Evaluation of ResistancePlus GC to Inform Ciprofloxacin Treatment of Gonorrhoea

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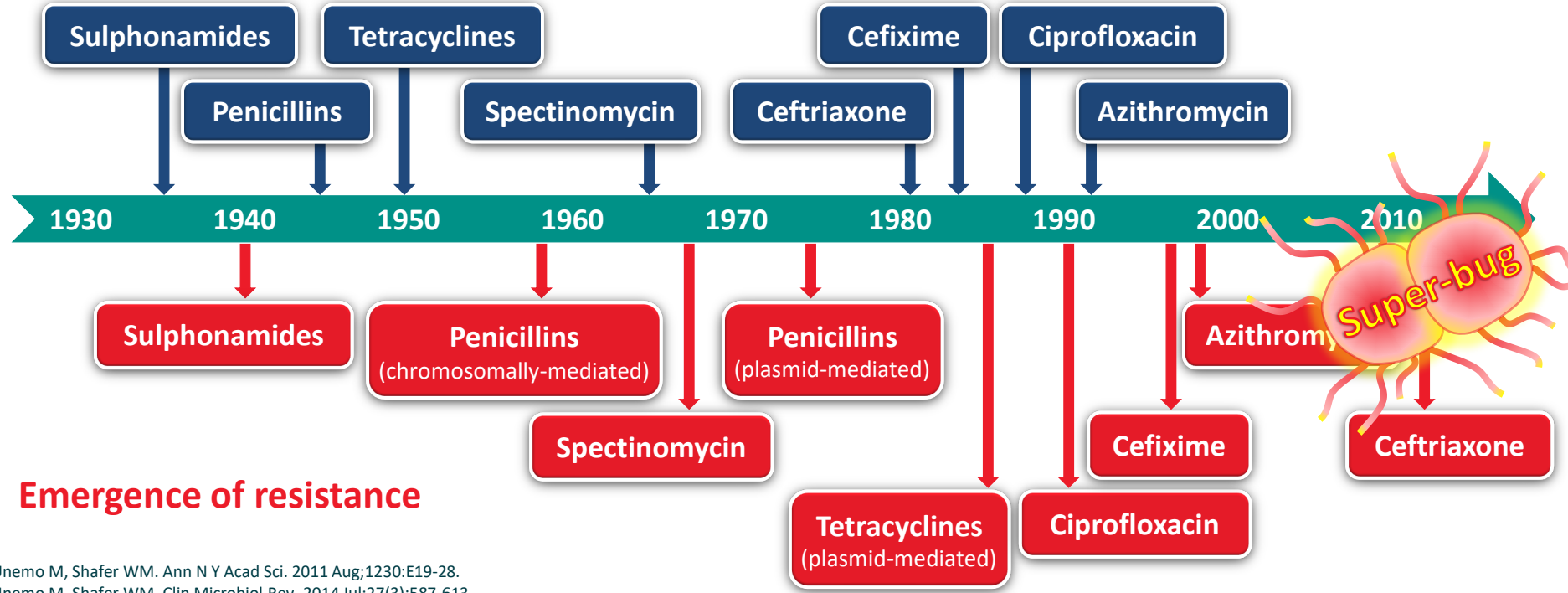
Evolution of Antimicrobial Resistance (AMR) in Neisseria gonorrhoeae (GC)

Antibiotics used for treatment



Emergence of resistance

Unemo M, Shafer WM. Ann N Y Acad Sci. 2011 Aug;1230:E19-28.
Unemo M, Shafer WM. Clin Microbiol Rev. 2014 Jul;27(3):587-613.

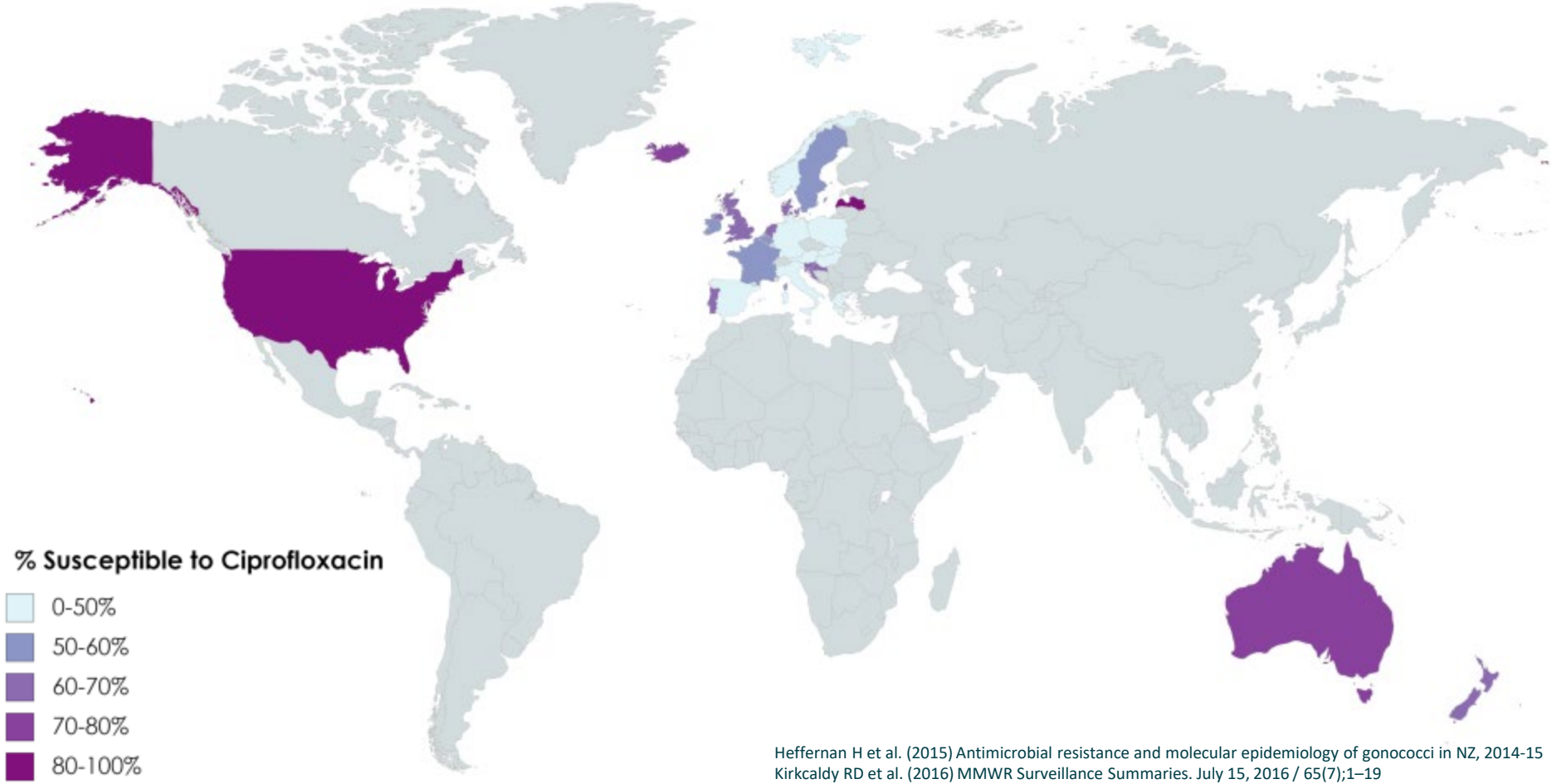
WHO Global Action Plan for AMR GC

- Increase awareness on correct antibiotic use
- Effective prevention, diagnosis and control
- Monitor treatment failures
- Strengthen AMR surveillance
- Effective drug regulations and prescription policies

- Newer molecular methods for monitoring and detecting AMR
- Alternative effective treatment regimens

Molecular detection of genetic markers for resistance/susceptibility could allow 'older' drugs to be used for gonorrhoea treatment

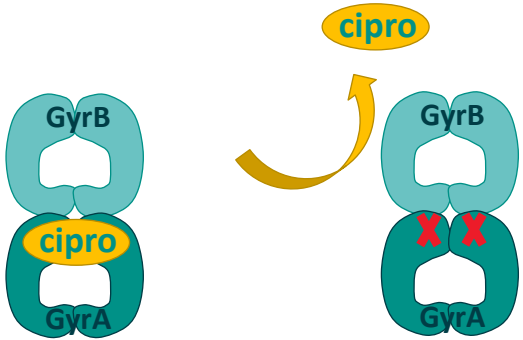
GC Susceptibility to Ciprofloxacin



Heffernan H et al. (2015) Antimicrobial resistance and molecular epidemiology of gonococci in NZ, 2014-15
Kirkcaldy RD et al. (2016) MMWR Surveillance Summaries. July 15, 2016 / 65(7);1–19
Lahra MM et al. (2017) Australian Gonococcal Surveillance Programme Annual Report, 2017

GC Mechanisms of Fluoroquinolone Resistance

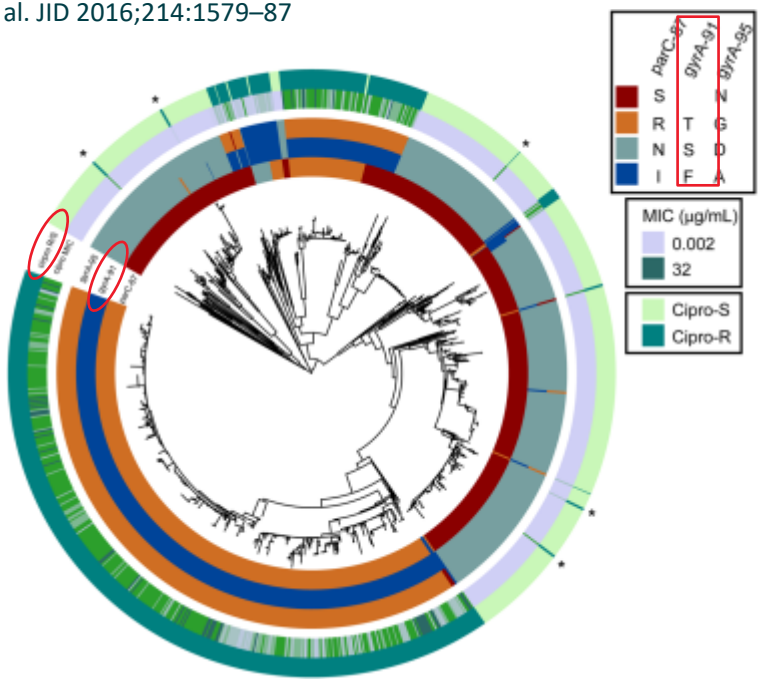
- Fluoroquinolones were predominant GC treatment in 1990s
  - 2007 - No longer recommended
- Quinolones act by inhibition of DNA gyrase and topoisomerase IV
  - DNA gyrase – heterotetramer of GyrA and GyrB
  - Topoisomerase IV – heterotetramer of ParC and ParE
- Bacteria develop resistance through mutations in the QRDR (quinolone resistance determining region)
  - GC: GyrA (aa positions 91, 95) and ParC (87)



GyrA Genotype Predicts Cipro Resistance in GC

Genomic Epidemiology in US, 2000-2013 isolates

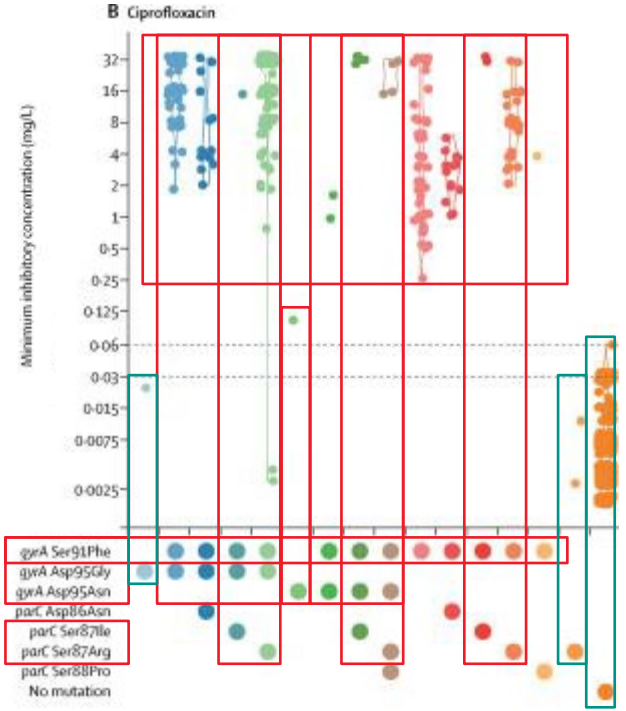
Grad et al. JID 2016;214:1579–87



GyrA Genotype	Predictive Value
S91F	PPV of Ciprofloxacin Resistance = 98%
S91 WT	NPV of Ciprofloxacin Susceptibility = 99%

Genomic Survey in Europe, 2013 isolates

Harris et al. Lancet Infect Dis, published online May 15, 2018



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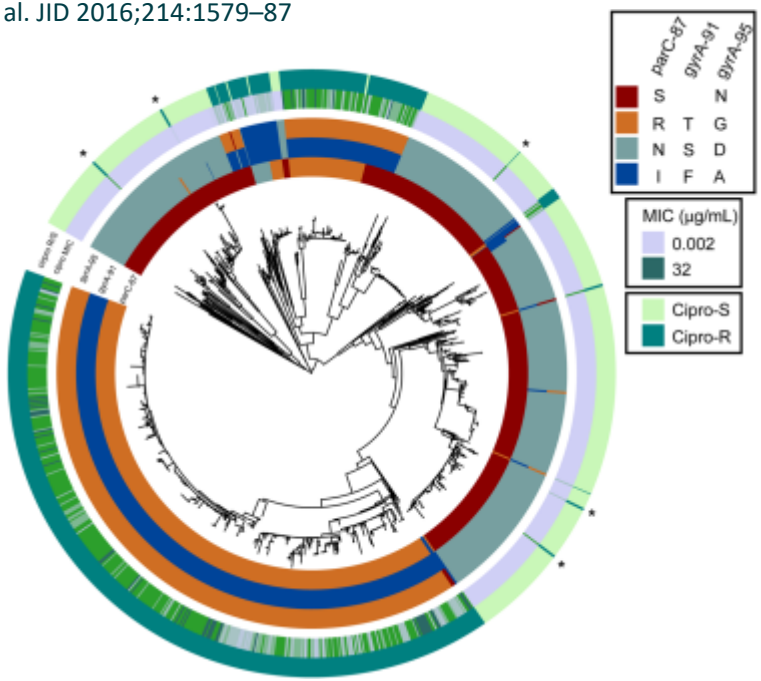

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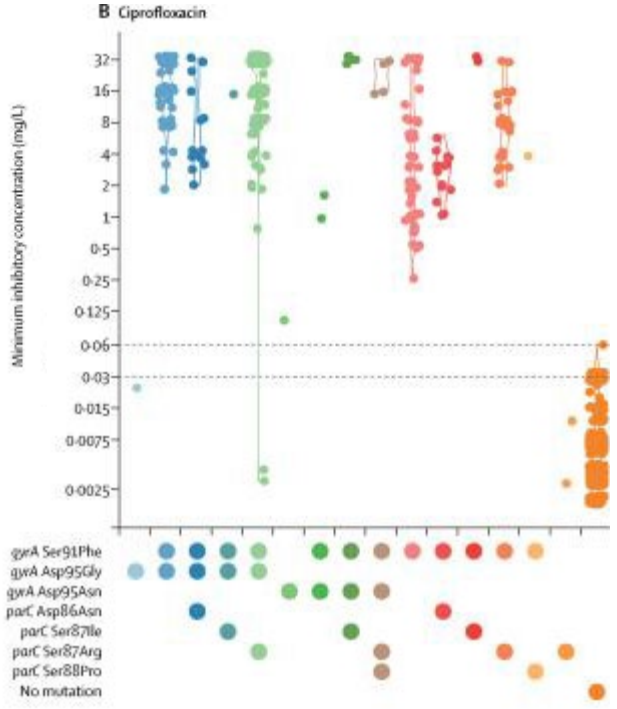


GyrA S91/S91F is highly predictive of ciprofloxacin susceptibility/resistance

GC Molecular Diagnostics With gyrA Genotyping Can Guide Ciprofloxacin Treatment

- Advantages
  - Oral treatment preferred
  - Antibiotic stewardship of ceftriaxone
  - Utilize existing drug while new drugs are still in development

- Pooled estimate of real-time PCR gyrA genotyping tests for predicting GC susceptibility to ciprofloxacin

  Allan-Blitz et al. Sex Transm Dis. 2017 May;44(5):261-265.

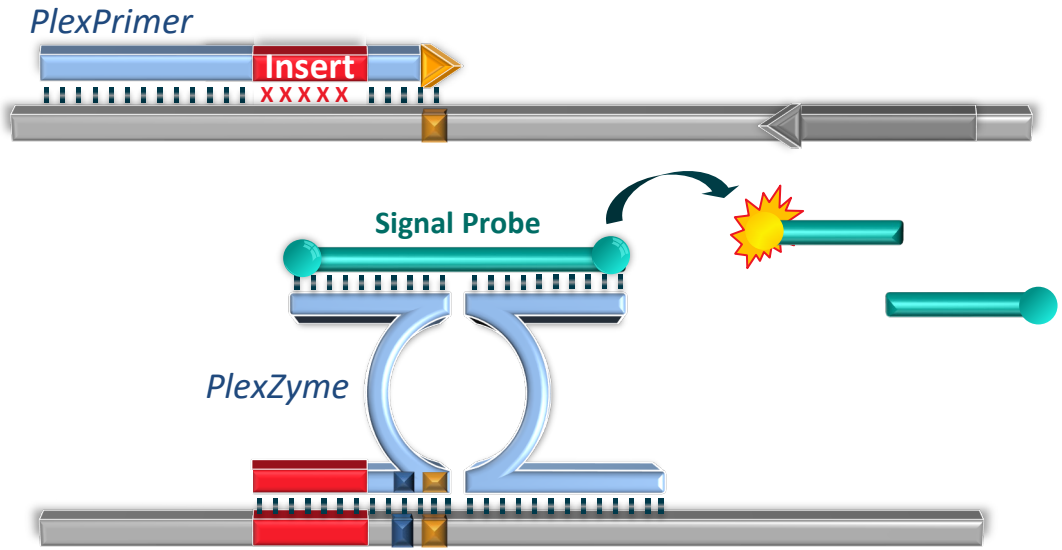
  - Sensitivity: 98.2% (95% CI, 96.5–99.1%), Specificity: 98.6% (95% CI, 97.0–99.3%)

- UCLA Health System has implemented a gyrA molecular assay to identify patients for ciprofloxacin treatment

  Allan-Blitz et al. Sex Transm Dis. 2018 Apr;45(4):e18.

  - 100% Ciprofloxacin cure rate for gyrA WT (n=25; 7 urethral, 7 pharyngeal, 7 rectal, 4 genital)

PlexPCR® Technical Advantages



Tan et al PLoS ONE (2017) and Patent: WO 2013/123552

- **Specific amplification and detection**
- **Universal probe for easy multiplexing**
- **Maintains sensitivity**

Ideal molecular diagnostic technology for multiplexed detection of resistance/susceptibility markers

ResistancePlus GC

- **GC detection and ciprofloxacin resistance/susceptibility information**
  Rapid qPCR format (<1.5 hours)
  Direct from clinical specimens
- **Specimen Types**
  Urine, Swabs

	Channel	Target
1 Well	1	***N. gonorrhoeae*** **(opa)**
	2	***N. gonorrhoeae*** **(porA)**
	3	**gyrA S91 wild type**
	4	**gyrA S91F mutation**
	5	Internal Control

ResistancePlus GC is a molecular diagnostic test for ciprofloxacin resistance/susceptibility

ResistancePlus GC (beta) Analytical Performance

- Analytical sensitivity
  - GC gyrA S91 wildtype – 15 geq/reaction
  - GC gyrA S91F mutant – 15 geq/reaction

- Analytical specificity
  - 100% specificity:
    - *Neisseria spp*. isolates
    - Other organisms found in genital/throat/rectal sites

ResistancePlus GC (beta) on GC Clinical Isolates

- Australian isolates (top 70 most common genotypes from 2012)

		WGS	
		S91F mut	S91 WT
SpeeDx	S91F mut	28	0
	S91 WT	0	42
	Total	**28**	**42**
Sensitivity		**100.0%** (95% CI 87.7-100.0%)	
Specificity		**100.0%** (95% CI 91.4-100.0%)	

		Ciprofloxacin AST	
		Resistant (R)	Susceptible (S)
SpeeDx	S91F mut	27	1*
	S91 WT	0	42
	Total	**27**	**43**
Sensitivity		**100.0%** (95% CI 87.7-100.0%)	
Specificity		**97.7%** (95% CI 87.7-99.9%)	

* LS – less susceptible (S91F mutation by WGS)

High concordance to gyrA genotype & ciprofloxacin R/S phenotype

ResistancePlus GC (beta) Clinical Performance

- University of Queensland Centre for Clinical Research (Brisbane, Australia)
- 416 specimens from 2017

	Urine	Genital	Throat	Rectal/ Anal	Other/ unknown	Total
Male	27	11	122	28	2	190
Female	18	184	22	1	1	226
Total	45	195	144	29	3	416

ResistancePlus GC (beta) Clinical Performance

- University of Queensland Centre for Clinical Research (Brisbane, Australia)

	GC detection	GC Clinical Results	
		+	-
SpeeDx	+	123	1
	-	4	288
	Total	**127**	**289**
	Sensitivity	**96.9%** (95% CI 92.1-99.1%)	
	Specificity	**99.7%** (95% CI 98.1-100.0%)	

	gyrA detection	In-house gyrA qPCR	
		Mutant	Wild type
SpeeDx	Mutant	20	0
	Wild type	0	70
	Indeterminate	0	1
	Total	**20**	**71**
	Sensitivity	**100.0%** (95% CI 83.2-100.0%)	
	Specificity	**98.6%** (95% CI 92.4-100.0%)	

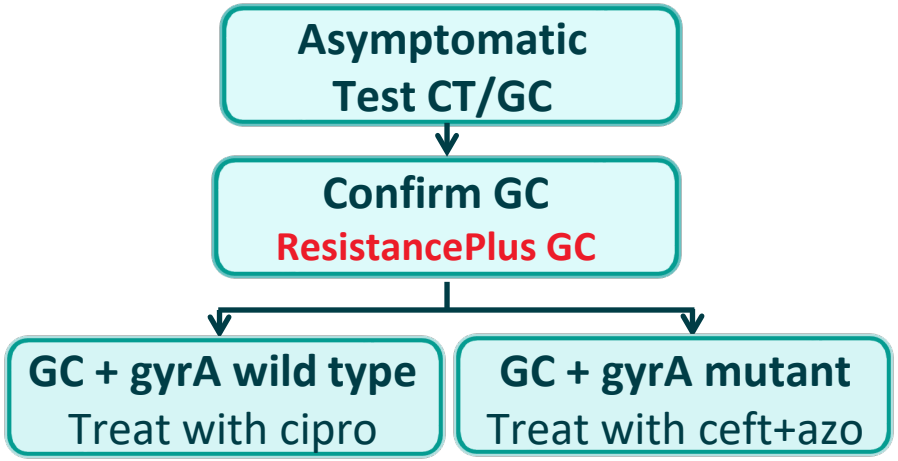
Excellent clinical sensitivity and specificity

ResistancePlus GC – Future work

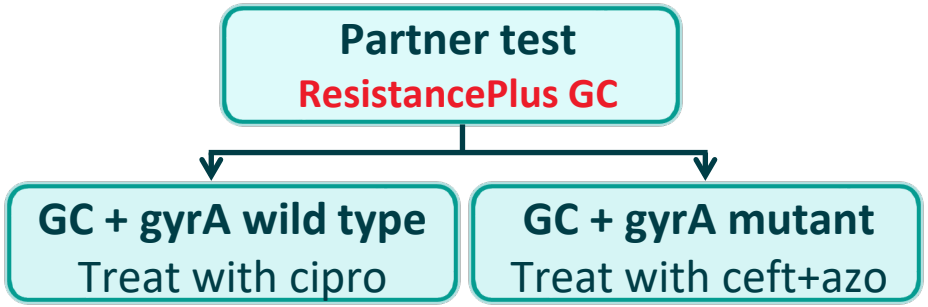
- Expected date for CE-IVD (Sept 2018)
- GRAND2 – GC clinical study

Potential Implementation Pathways

Screening or postal service



Partner testing



Acknowledgements

UQCCR, Australia

- David Whiley
- Lebogang Mhango
- Ella Trembizki

SpeeDx, Australia

- Samantha Walker
- Madeline Windsor
- Simon Erskine
- Elisa Mokany
- Colin Denver

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