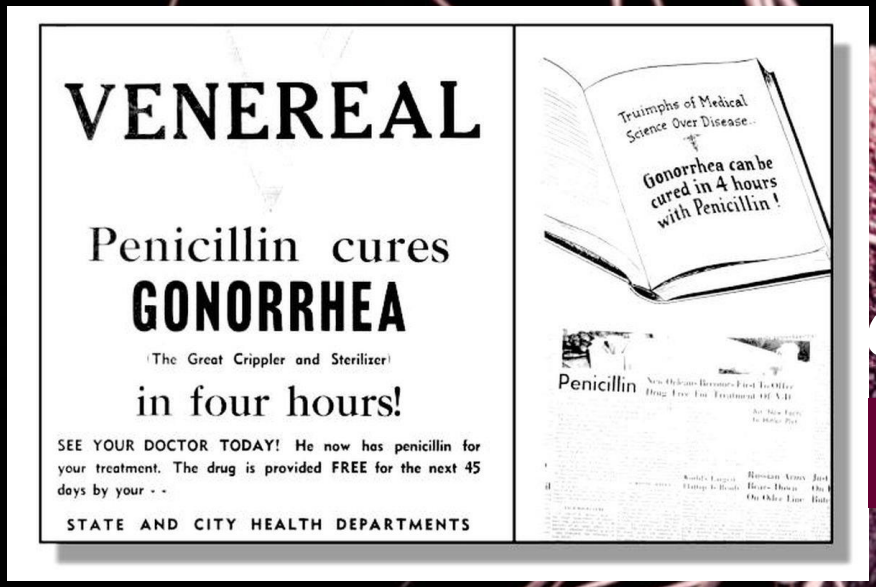
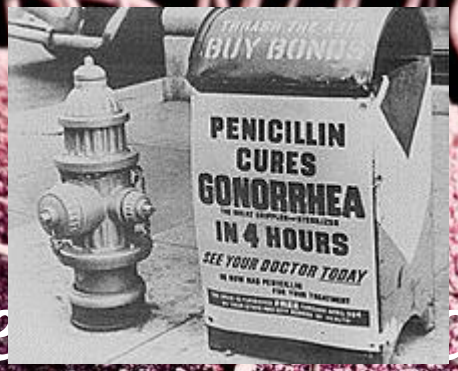
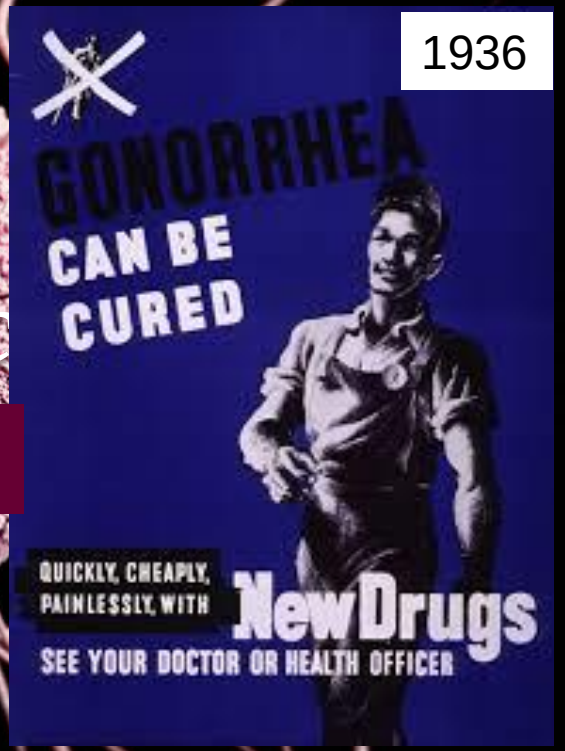
Neisseria gonorrhoeae







20th century

David Speers
Sir Charles Gairdner Hospital
PathWest Laboratory Medicine
University of WA

21st century

Gonorrhea Is Becoming Resistant to the Last Drugs That Treat It

TIME HEALTH

Antibiotic Resistance Leads To 'Super Gonorrhea'; Treatment May Soon Be Ineffective

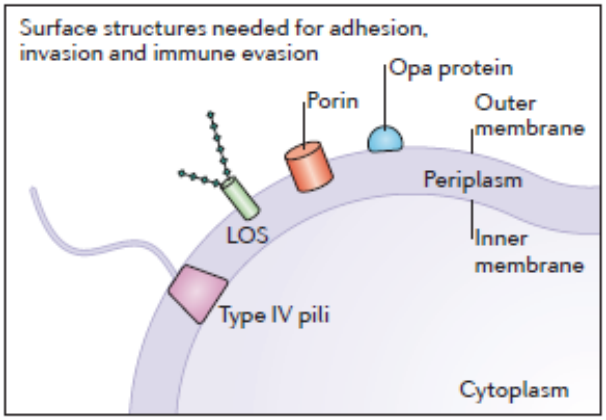
The Grapevine

Gonococci are about transmission and mucosal colonisation

- *N. meningitidis* (NM) and *N. gonorrhoeae* (NG) evolved from common ancestor in oropharynx
  - obligate human pathogens
  - NG gained genetic machinery for anaerobic growth to survive in urogenital tract
  - lost capsule expression
  - Changed lipopolysaccharide (LPS) to lipooligosaccharide (LOS)

- Transmission
  - NM can withstand dehydration
    - Respiratory droplet spread
  - NG non-viable if dehydrated or temperature change
    - Intimate contact for spread
  - ♀ to ♂: vaginal bacteria modify LOS to enable binding to male urethra
  - ♂ transmission: NG adheres to sperm transmitting high numbers through ejaculate

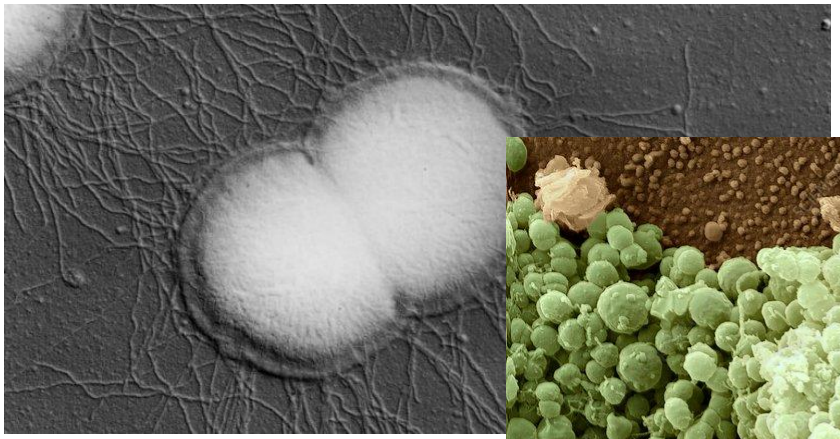
- Colonisation is about urethral cell adherence
  - Mediated by pili
  - Opa
  - LOS
  - major outer membrane protein (PorB)



doi: 10.1038/nrmicro.2017.169

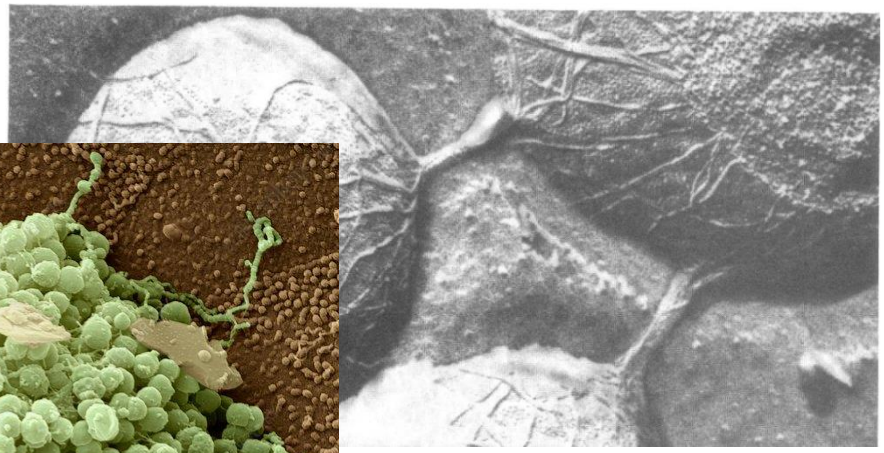
Gonococcal adherence and colonisation

Gonococci adhere, crawl, and communicate via pili



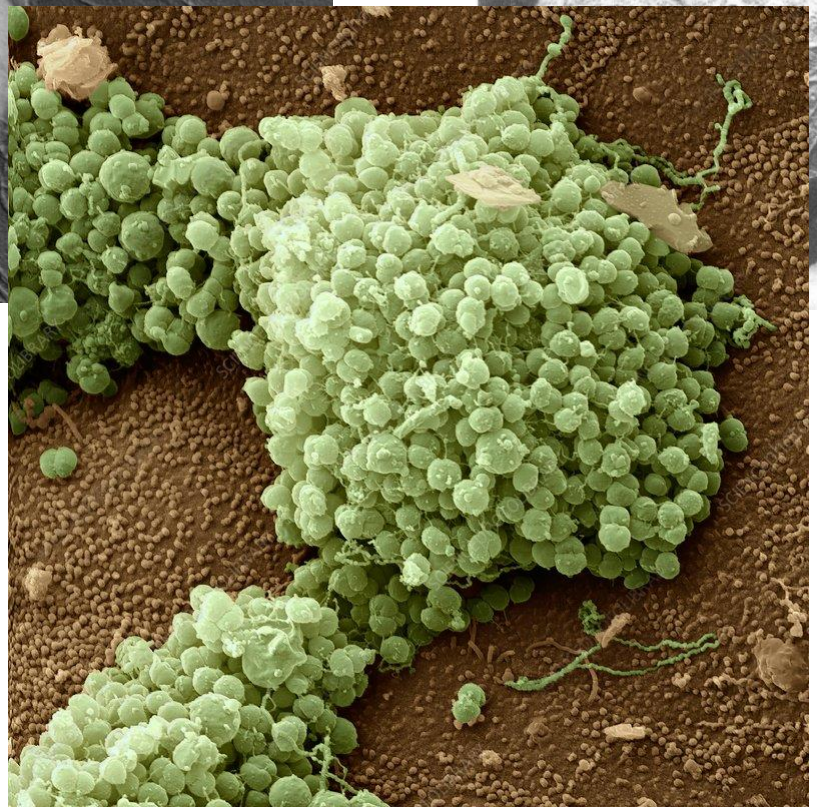
Bio.libretexts.org

Gonococci take up DNA and via pili



www.semanticscholar.org

Biofilm protects bacteria from antibiotics



Sciencephoto.com.org

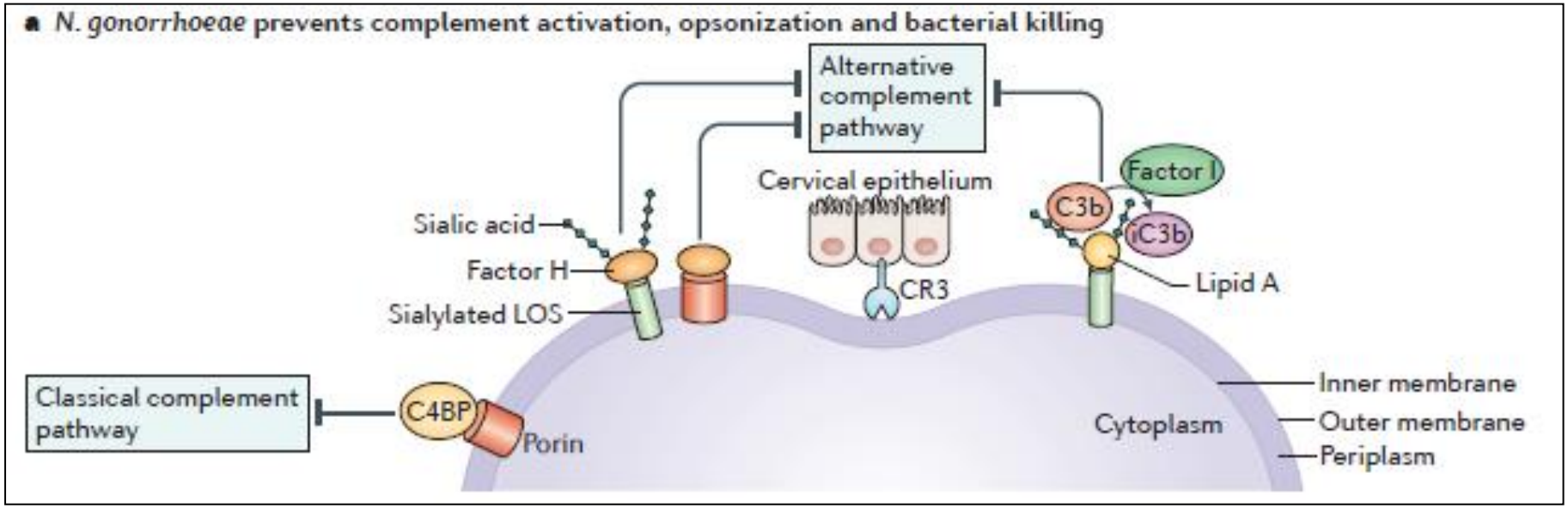
Gonococci have many survival factors to evade the innate immune system

- Avoid immune destruction to survive and replicate within neutrophils
  - Negative surface charge to block phagocytosis
  - Porins block phagolysosome fusion
  - Detoxifies reactive O_2 species from neutrophils
  - Efflux pump (MtrCDE) exports antimicrobial peptides from neutrophil degranulation

- Damage at mucosal sites is due to neutrophil response of host as NG has no potent exotoxin
  - Neutrophils produce reactive O_2 species, enzymes and antimicrobial peptides
  - The inflammatory response is the same for men and women but the difference in symptoms in women c.f. men is due to anatomical differences
  - Immune cell influx and inflammation causes symptoms
    - purulent exudate visible in men c.f. cervical discharge
    - Painful urination in men but not women

Gonococci evade the adaptive immune system

- Biofilm formation
- Antigenic variation of pili and Opa
- Proteins to block bactericidal secretory IgA antibodies
- Inactivates neutrophils and macrophages blocking immune cell recruitment
- Evades complement:



doi: 10.1038/nrmicro.2017.169

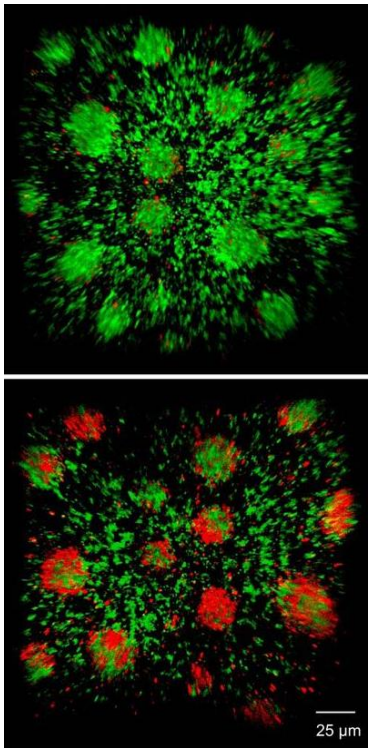
Immune evasion is achieved by DNA recombination (sexual behaviour)

- Genetic change more frequent in NG through recombination than by mutation
  - Feature of obligate human pathogenic bacteria
  - Unregulated recombination in NG is a powerful mechanism for antigenic variation

- Recombination was primarily a mechanism of DNA repair
  - Enables repair from oxidative damage from phagocytic cells
  - NG rich (2,000 copies) in DNA uptake sequences (DUS)

- NG is promiscuous recombining DNA with other species
  - spreads other advantageous genes when selective pressure
    - antimicrobial resistance genes
      - *penA* gene (ceftriaxone resistance) from commensal *Neisseria*
  - Pharynx may be the site for this
    - a partially protected site from antibiotics

Gonococci can take up free DNA - transformation

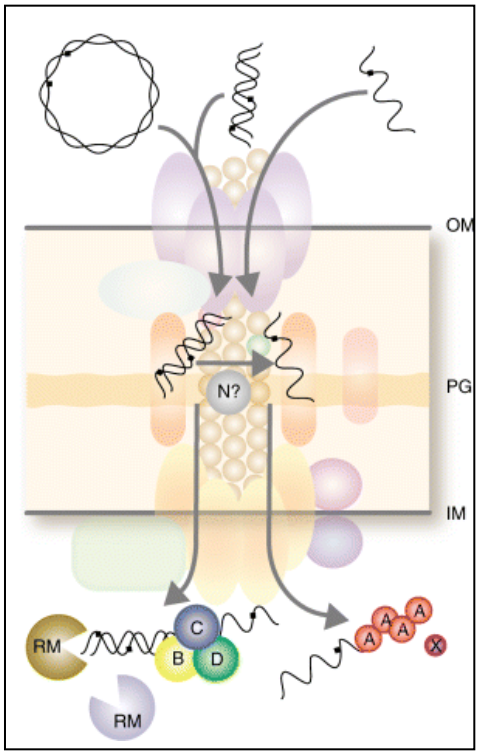


24 hr

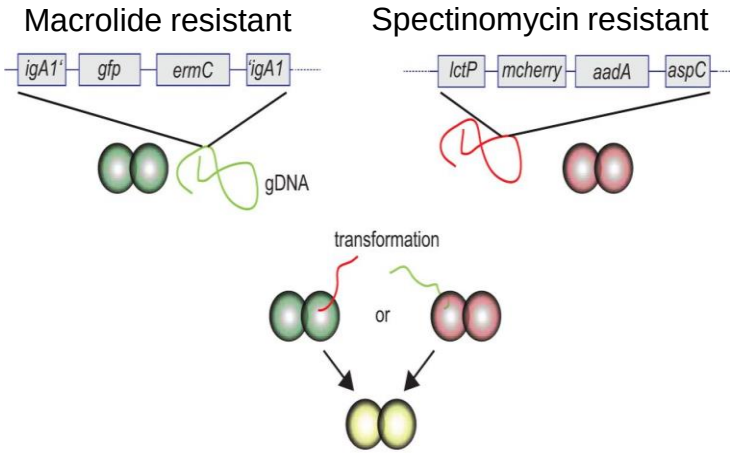
46 hr

confocal microscopy

Green: gonococcal bacteria
Red: free DNA



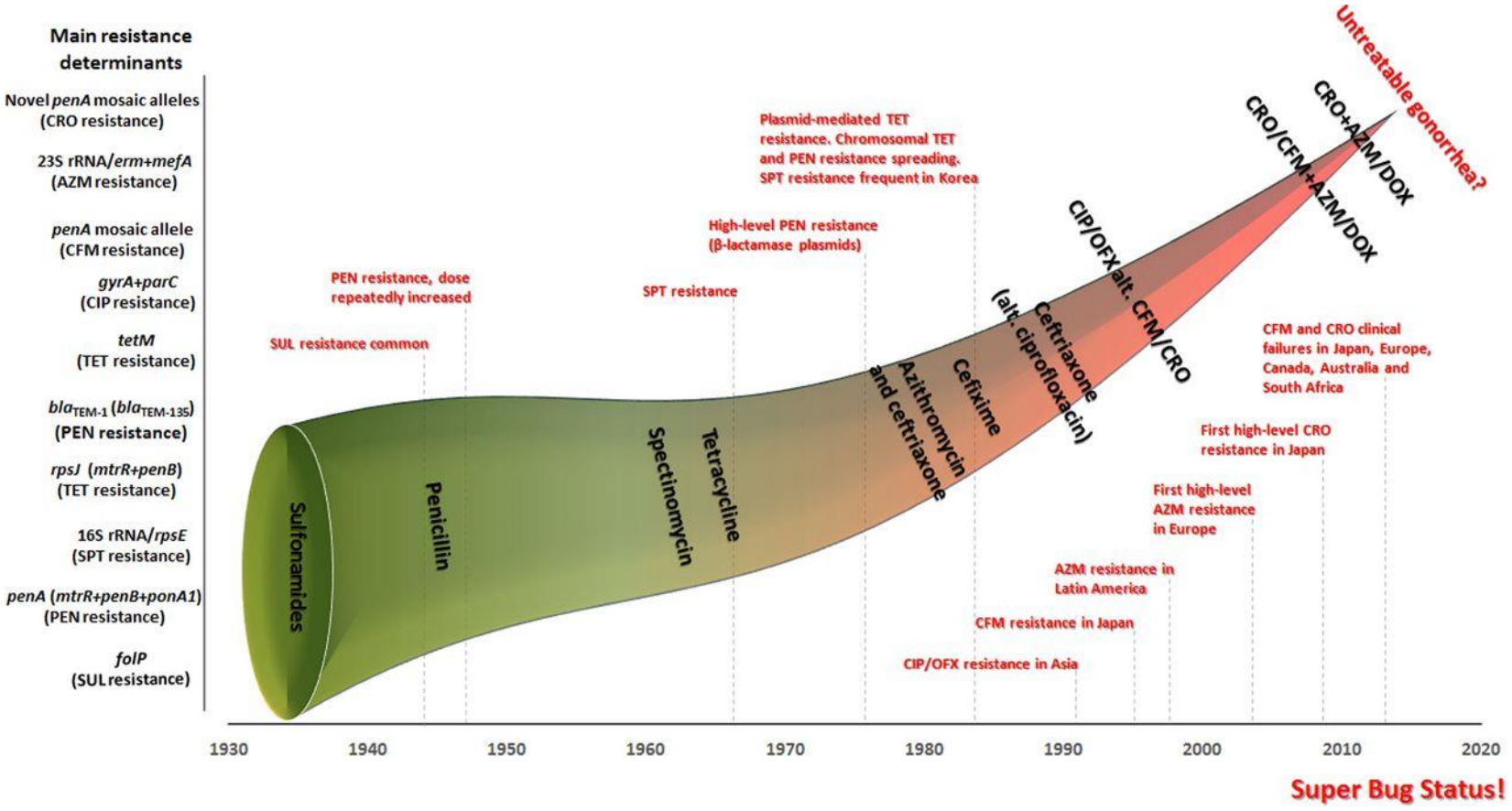
Hamilton and Dillard. Mol Microbiol Dec 2005



Macrolide and spectinomycin resistant

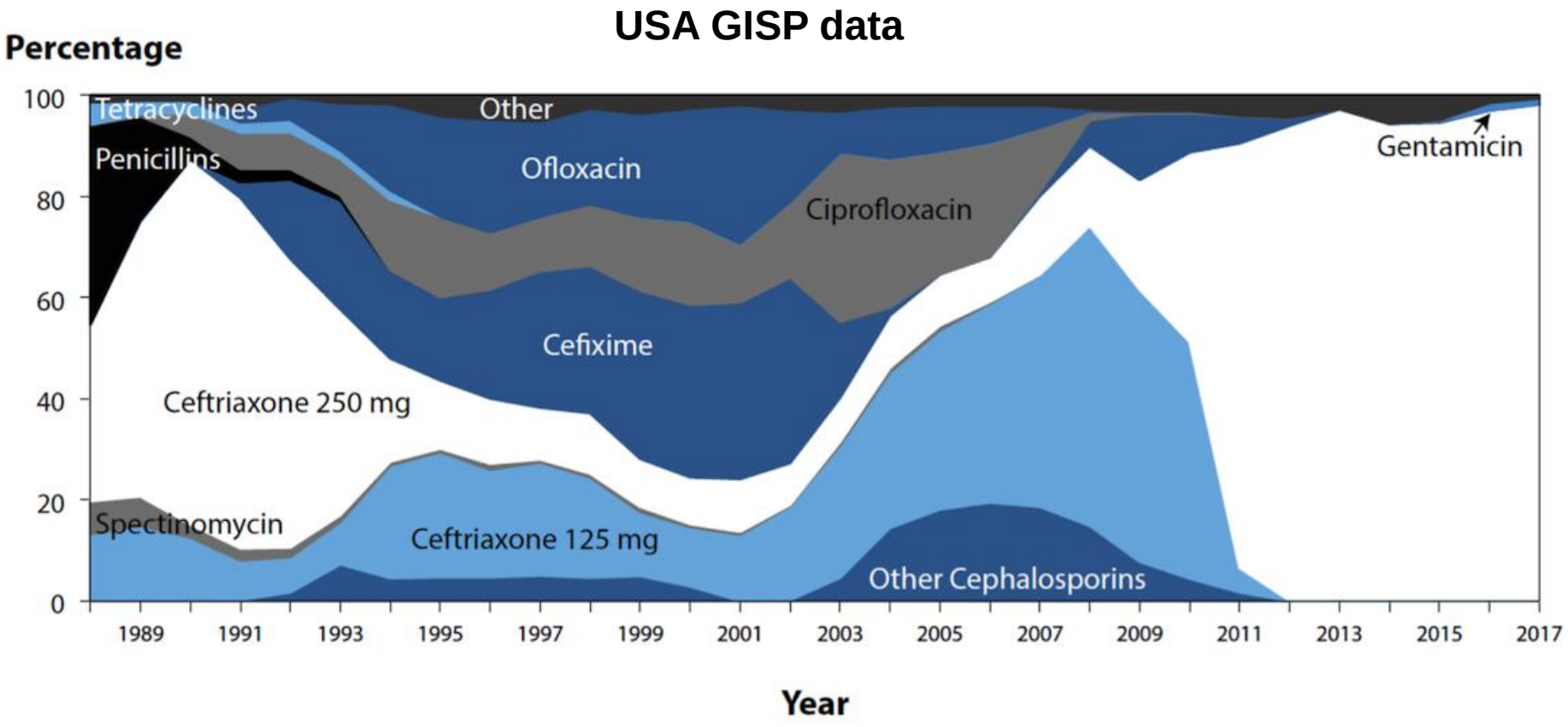
Kouzel et al J Bact 2015,197:2422

Evolution of resistance in *Neisseria gonorrhoeae*

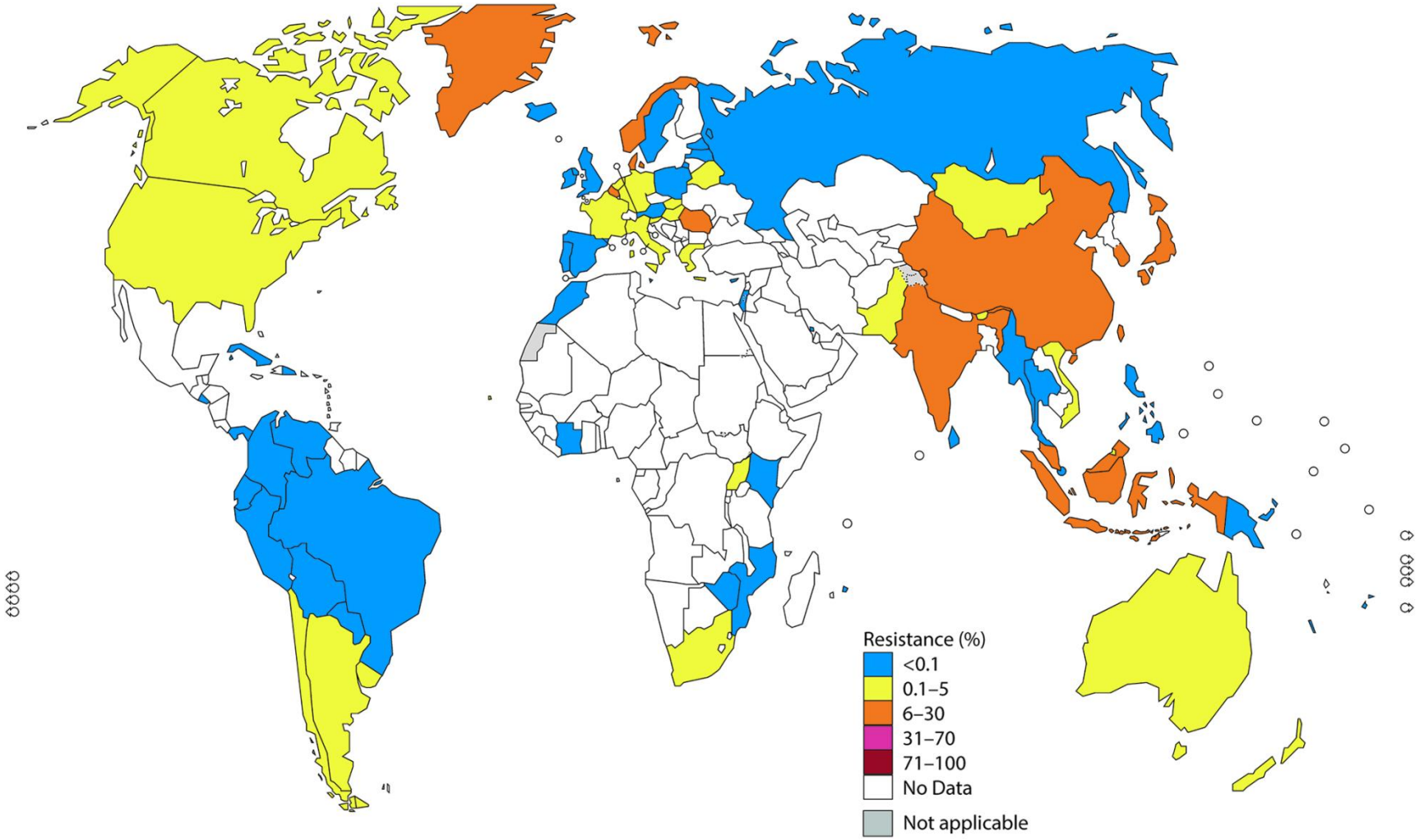


Magnus Unemo, and William M. Shafer Clin. Microbiol. Rev. 2014; doi:10.1128/CMR.00010-14

Ceftriaxone entered widespread use in 1986

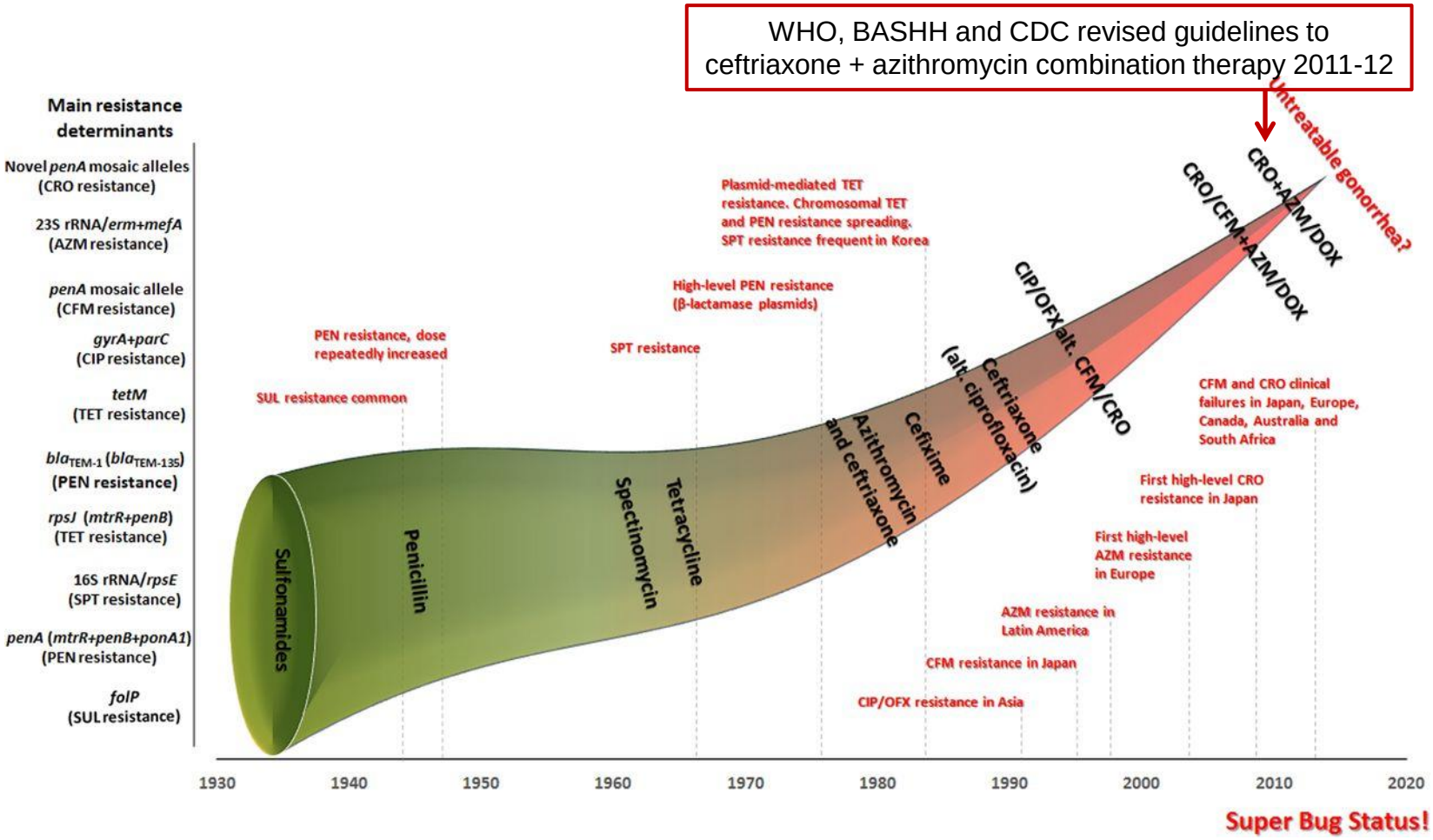


Decreased susceptibility to extended spectrum cephalosporins (cefixime and/or ceftriaxone) 2011-2014



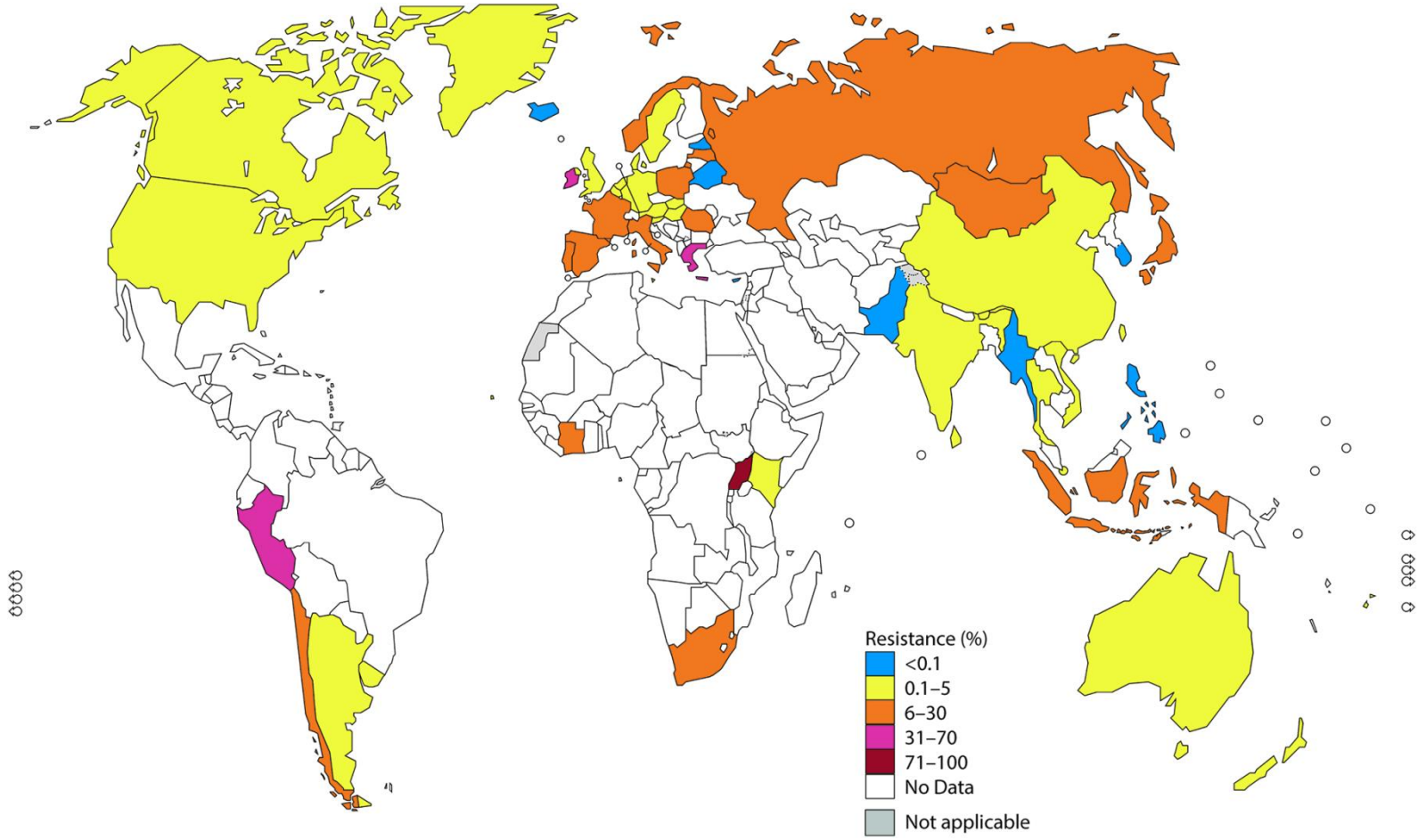
Wi et al. PLoS Med. 2017 Jul; 14(7): e1002344.

Evolution of resistance in *Neisseria gonorrhoeae*



Magnus Unemo, and William M. Shafer Clin. Microbiol. Rev. 2014; doi:10.1128/CMR.00010-14

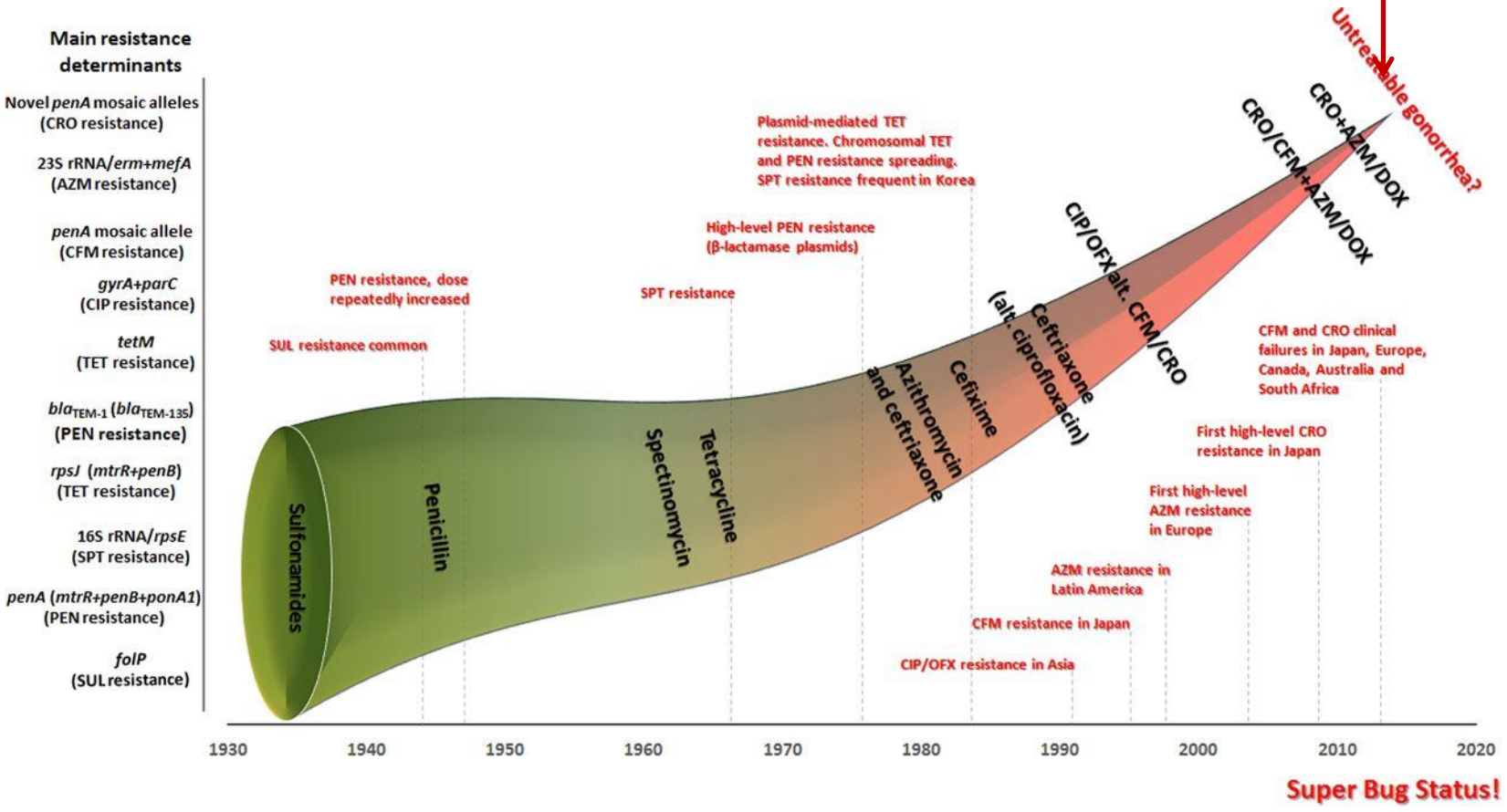
Azithromycin low level resistance 2011-2014



Wi et al. PLoS Med. 2017 Jul; 14(7): e1002344.

Evolution of resistance in *Neisseria gonorrhoeae*

BASHH revised guidelines back to ceftriaxone 1g monotherapy 2019



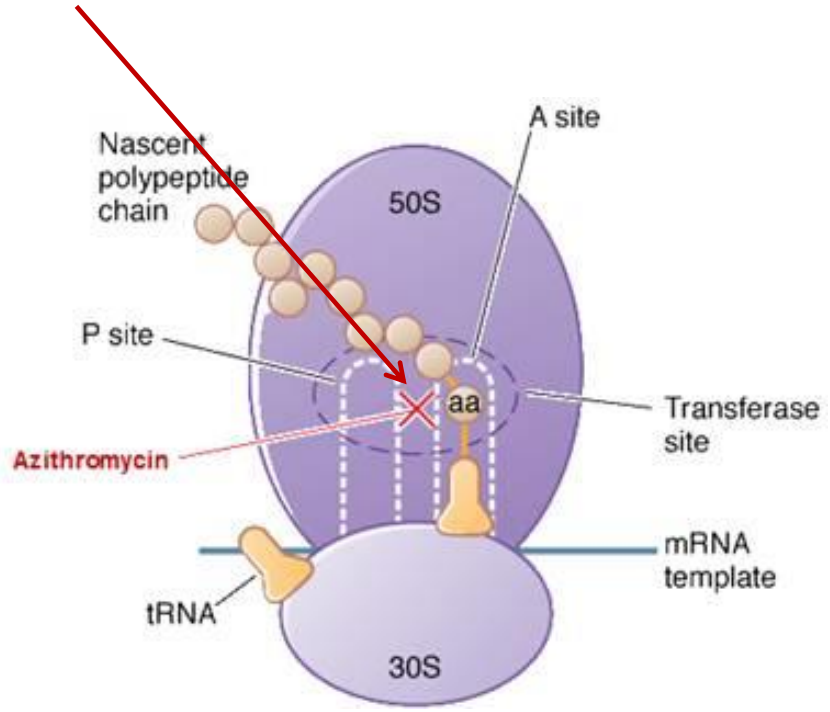
Magnus Unemo, and William M. Shafer Clin. Microbiol. Rev. 2014; doi:10.1128/CMR.00010-14

Mechanisms of gonococcal azithromycin resistance

Azithromycin binds the 23S rRNA blocking the tRNA moving through the ribosome

Commonest mechanism of resistance in NG is mutations in the four copies (alleles) of the 23S rRNA genes of the ribosome (stops azithromycin binding to the macrolide binding pocket)

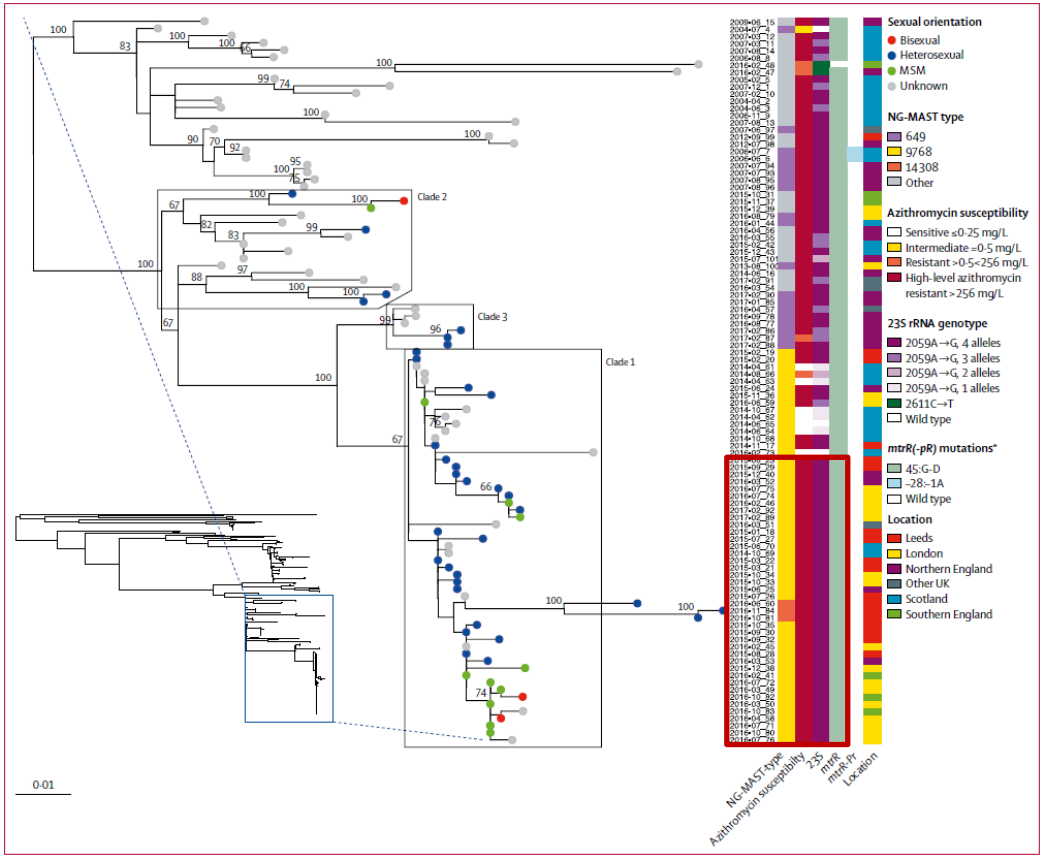
- **Low level resistance**
  - A2059G change (from adenosine to guanine) in one or two copies
  - mutations at other 23sRNA sites e.g. C2611T
  - efflux pump (*mtrR*) mutations (as for penicillin and ceftriaxone)
- **High level resistance**
  - A2059G change in three or all four copies



Goodman and Gilman's Manual of Pharmacology and Therapeutics

High level azithromycin resistance

- Small outbreak in Hawaii
- Larger (>100 cases) outbreak in England 2014
  - Clonal outbreak of descendants from low level azithromycin resistant isolates
    - ST9768
  - All ceftriaxone susceptible



Fifer et al. Lancet Infect Dis. 2018;18:573-81

Move from ceftriaxone decreased susceptibility to resistance

- 2009 – 1 XDR case Japan (H041 clone)
- 2010/11 – 3 XDR cases in France, Spain (F89 clone)
- 2013-15 – 4 cases in Australia (A8806), Japan (GU140106, FC428, FC460)

- 2015-17 Denmark, Canada, Australia, France (FC428 clone)
  - Altered PBP (*PenA*60 allele), azithro susceptible

- 2018 - 1 XDR England, 2 XDR Australia (SEA exposure for two)
  - **high level azithromycin resistance**
  - English case:
    - Failed pharyngeal clearance with ceftriaxone/spectinomycin
    - Cleared with ertapenem
  - Australian cases (A2543 clone with *penA*60 allele of FC428 clone):
    - Case 1: Treated with ciproxin/ceftriaxone/azithromycin
    - Case 2: treated with gentamicin

Is dual therapy driving azithromycin resistance?

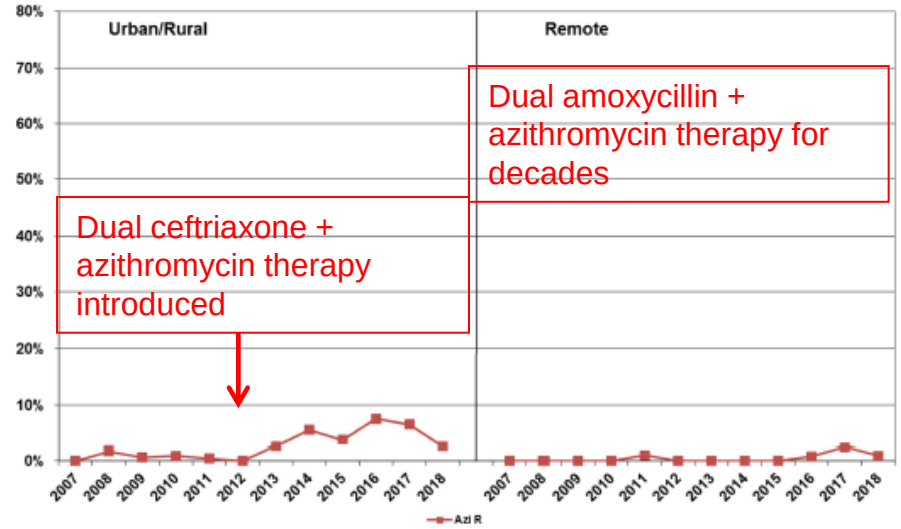


Figure 2. Azithromycin resistance (MIC ≥1 mg/L), 2007 to 2018

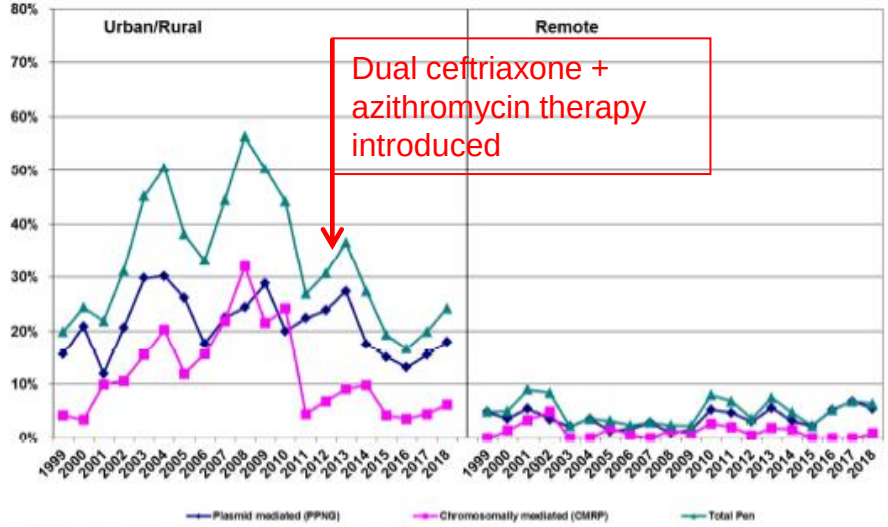
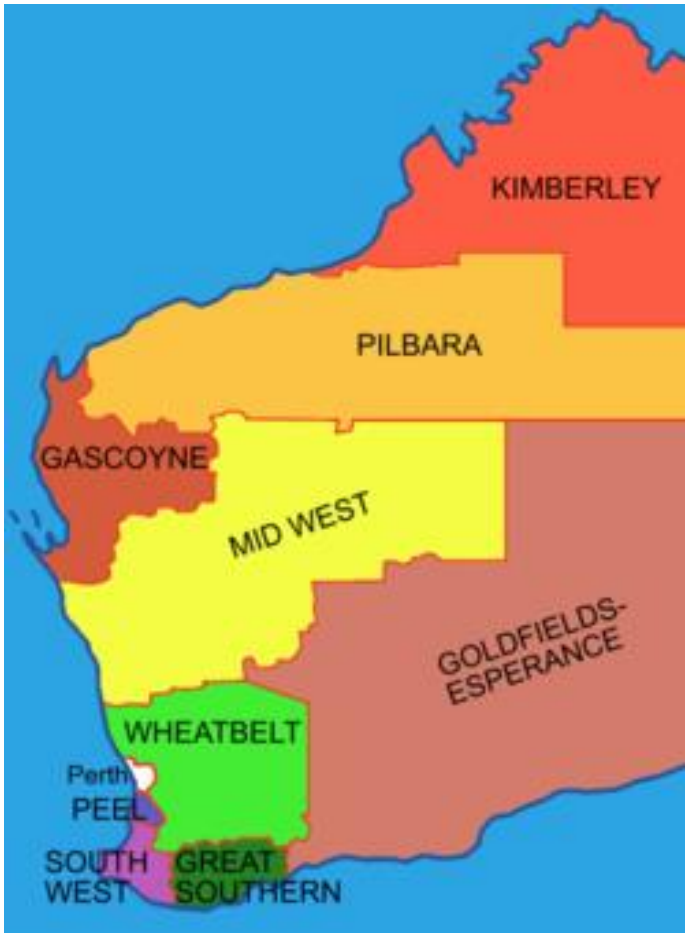


Figure 3. Penicillin resistance (PPNG + CMRP), 1999 to 2018



WAGSP 2018

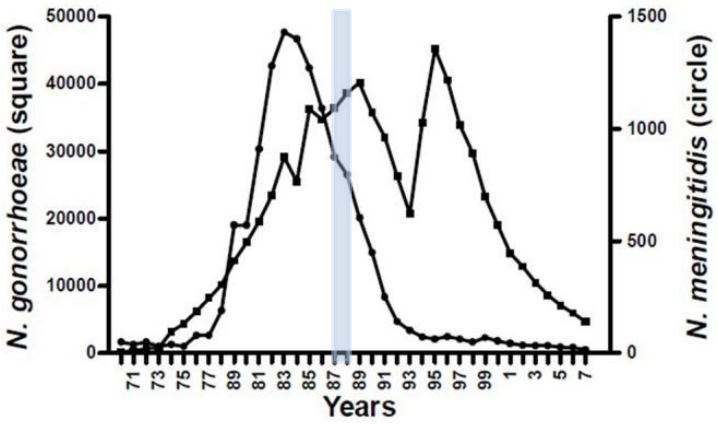
Where to from here?

- Prevention so don't have to treat
  - Vaccines
  - POCT to facilitate early treatment and contact tracing
- Treatment in an increasing AMR environment
  - Old drugs
    - Resurrecting: spectinomycin (currently SAS approval)
    - Repurposing: ertapenem, gentamicin, rifampicin
  - New drugs
- Increased surveillance
  - More isolates for phenotypic AST
  - Molecular AMR tests
    - Rapid resistance testing and reporting in real-time for case Mx
    - Molecular AMR surveillance to prevent empiric treatment failure

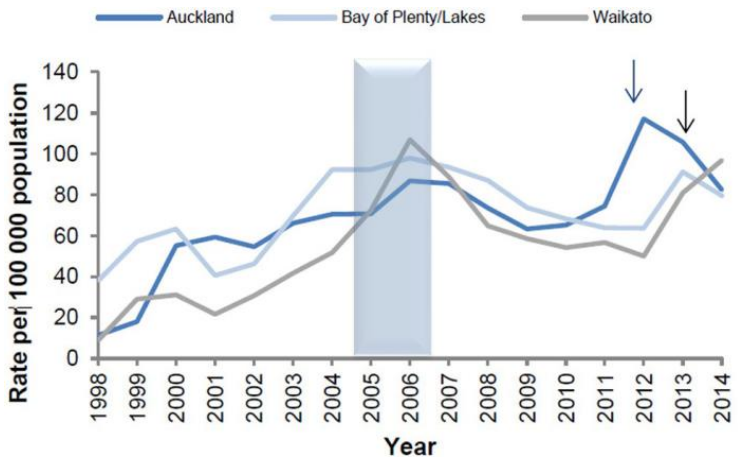
Gonococcal vaccines

- Significant antigenic variation makes vaccine development difficult
  - A moderately effective vaccine could have significant impact:
    - Measles R_0 12-18 needs population immunity of 95%
    - Influenza has R_0 1.4 to 4 – needs 30-75% population immunity to prevent transmission
    - Gonorrhoea R_0 estimated to be 1.18 to 3.6

- OMV vaccines for Group B *N. meningitidis*:
  - Calculated vaccine effectiveness for gonorrhoea was 24-47%



Cuba



New Zealand

Petousis-Harris H and Radcliff FJ (2019) Front. Immunol. 10:683.

Gonorrhoea Point-of-care Testing

- TTANGO2
  - 32 clinics across WA, SA, NT, QLD
  - 11,080 tests performed
  - Quality
    - GeneXpert operator training
    - Competency assessments
    - Internal QC program monthly
    - Aboriginal and Torres Strait Islander Reference Group

  - Excellent concordance with routine testing

Causer L, Guy R, Tabrizi S, Whiley D, Speers D, Ward J, Tangey A, Badman S, Hengel B, Natoli L, Anderson D, Wand H, Wilson D, Regan D, Shephard M, Donovan B, Fairley C, Kaldor J.. 2018 Sex Transm Infect.

Detection of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* from pooled rectal, pharyngeal and urine specimens in men who have sex with men

David John Speers,[1,2] I-Ly Joanna Chua,[1] Justin Manuel,[3] Lewis Marshall[2,3]

Table 1 Comparison between GeneXpert pooled sample and cobas individual sample testing for detection of NG and CT

Organism			Assay comparison		
			cobas		
			Detected	Not detected	Total
Chlamydia trachomatis	GeneXpert	Detected	14	2	16
		Not detected	4	89	93
		Total	18	91	93
Neisseria gonorrhoeae	GeneXpert	Detected	34	0	34
		Not detected	0	75	75
		Total	34	75	109

CT, *Chlamydia trachomatis*; NG, *Neisseria gonorrhoeae*.

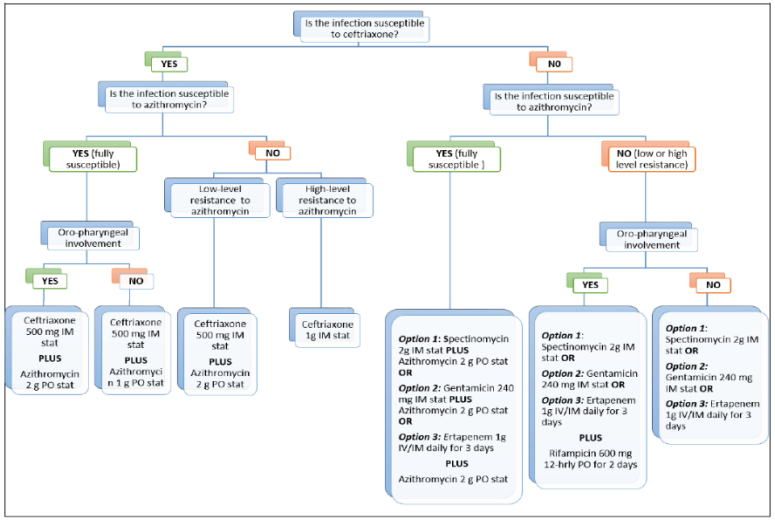
Speers DJ, Chua I, Manuel J, Marshall L. Sex Transm Infect. 2018;94:293-297

Treatment Guidelines

RECOMMENDATIONS FOR TREATMENT OF GONOCOCCAL INFECTIONS IN THE ERA OF MDR/XDR GONORRHOEA (Document for Sexual Health and Infectious Disease Specialists)

Summary Document of Discussions held by a Working Group established to report to the Communicable Diseases Network Australia on recommendations for treatment of gonococcal infections in a new era of extensively drug resistant gonorrhoea.

Working Group members: Christopher Bourne, Marcus Chen, Monica Lahra, David Lewis, Lewis Marshall, David Paterson, Tim Read, David Speers



New antibiotics

- Trial efficacy recommendations from early 1990s for new agents for gonorrhoea:
  - 95% efficacy for anogenital infection
  - 90% efficacy for pharyngeal infection

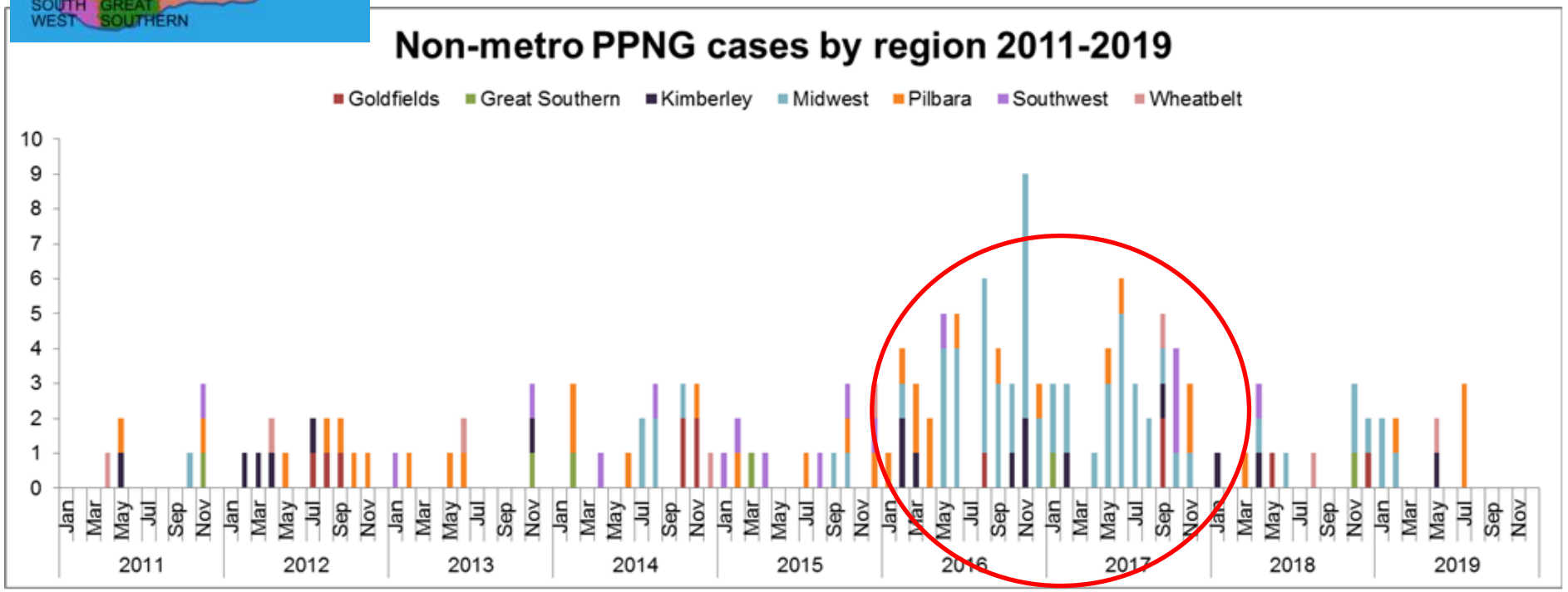
- Phase 3 trials
  - Solithromycin (macrolide) and Delafloxacin 900mg (fluoroquinolone)
    - Both failed noninferiority margin for uncomplicated gonorrhoea against ceftriaxone

Phase 2 trials

- Zoliflodacin (a piropyrimidinetrione antibiotic)
  - Novel action: blocks the gyrase complex and formation of circular DNA
  - shown activity against fluoroquinolone and ceftriaxone resistant NG
  - 2g (49) cured 98% and 3g (47) cured 100% c.f. 100% (21) for 500mg ceftriaxone
  - 67-78% effective for pharyngeal NG (15) c.f. 100% for ceftriaxone (4)
  - Multinational phase 3 trial this year with FDA fast tack status

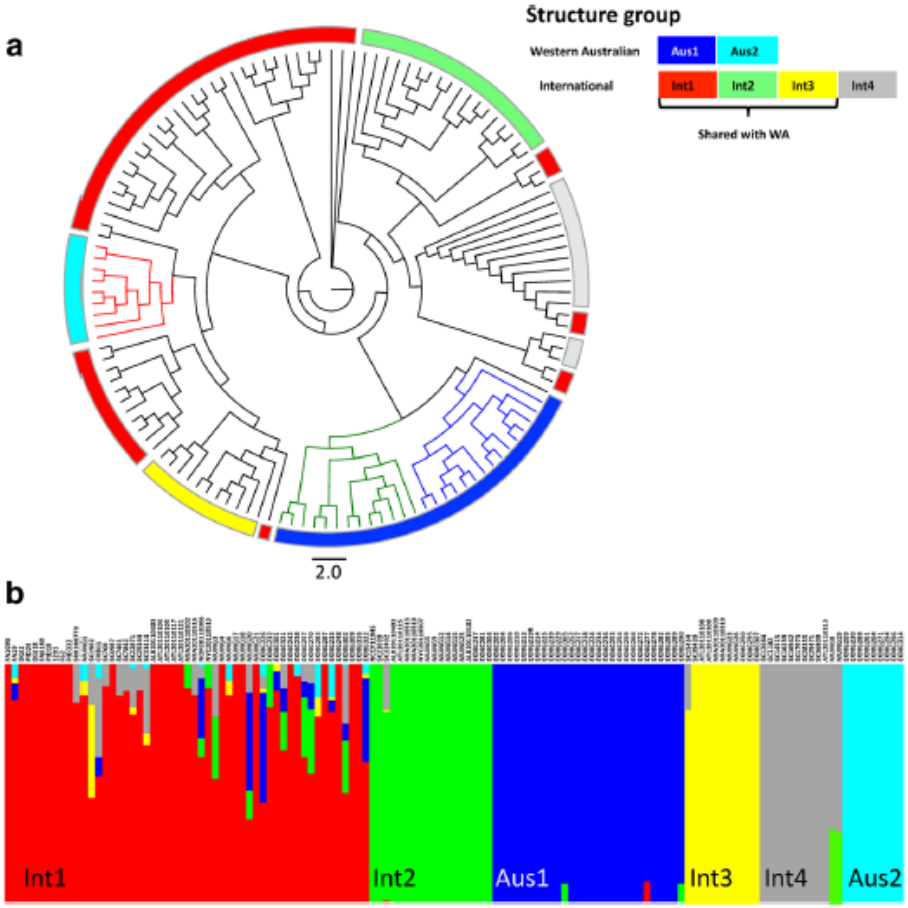
- Gepotidacin (a triazaacenaphthylene antibiotic)
  - topoisomerase inhibitor blocking DNA synthesis
  - shown activity against ciprofloxacin and azithromycin resistant strains
  - 1.5-3g (69 subjects) with 96% cure for urogenital, 1 of 2 pharyngeal infections
  - Phase 3 trial this year of 600 subjects vs ceftriaxone + azithromycin for uncomplicated urogenital NG

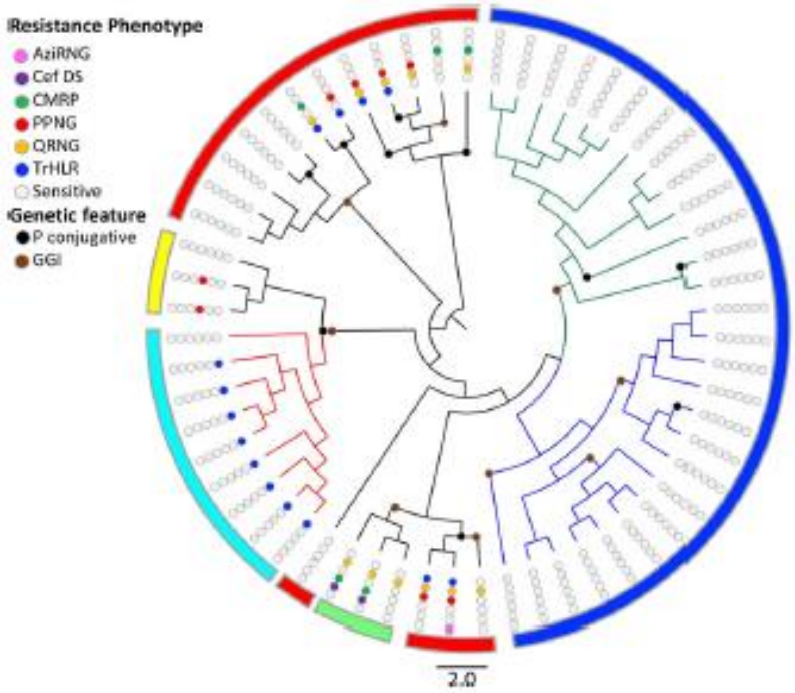
AMR Surveillance
- PathWest PPNG Surveillance



Speers D, Fisk R, Goire N, Mak D.. J. Antimicrob. Chemother. 2014;69(5)1243-7.

Not all gonococci are the same
- WA remote region NG





Suwayyid B, Coombs G, Speers DJ, Pearson J, Wise M, Kahler C. Genomic epidemiology and population structure of Neisseria gonorrhoeae from remote highly endemic Western Australian populations. BMC Genomics. 2018 Feb 27;19(1):165.