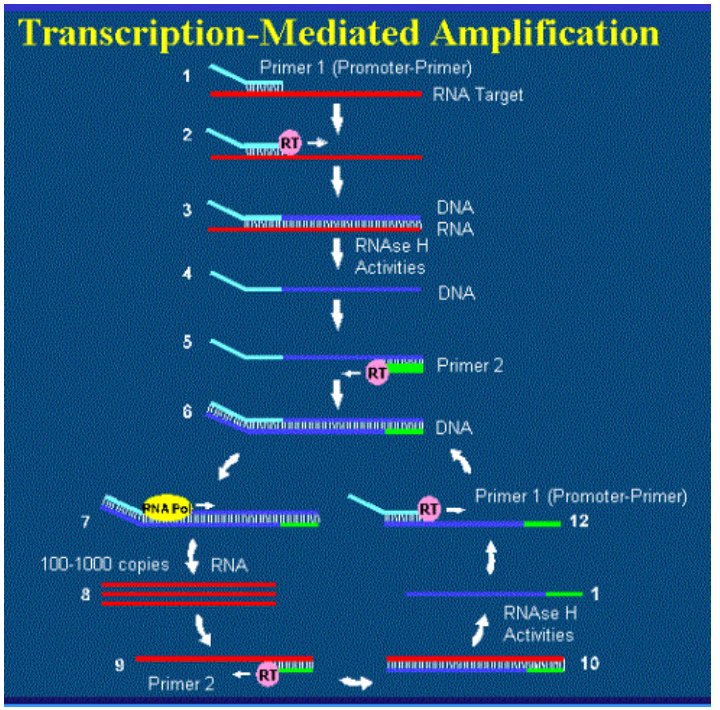
"NAATs" – performance characteristics sufficient (sensitivity, specificity, negative and positive predictive value)?

assays – validate and quality assure in your setting!

Jensen and Unemo. WHO Manual. 2013

- Sample preparation and assay sensitivity should be optimal for *M. genitalium* testing, as this pathogen is present in 100-fold lower concentrations than *C. trachomatis*. Most multiplex assays have a slightly lower sensitivity than assays with only one target.
- NAATs targeting the *M. genitalium* MgPa gene should be carefully designed to avoid variable regions of the gene. (*+16S rRNA* gene homology with *M. pneumoniae*)





APTIMA *Mycoplasma genitalium* Assay (Hologic), now CE-IVD
(16S rRNA; Target capture, TMA, and HPA)

Clinical and analytical evaluation of the new APTIMA *M. genitalium* assay and AMR in *M. genitalium* in Scandinavia in 2016

Principal investigator:

Magnus Unemo

Co-investigators:

Jørgen Skov Jensen, Statens Serum Institut, Copenhagen, Denmark.

Anne Olaug Olsen and Harald Moi, Olafia Clinic, Oslo, Norway.

Henrik Westh, Hvidovre University Hospital, Copenhagen, Denmark.

Hologic main contact persons:

Thomas Schelhorn and Sven Schaffer, Scientific Affairs & Clinical Dx EU, Marketing, Hologic, Wiesbaden, Germany

Other with main involvement:

Marit Hansen, My Falk, Christian Steczko-Nilsson, Kirsten Salado-Rasmussen, Carita Stenudd, Lotte Bjerregaard…….

Cure rate for azithromycin 1 g decreasing (resistance rapidly selected!)!

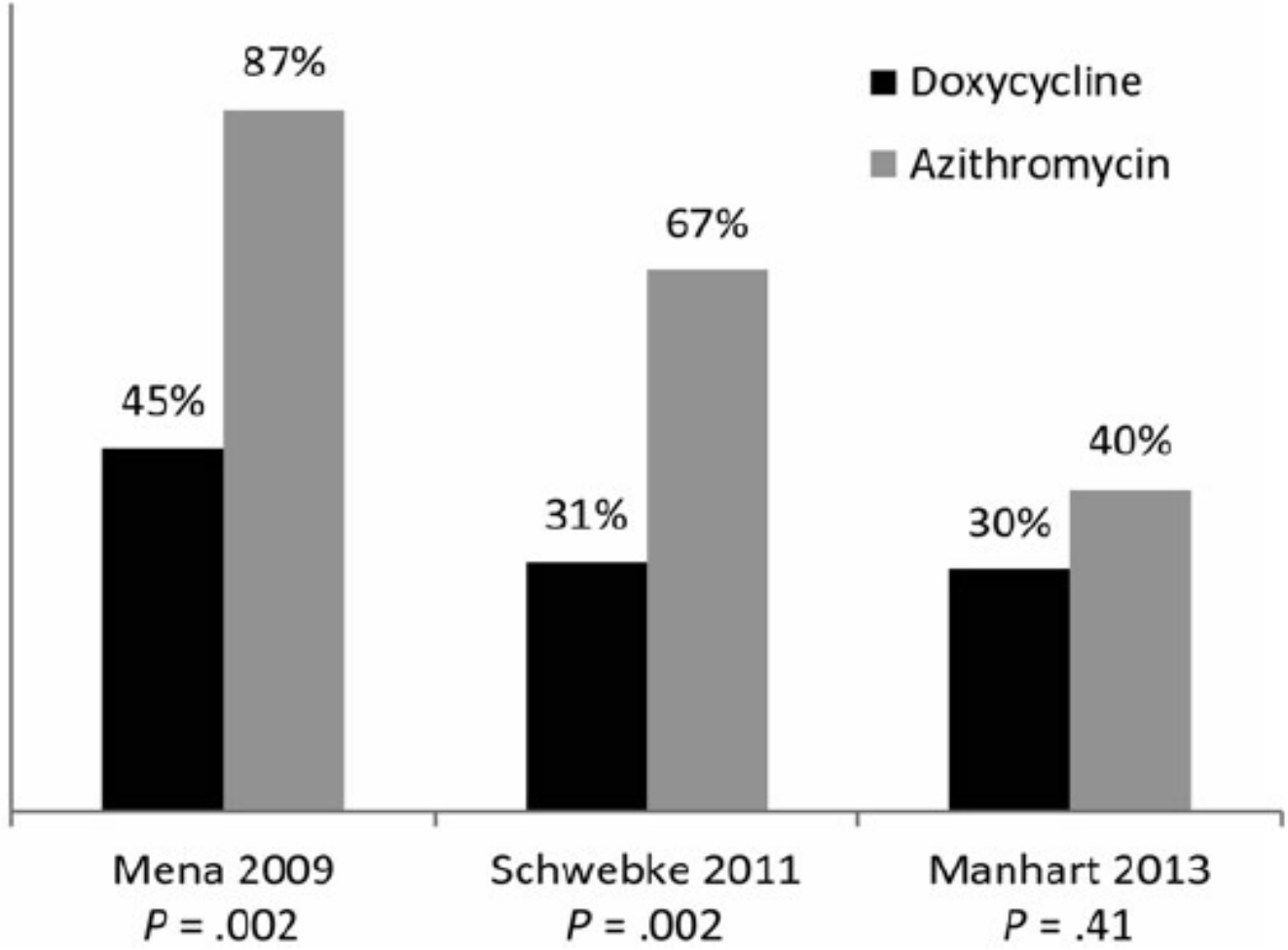
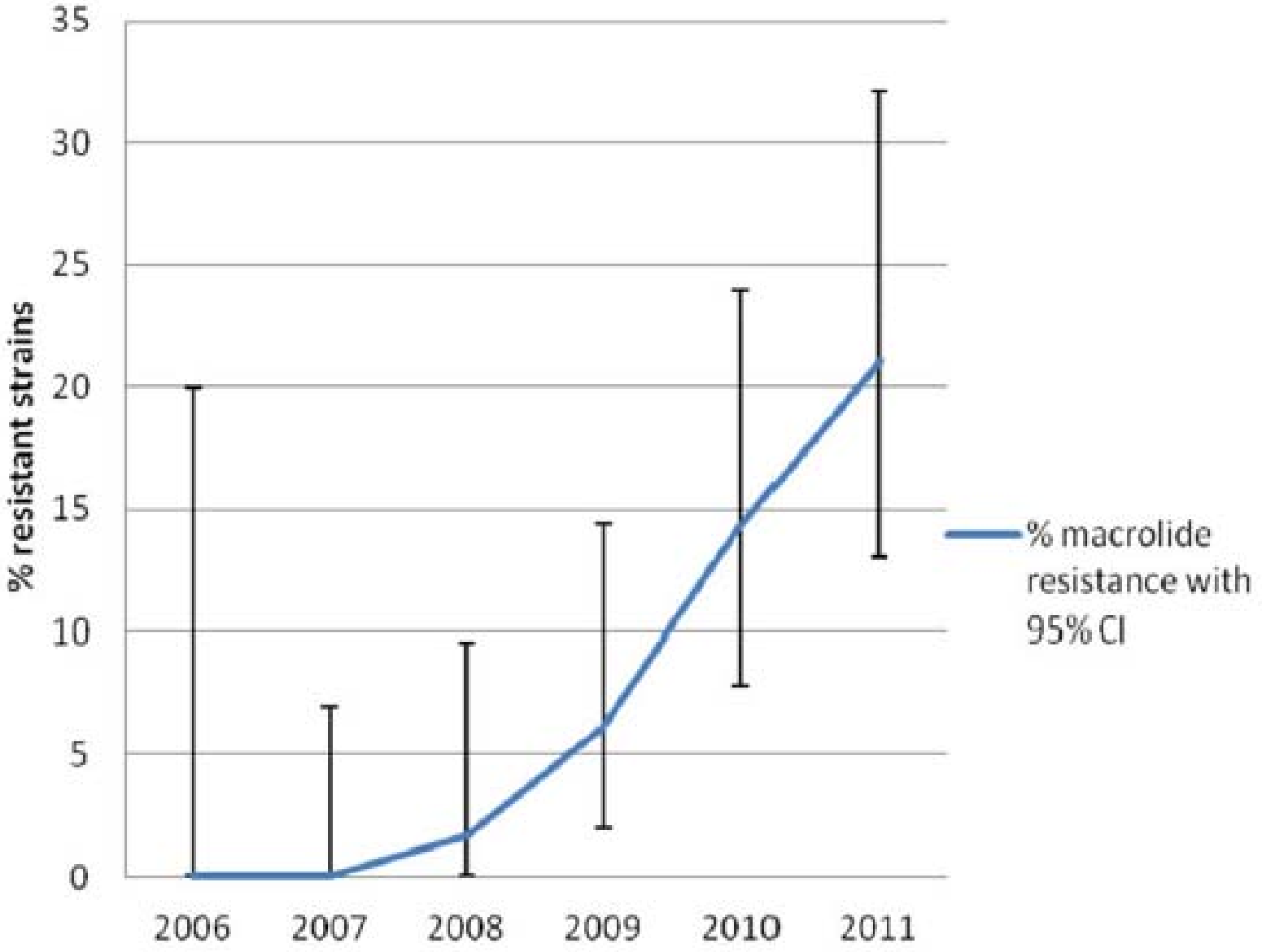


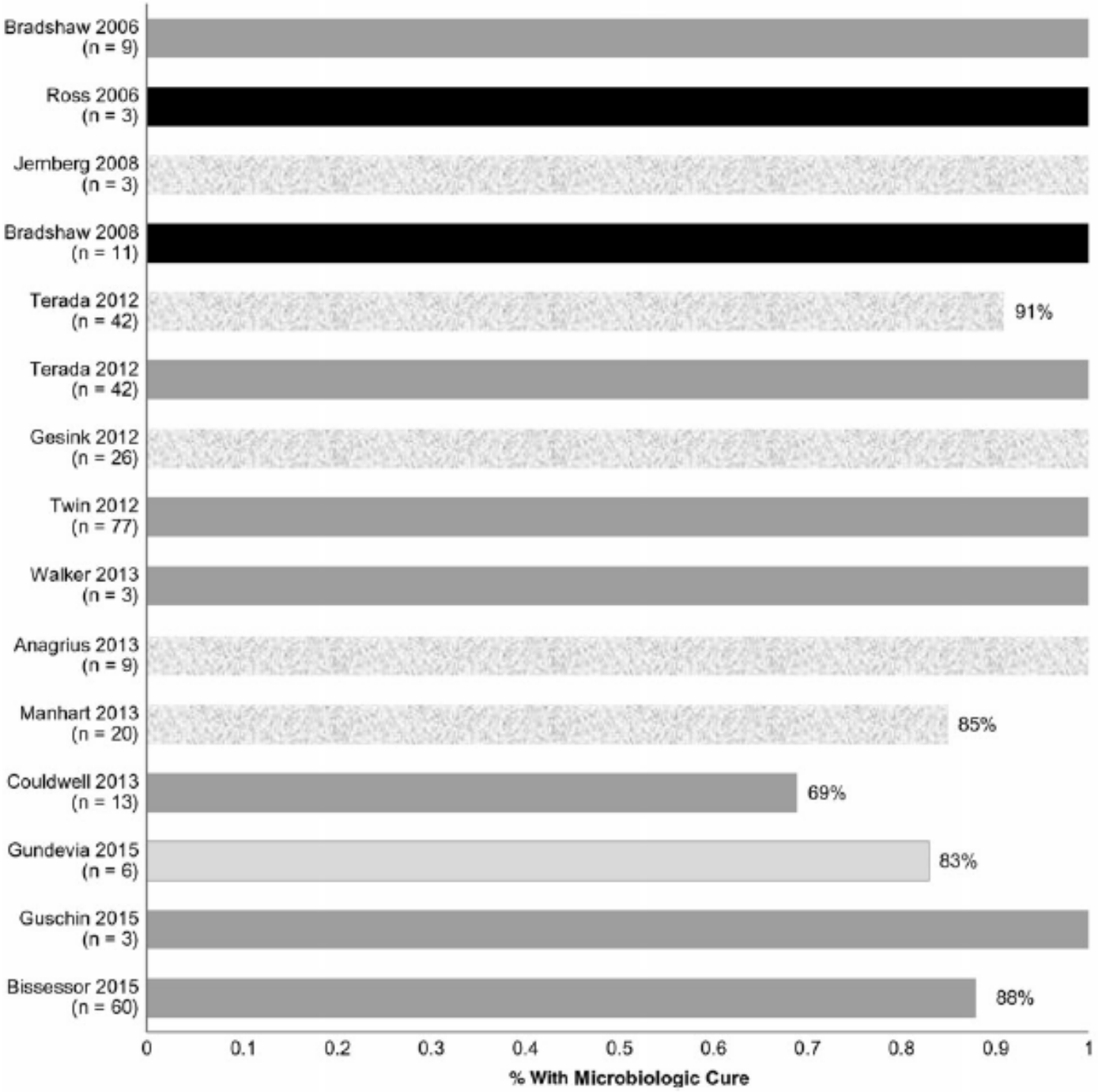
Figure 1. Randomized controlled trials comparing the efficacy of doxycycline (100 mg bid x 7 days) vs azithromycin (1 g single dose) for the treatment of *Mycoplasma genitalium* infections.

Manhart et al. CID. 2015

Increase in macrolide resistant *M. genitalium* (23S rRNA gene mutations) in Falun, Sweden



Anagrius et al. PLOS One. 2013



Moxifloxacin

- 14 days
- 10 days
- 7 days
- Not specified

Resistance more prevalent in Asia-Pacific!

Figure 2. Summary of treatment outcomes after moxifloxacin (400 mg ×7, 10, or 14 days), n = total number given moxifloxacin.

Manhart et al. CID. 2015

Recommended third line treatment for persistent *M. genitalium* infection after azithromycin and moxifloxacin *[III;B]*

- Doxycycline 100 mg two times daily for 14 days can be tried and will eradicate *M. genitalium* from approximately 30% of the patients, but the patient must be informed about the poor eradication rate and accept to comply with advice regarding sexual abstinence or condom use.
- Pristinamycin 1g four times daily for 10 days (oral). The patient should be informed about the need to comply strictly with the dosage scheme.

=streptogramin: registered only in France, Algeria, Morocco and Tunisia (special license needed in other countries)

Future options?: solithromycin, sitafloxacin, ETX0914, Lefamulin/BC-3781 (pleuromutilin), dual antimicrobial therapy?