THE UNIVERSITY OF QUEENSLAND
AUSTRALIA
CREATE CHANGE

The science of *Mycoplasma genitalium*

Dr Emma Sweeney

Funding disclosure

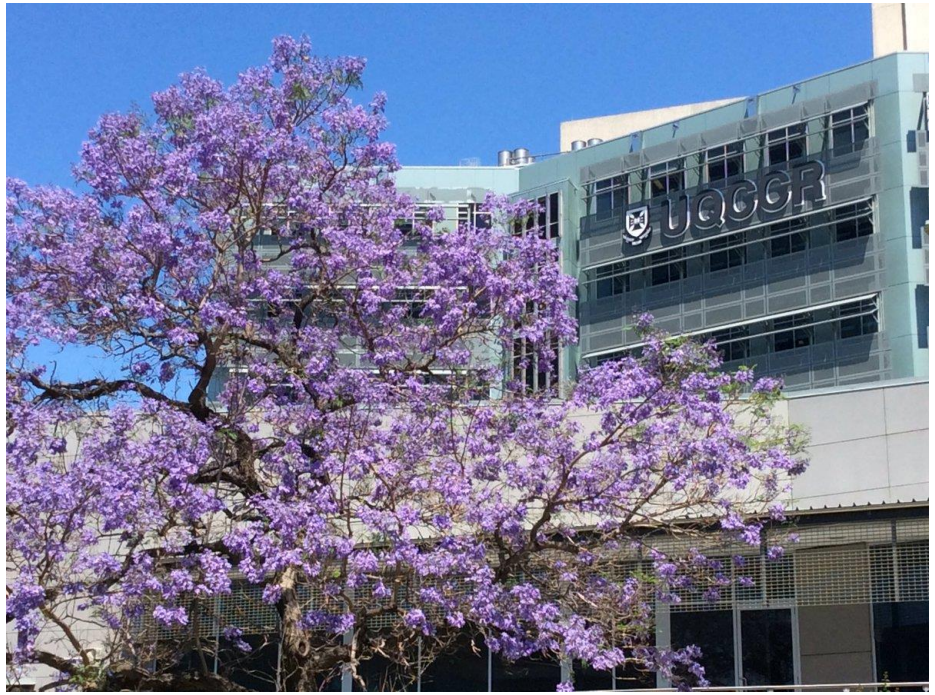
- SpeeDx

University of Queensland Centre for Clinical Research (UQ-CCR), RBWH campus

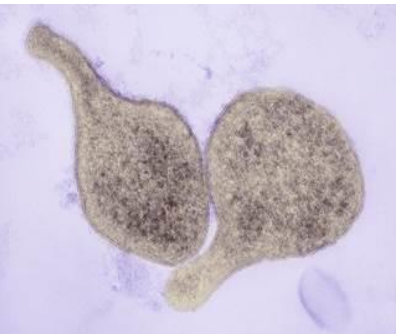
Molecular Diagnostics and Characterisation Group

Led by **A/Prof David Whiley**

- Dr Emma Sweeney
  - *M. genitalium* antibiotic resistance
  - Also working on *Treponema pallidum*
- Dr Ella Trembizki
  - *Neisseria gonorrhoeae* molecular diagnostics and antimicrobial resistance
- Cameron Buckley
  - *Neisseria gonorrhoeae* whole genome sequencing/epidemiology

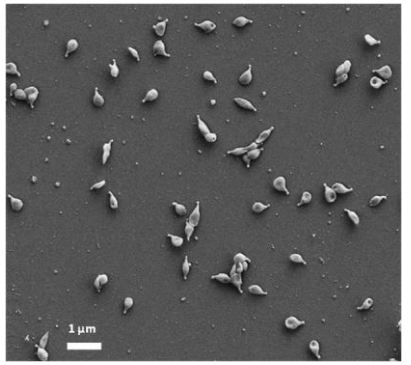


What is *Mycoplasma genitalium*?



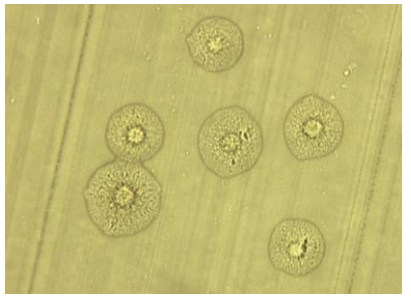
Fastidious bacteria

- No cell wall
- Inherently resistant to penicillins
- **Culture can take up to 6 months**
  - Colonies can’t be seen with the naked eye



Culture not feasible in diagnostic setting

- **PCR** is the **gold standard** for diagnosis (and AMR)



Mycoplasma genitalium prevalence

- 1 – 3% in low risk persons (Jensen & Taylor-Robinson 2011 Clin Micro Rev)
  - Compared to *Chlamydia* (4%) and *Neisseria gonorrhoeae* (0.5%)

BUT, is more common in clinical populations (Baumann *et al.* 2018 Sex Transm Infect)

- young people

- MSM

	Infected (% [95% CI])	
Organism	Female	Male
M. genitalium	16.3 (13.4–19.8)[a]	17.2 (13.9–21)[b]
C. trachomatis	9.3 (7.1–12.1)	17.8 (14.5–21.8)
N. gonorrhoeae	1.9 (1.1–3.5)	4.2 (2.7–6.5)
T. vaginalis	25.2 (21.7–29.2)	5.6 (3.8–8.2)

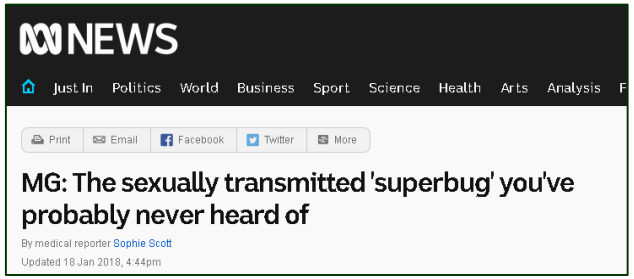
[a] OR of 1.75 ($P = 0.0035$) versus *C. trachomatis*, OR of 8.4 ($P < 0.0001$) versus *N. gonorrhoeae*, and OR of 0.646 ($P < 0.0044$) versus *T. vaginalis*.
[b] OR of 4.11 ($P < 0.0001$) versus *N. gonorrhoeae* and OR of 3.08 ($P < 0.0001$) versus *T. vaginalis*.

Table from Getman *et al.* 2016

Mycoplasma genitalium is an emerging STI threat

In 2015 was listed as an important 'STI threat' by CDC

Unlike other STIs, like *Neisseria gonorrhoeae*, whose antimicrobial resistance has increased steadily over the past decade; **antimicrobial resistance in *M. genitalium* has increased rapidly → treatment failure now relatively common**



Successful treatment of *M. genitalium* is becoming very difficult

Without appropriate treatment, *M. genitalium* infections are often chronic

- **25% of infections persist for >12 months;** some infections in females persist for 2-3 years (Vandepitte *et al.* 2014 Sex Transm Dis)

- More recently, in men it was found that *M. genitalium* infections persisted for ~5 months (range 21 – 228 days) in the absence of curative therapy (Romano *et al.* 2018 Clin Infect Dis)

Selecting an appropriate antimicrobial therapy is crucial

Mycoplasma genitalium antimicrobial resistance

Tetracyclines (e.g. doxycycline)

- No known mechanisms of resistance in *M. genitalium*
- BUT, clinically **up to two thirds** of patients are **not cured** following treatment (Wilkstrom & Jensen, 2006)

Macrolides (e.g. azithromycin)

- Rapid decline in efficacy → mutations in **23S rRNA** gene
- Five single nucleotide mutations at positions 2058 and 2059
  - **A2058G/C/T, A2059G/C**
- Macrolide resistance exceeds 50% in most urban centres across Australia

Quinolones (e.g. moxifloxacin)

- Prior to 2010, 100% efficacy; treatment failures now being reported
- Four genes contribute to quinolone resistance (gyrA, gyrB, parC, parE)
- A range of single nucleotide mutations in ***parC*** gene associated with resistance
  - Prevalence of these mutations varies worldwide (2 – 38%)

S83I
S83N
D87H
D87Y
D87N
S84P

High MIC and/or treatment failure

May be associated with treatment failure

Antimicrobial resistance in Queensland

Sweeney *et al.* (2019) J Clin Micro; n = 477

- 62% of all samples had evidence of macrolide resistance mutations
- 10.5% of all samples had evidence of quinolone resistance mutations
- **7.8% had evidence of resistance mutations to BOTH macrolides and quinolones**

Region	Macrolide resistance mutations	Quinolone resistance mutations	Dual resistance mutations
SEQ (n = 209)	136, 65.1%	39, 18.7%	28, 13.4%
Male (n = 159)	109, 68.5%	28, 17.6%	22, 13.8%
Female (n = 50)	27, 54.0%	11, 22%	6, 12%
NQ (n = 238)	141, 59.2%	8, 3.4%	7, 3.0%
Male (n = 110)	68, 61.8%	6, 5.5%	5, 4.5%
Female (n = 126)	71, 56.3%	2, 1.6%	2, 1.6%
Undisclosed (n = 2)	2, 100.0%	0, 0.0%	0, 0.0%

Levels of antimicrobial resistance by site (SEQ)

Site	Macrolide resistance mutations	Quinolone resistance mutations	Dual resistance mutations
AMU (n = 13)	10/13 (77%)	2/13 (15%)	1/13 (8%)
GCSH (n = 71)	44/71 (62%)	7/71 (10%)	5/71 (7%)
PA (n = 12)	10/12 (83%)	2/12 (17%)	2/12 (17%)
PASH (n = 67)	49/67 (73%)	14/67 (21%)	11/67 (16%)
RDCH (n = 4)	3/4 (75%)	2/4 (50%)	2/4 (50%)
RK (n = 10)	4/10 (40%)	2/10 (20%)	0/10 (0%)
SCSH (n = 12)	5/12 (42%)	5/12 (42%)	4/12 (33%)
SHHS (n = 12)	7/12 (58%)	3/12 (25%)	3/12 (25%)

Levels of antimicrobial resistance by site (NQ)

Site	Macrolide resistance mutations	Quinolone resistance mutations	Dual resistance mutations
BAM (n = 8)	6/8 (75%)	0/8 (0%)	0/8 (0%)
CNSH (n = 41)	23/41 (56%)	3/41 (7%)	3/41 (7%)
DOOM (n = 7)	6/7 (86%)	0/7 (0%)	0/7 (0%)
MI (n = 24)	14/24 (58%)	0/24 (0%)	0/24 (0%)
MORN (n = 8)	3/8 (38%)	0/8 (0%)	0/8 (0%)
MSHS (n = 19)	10/19 (53%)	0/19 (0%)	0/19 (0%)
OR (n = 7)	4/7 (57%)	0/7 (0%)	0/7 (0%)
ORN (n = 6)	4/6 (67%)	0/6 (0%)	0/6 (0%)
PI (n = 28)	17/28 (61%)	0/28 (0%)	0/28 (0%)
TI (n = 8)	3/8 (38%)	0/8 (0%)	0/8 (0%)
TN/TNSH (n = 72)	45/72 (63%)	5/72 (7%)	4/72 (6%)

What can we do next to improve diagnostics?

Diagnostic and point-of-care tests for *M. genitalium*

- Discussions with Pathology Queensland to implement SpeeDx ResistancePlus MG (detect macrolide resistance)
- **Point of care (POC) tests to detect macrolide resistance at the site of care (SpeeDx/Cepheid)**

Developing new diagnostic tests → commercial tests to detect quinolone resistance

- Currently the 'gold standard' assays involve PCR amplification of gene fragments and sequencing to detect mutations → turn around time for this is very slow (~5-10 days)

- **Working with SpeeDx to validate parC "beta" kit that can detect the most common mutations associated with quinolone resistance**

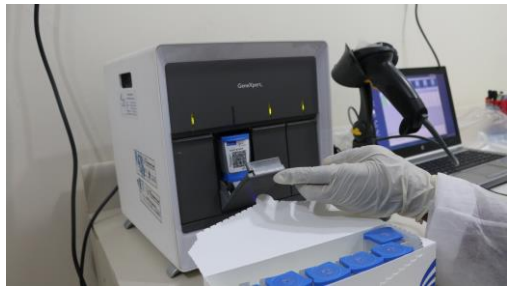
Point of care tests → GeneXpert

Testing patients and receiving results at the point of care – reduce time to diagnosis and initiate tailored treatment

- GeneXpert FleXible cartridge paired with SpeeDx ResistancePlus MG test (detects *M. genitalium* and 23S rRNA mutations)

- 'Raw' sample (1mL) added to cartridge → **"hands off" workflow**
  - Sample preparation, target detection by PCR and reporting of result all completed by machine
  - **~2 hrs from start (adding sample) to finish (report generated)**

- Testing/validation of the cartridges are underway in our laboratory, **results are looking very promising!**



ResistancePlus® M

FleXible for the GeneXper

COMING SOON

Advance Resistance Guided Therapy

- Healthcare Associated Infections
  - Xpert MRSA NxG
  - Xpert SA Nasal Complete
  - Xpert MRSA/SA BC
  - Xpert MRSA/SA SSTI
  - Xpert C. difficile
  - Xpert C. difficile/Epi
  - Xpert Carba-R
  - Xpert vanA
  - Xpert Norovirus
- Critical Infectious Diseases
  - Xpert Xpress Strep A
  - Xpert Xpress Flu/RSV
  - Xpert Xpress Flu
  - Xpert Flu/RSV XC
  - Xpert MTB/RIF
  - Xpert EV
- Sexual Health
  - Xpert TV
  - Xpert CT/NG
  - Xpert GBS
  - Xpert GBS LB

*not yet commercially available

Conclusions

Antimicrobial resistance is high in *M. genitalium* in Queensland

- Macrolide resistance **exceeds 50%** in most urban centres of the world
- Quinolone resistance increasing, levels vary between **20 – 40% in Asia Pacific** but are lower in Europe (~5%)

Molecular assays to guide successful treatment are needed

- Some currently available - more in development

Working towards resistance-guided treatment

- Not possible without molecular assays to detect resistance mutations
- Could be implemented into pathology laboratories and/or clinical sites (for POC tests) – further reduce overuse of particular antimicrobials and tailor treatment

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