Molecular testing for gonorrhoea resistance and its role in gonorrhoea control

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Disclosure:

Research funding from SpeeDx Pty Ltd, Australia.

2013

HEALTH

‘Super’ strain of drug-resistant gonorrhea concerns U.S. officials

A Canadian study showed the sexually transmitted disease is becoming increasingly resistant to the only oral antibiotic currently used to treat it.

REUTERS

WEDNESD

CDC Warns of Super-Gonorrhea

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By ABC

Pucker-Up: Super-Gonorrhea Just in Time for Valentine’s Day

Super-Bug on its Way to USA

February 14, 2013

New Gonorrhoea Strain 'Worse Than Aids'

Doctors in the US warn that a new drug-resistant strain of the sexually-transmitted disease is "potentially disastrous".

1:19pm UK, Monday 06 May 2013

2017

7NEWS LATEST NATIONAL WORLD VII

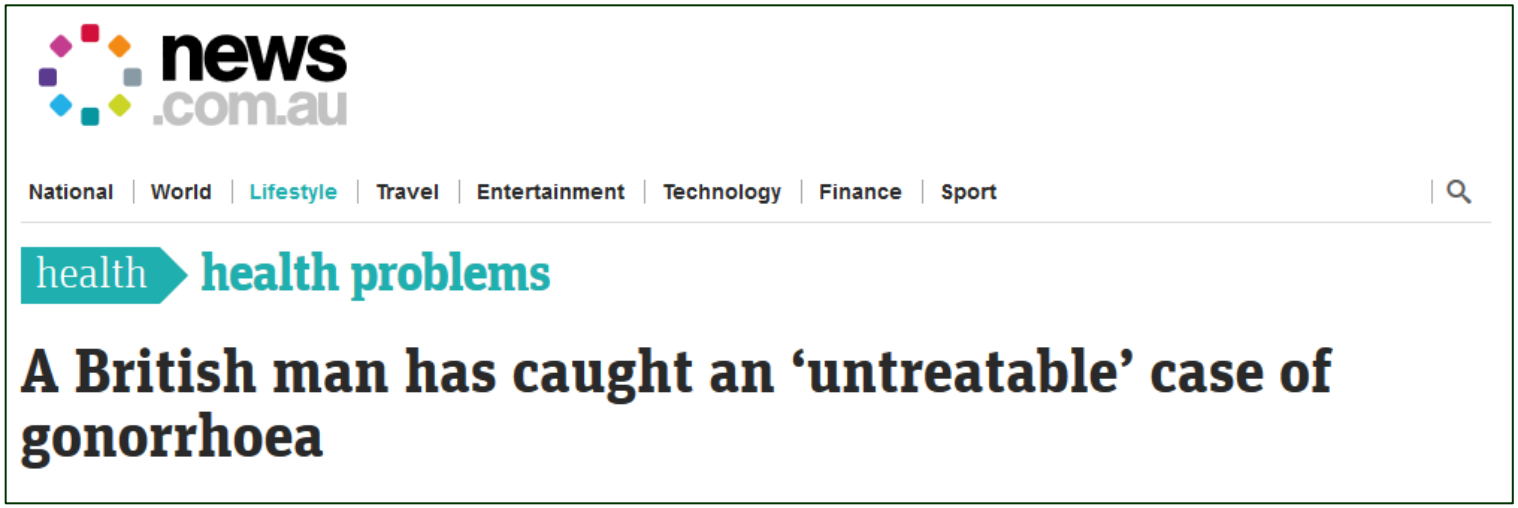
'Nightmare' new gonorrhoea superbug may soon be untreatable in Australia

Yahoo7 on July 19, 2017, 4:50 pm

SHARE TWEET EMAIL ...

A new health report published on Wednesday warns that antibiotic-resistant "superbugs" are on the rise in Australia.

2018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A British man has caught an 'untreatable' case of gonorrhoea


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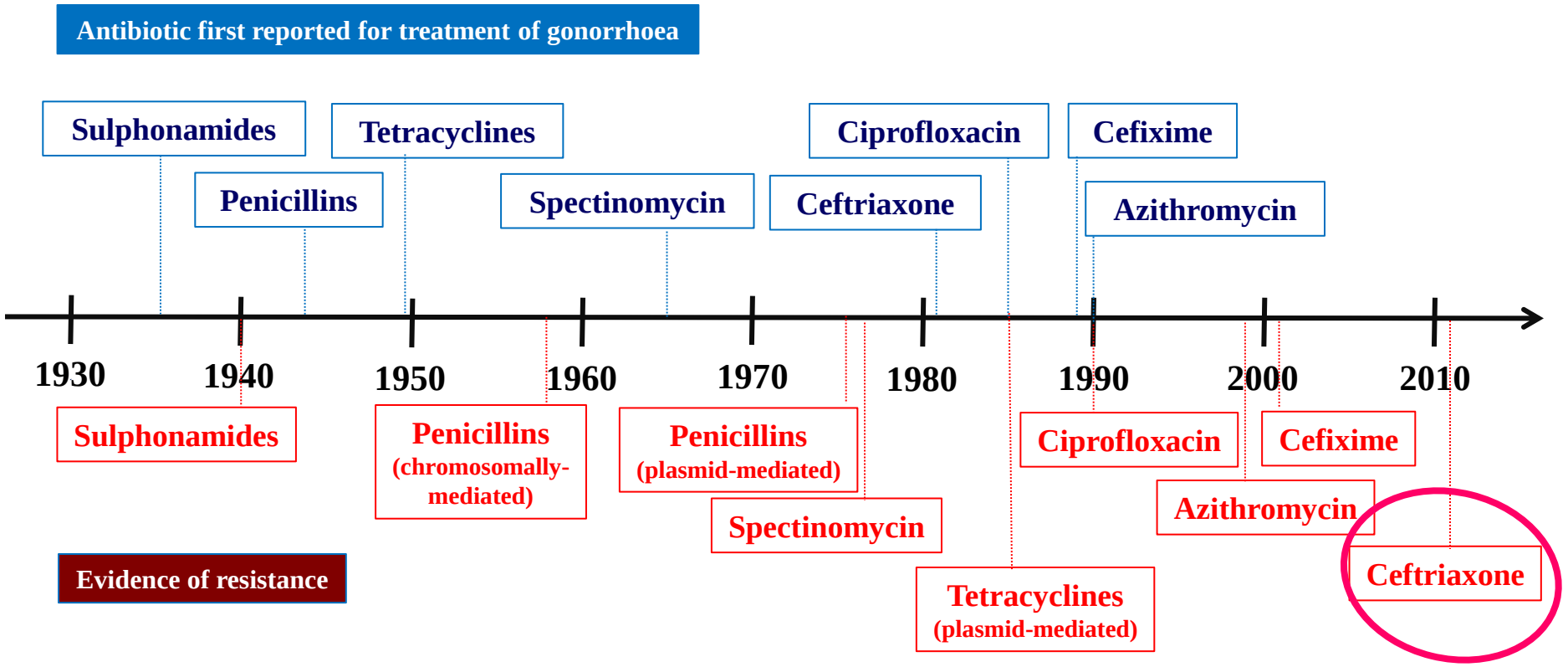
Cases of 'super gonorrhoea' detected in WA and Queensland

Neisseria gonorrhoeae has developed resistance to multiple classes of antimicrobials:



WHO = 'High Priority'
CDC = 'Urgent Threat'

Neisseria gonorrhoeae has developed resistance to multiple classes of antimicrobials:

Ceftriaxone –

- **Decreased-susceptibility (MICs 0.06-0.125mg/l):**
  - ❖ Increasing numbers of gonococci with decreased-susceptibility to ceftriaxone observed worldwide over several years.
  - ❖ Have been associated with a few pharyngeal treatment failures, but not genital infections.

- **Ceftriaxone-resistance (MICs >0.125mg/l):**
  - ❖ H041 strain: Japan 2009 *(Ohnishi et al. Emerg Infect Dis. 2011)*
  - ❖ F89 strain: France 2010 & Spain 2011
    (Unemo et al. AAC. 2012 & Cámara J et al. JAC2012)
  - ❖ A8806 strain: **Australia 2013** *(Lahra, Ryder, Whiley. NEJM. 2014)*

  sporadic

  - ❖ **'Untreatable' A2543 strain:** UK 2018, Australia 2018 (2 cases)
    - Also exhibited high-level resistance to azithromycin!
  - ❖ **FC428 strain**: Japan 2015, China 2016, **Australia 2017**, Canada 2017, Denmark 2017, France 2018, UK 2018, Ireland 2018, China 2019, France 2019, **Australia 2019**
    - Same ceftriaxone resistance mechanism as A2543 ➡ penA-60

So what is being done to combat antibiotic-resistant gonorrhoea?

Key components of WHO and CDC action plans:

- advocacy for increased awareness on correct use of antibiotics.
- effective drug regulations and prescription policies
- effective prevention, diagnosis and control of gonococcal infections.
- research into alternative effective treatment regimens.
- strengthened AMR surveillance, including developing NAAT for detecting AMR
- developing procedures for systematic monitoring of treatment failures.

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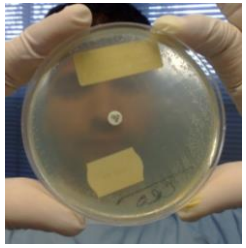
THE GONORRHOEA RESISTANCE ASSESSMENT VIA NUCLEIC ACID DETECTION PROJECT

NHMRC-funded project.
2012 - 2015

<u>Phase 1:</u> To better understand the spread of NG and resistance.

- Characterising isolates from throughout Australia.



<u>Phase 2:</u> Develop and apply molecular AMR methods (PCR) for direct testing of NG NAAT-positive clinical samples.

<u>Key aims</u>

- To enhance surveillance.
- To explore other treatment strategies.

Molecular NG resistance testing is already underway in Australia;

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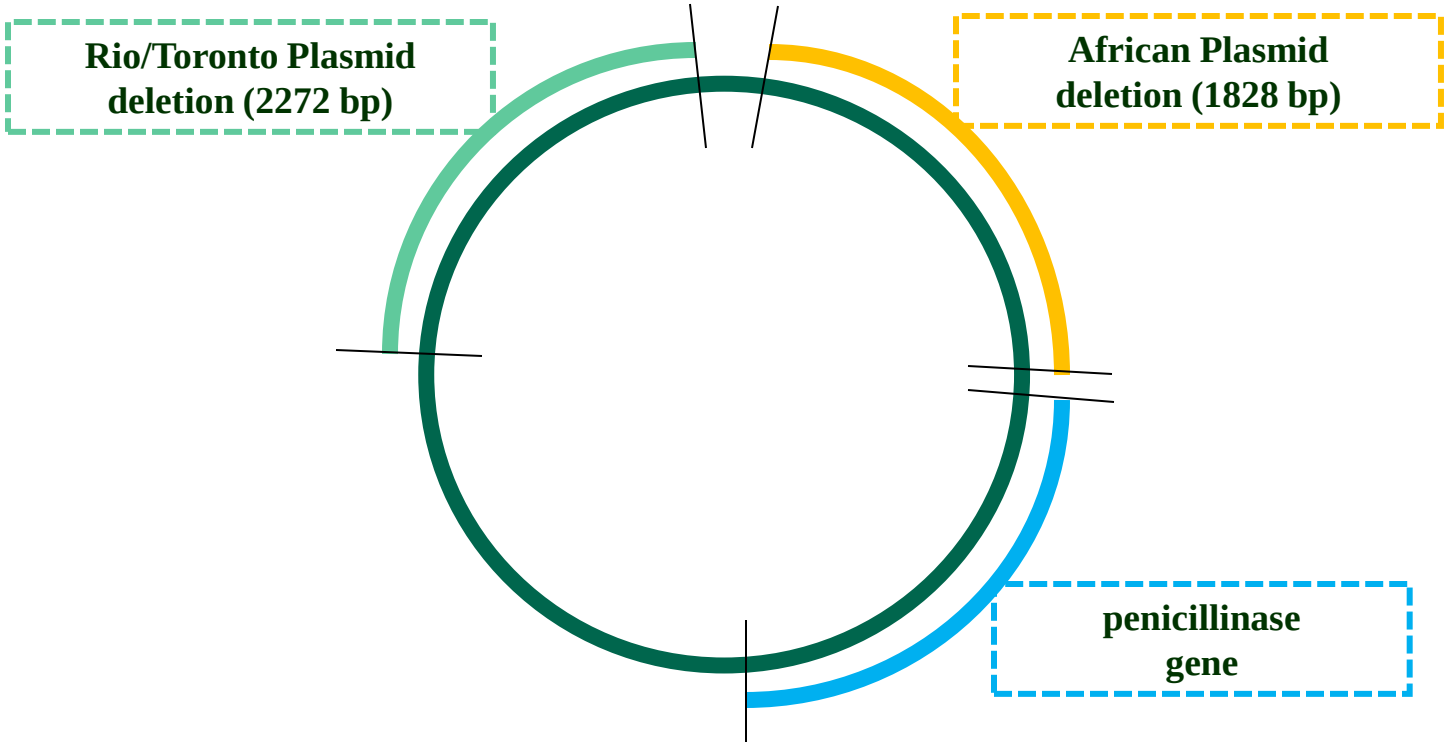
PPNG-PCR:

- a PCR to detect penicillinase-producing *N.gonorrhoeae* (PPNG)
- targets gonococcal plasmid(s) carrying penicillinase gene.

(Goire et al. J Clin Microbiol. 2011 Feb;49(2):513-8.)



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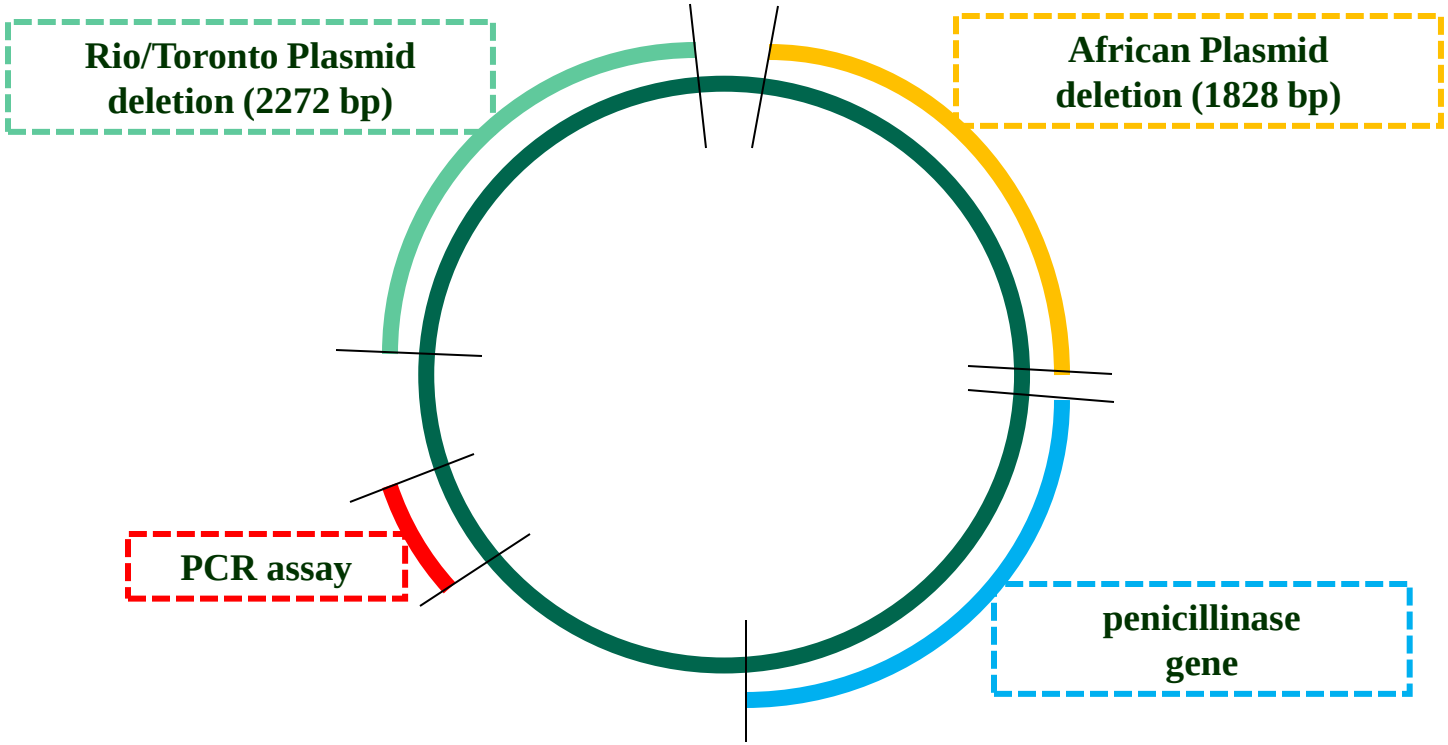

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- **Highly predictive of penicillin resistance (PPNG only, but misses CMRP)**

Application:

- **Remote settings in Australia.**
  - Penicillin still used for NG treatment in many remote communities.
  - PPNG-PCR provides enhanced surveillance for penicillin resistance only.
    - ❖ **used to inform gonorrhoea management guidelines**

(Speers et al. J Antimicrob Chemother. 2014 May;69(5):1243-7.)

Provides a model:

- A single PCR assay can be used to *enhance* bacterial culture-based surveillance.

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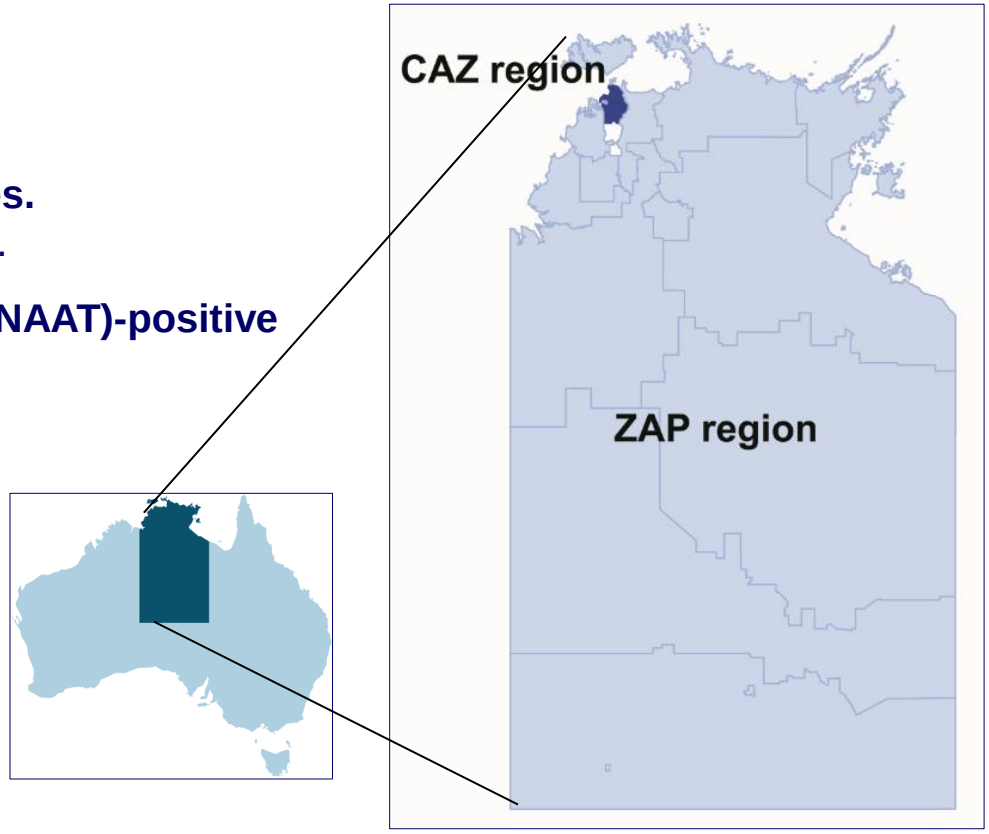
Pilot study: The Northern Territory, Australia.

Two different first-line therapies are used for gonorrhoea treatment:

- **Ceftriaxone + azithromycin (CAZ)**
- **Amoxicillin + azithromycin (ZAP)**

Year 2014:

- **GC bacterial culture; n = 222 isolates.**
  - Subjected to phenotypic AMR testing.
- **GC nucleic-acid-amplification-test (NAAT)-positive samples; n = 1,629 samples.**
  - Tested by the GC AMR PCR assays from GRAND.



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Pilot study: The Northern Territory, Australia.

	CAZ region (95% CI)		**ZAP region** (95% CI)		**Total** (95% CI)	
	Culture	PCR	Culture	PCR	Culture	PCR
Azithromycin R	**0.0%** (0.0–4.5)	**0%** (0–1.2)	**0.0%** (0.0–2.7)	**0.2%** (0.1–0.8)	**0.0%** (0.0–1.7)	**0.2%** (0.1–0.6)
Ceftriaxone DS	**3.7%** (1.3–10.3)	**2.3%** (1.3–10.3)	**0.0%** (0.0–2.7)	**0.0%** (0.0–0.3)	**1.4%** (0.5–3.9)	**0.6%** (0.3–1.1)
Ciprofloxacin R	**30.9%** (21.9–41.6)	**20.9%** (16.6–25.9)	**2.1%** (0.7–6.1)	**1.0%** (0.5–1.8)	**12.6%** (8.9–17.6)	**5.8%** (4.6–7.2)
Penicillin R	**25.9%** (17.6–36.4)	**21.3%** (17–26.4)	**2.1%** (0.7–6.1)	**1.6%** (0.9–2.6)	**10.8%** (7.4–15.6)	**6.7%** (5.3–8.3)
PPNG	**18.5%** (11.6-28.3)	**11.9%** (8.6-16.2)	**2.1%** (0.7-6.1)	**1.0%** (0.5-1.9)	**8.1%** (5.2-12.5)	**3.7%** (2.7-4.9)

Whiley et al. EID2017

$p<0.01$

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How can we exploit these markers for individualised therapy?

Ciprofloxacin resistance & GyrA91

Investigating GyrA 91 in **Australian** NG isolates:

n = 3,274 isolates		Culture (ciprofloxacin)	
		Susceptible	**Resistant**
GyrA 91	**wildtype**	**2,173**	**13**
	S91F	**22**	**1,066**

	sensitivity	**specificity**
Prediction of susceptibility (wildtype):	**99.0%**	**99.4%**
Prediction of resistance (S91F):	**98.8%**	**98.0%**

(Trembizki *et al.* Lancet Infect Dis. 2016 Sep;16(9):1005-6.)

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Sex Transm Dis. 2017 May;44(5):261-265.
Allan-Blitz LT1, Wang X, Klausner JD.

A systematic review of 31 studies from 16 countries. The pooled estimate of sensitivity and specificity of gyrA genotype results for the prediction of N. gonorrhoeae susceptibility to ciprofloxacin were 98.2% and 98.6%, respectively.

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Ciprofloxacin resistance & GyrA91

How can we use this GyrA marker?

Beyond surveillance ….individualised treatment of gonorrhoea.

- Where a PCR test is used to detect ciprofloxacin susceptibility at the individual patient level.
  - ❖ Ciprofloxacin is highly effective as a single oral dose (against sensitive strains)
  - ❖ If treatment decision-making were based this GyrA marker then 98% of patients could correctly receive ciprofloxacin treatment.
  - ❖ Large proportions of cases remain ciprofloxacin-susceptible.

    Eg. 70% of Australian gonorrhoea cases. AGSP

    50% of cases in Europe Euro-GASP 2014
- Potential benefits:
  - ❖ Antibiotic stewardship - spare use of ceftriaxone.
  - ❖ Expand oral treatment options for gonorrhoea.

Ciprofloxacin resistance & GyrA91

How can we use this GyrA marker?

Individualised treatment of gonorrhoea.

USA:

CID 2017
Implementation of a Rapid Genotypic Assay to Promote Targeted Ciprofloxacin Therapy of Neisseria gonorrhoeae in a Large Health System.
Allan-Blitz LT ... Klausner JD.
"We found a significant reduction in ceftriaxone use with a concomitant increase in targeted therapy."

Australia:

Study just commencing….

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- Will involve 9 sex clinics and associated labs
- Partnered with SpeeDx Pty Ltd
- Primary objectives: (1) **cure rate** (2) **acceptability.**

Ciprofloxacin resistance & GyrA91

ResistancePlus GC

	Channel	Target
1 Well	1	*N. gonorrhoeae* (*opa*)
	2	*N. gonorrhoeae* (*porA*)
	3	gyrA S91S wild type
	4	gyrA S91F mutation
	5	Internal Control

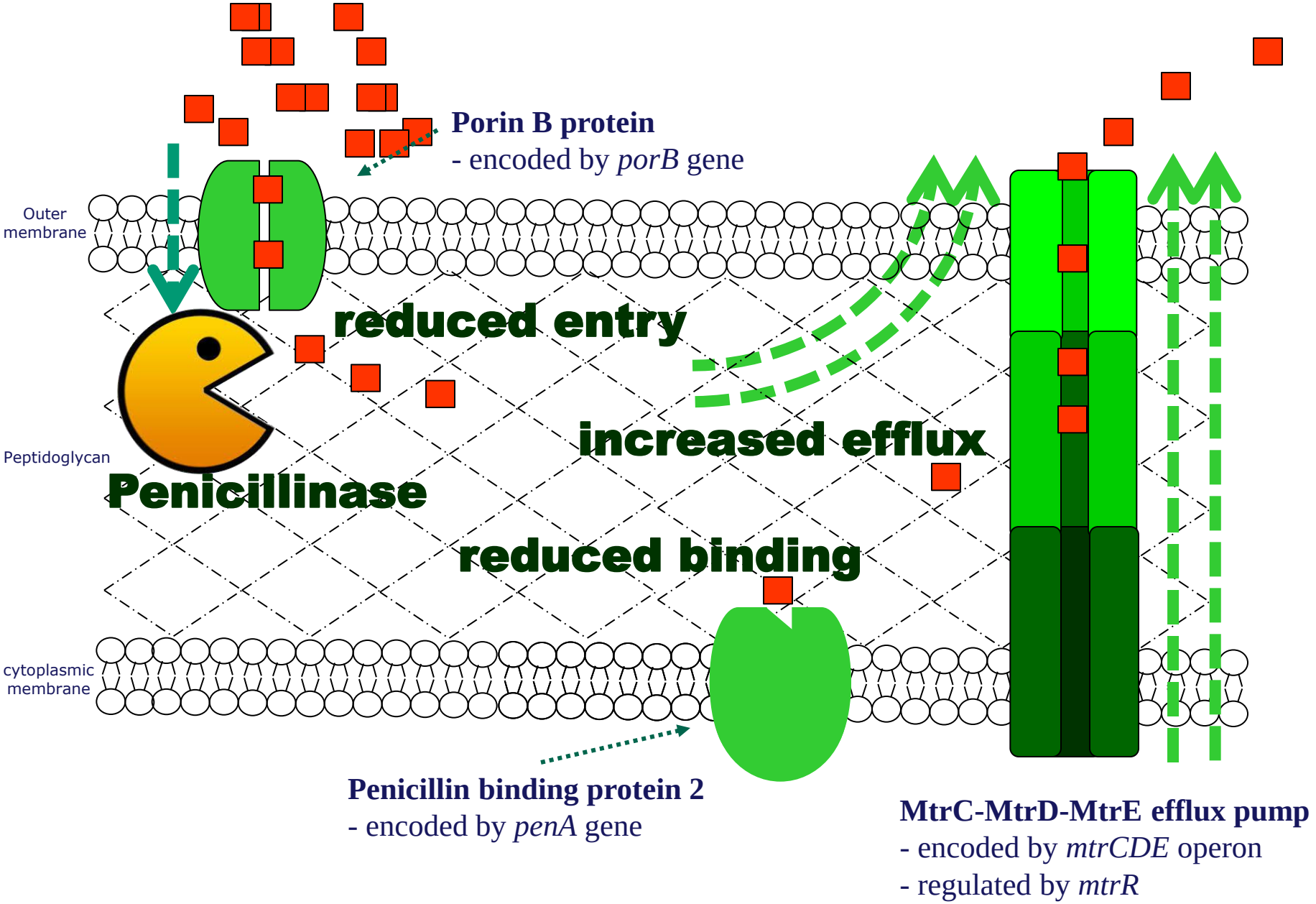
TGA Cleared
(AUSTRALIA)

CE IVD
(EUROPE)

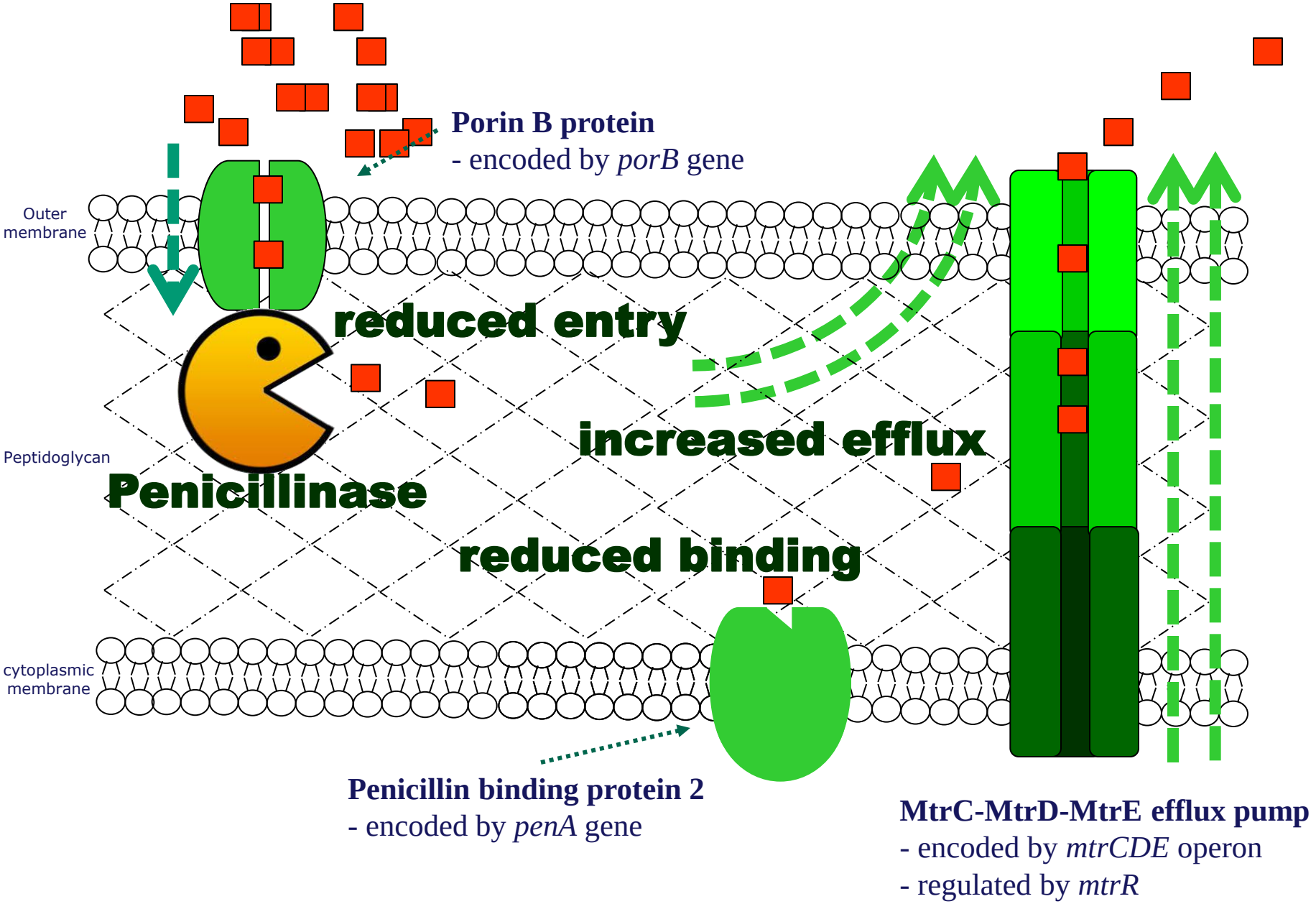
- Real-time PCR, single well assay, rapid qPCR results (<1.5 hours)
- Reflex test off GC positive result confirms GC with 2 targets, and additional the presence/absence of gyrA mutation
- Specimen Types – urogenital and extra-genital specimens

Can we individualise therapy for other antibiotics? Eg. Beta-lactams?

Yes, but is a little more complicated…



***N. gonorrhoeae* beta-lactam resistance**



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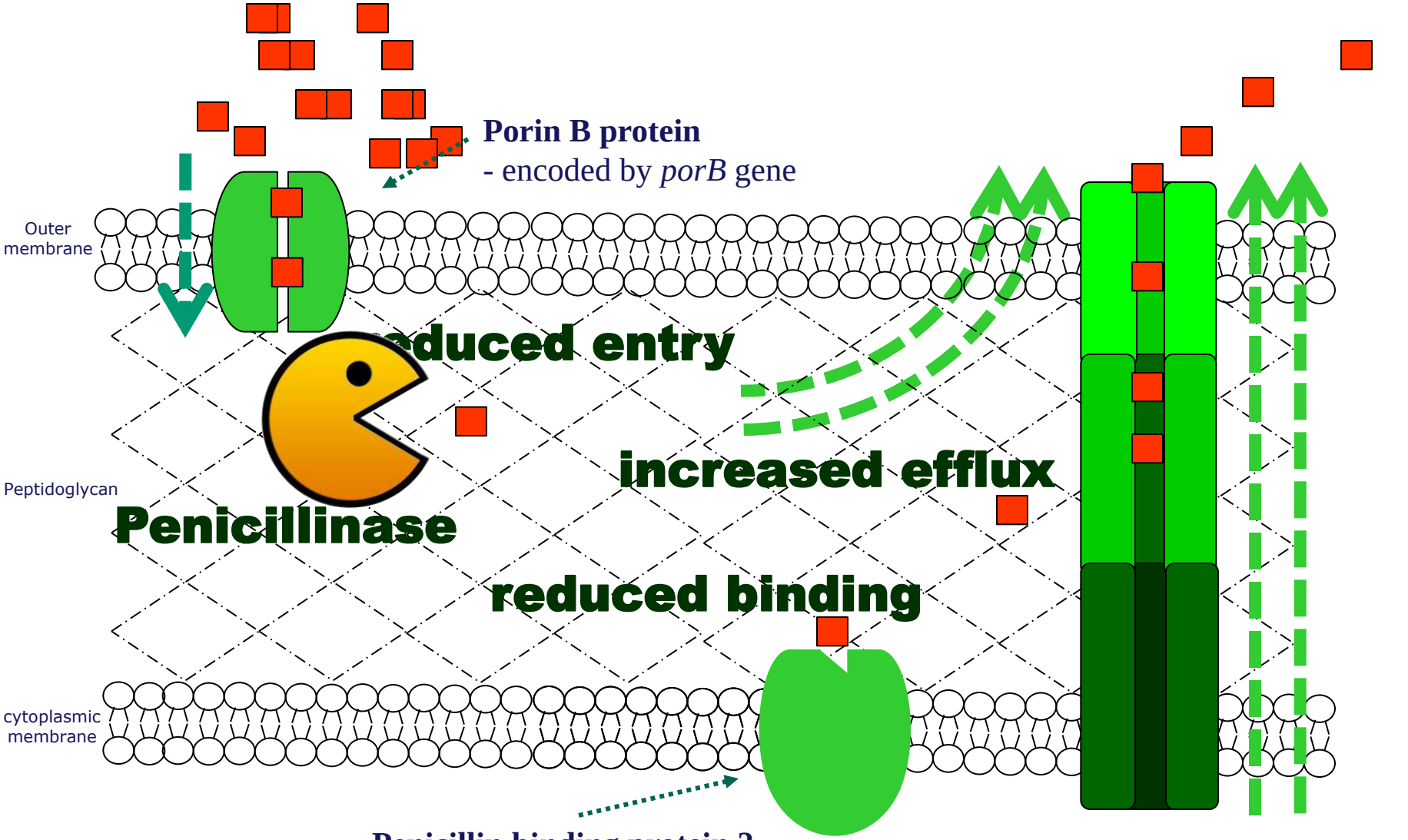

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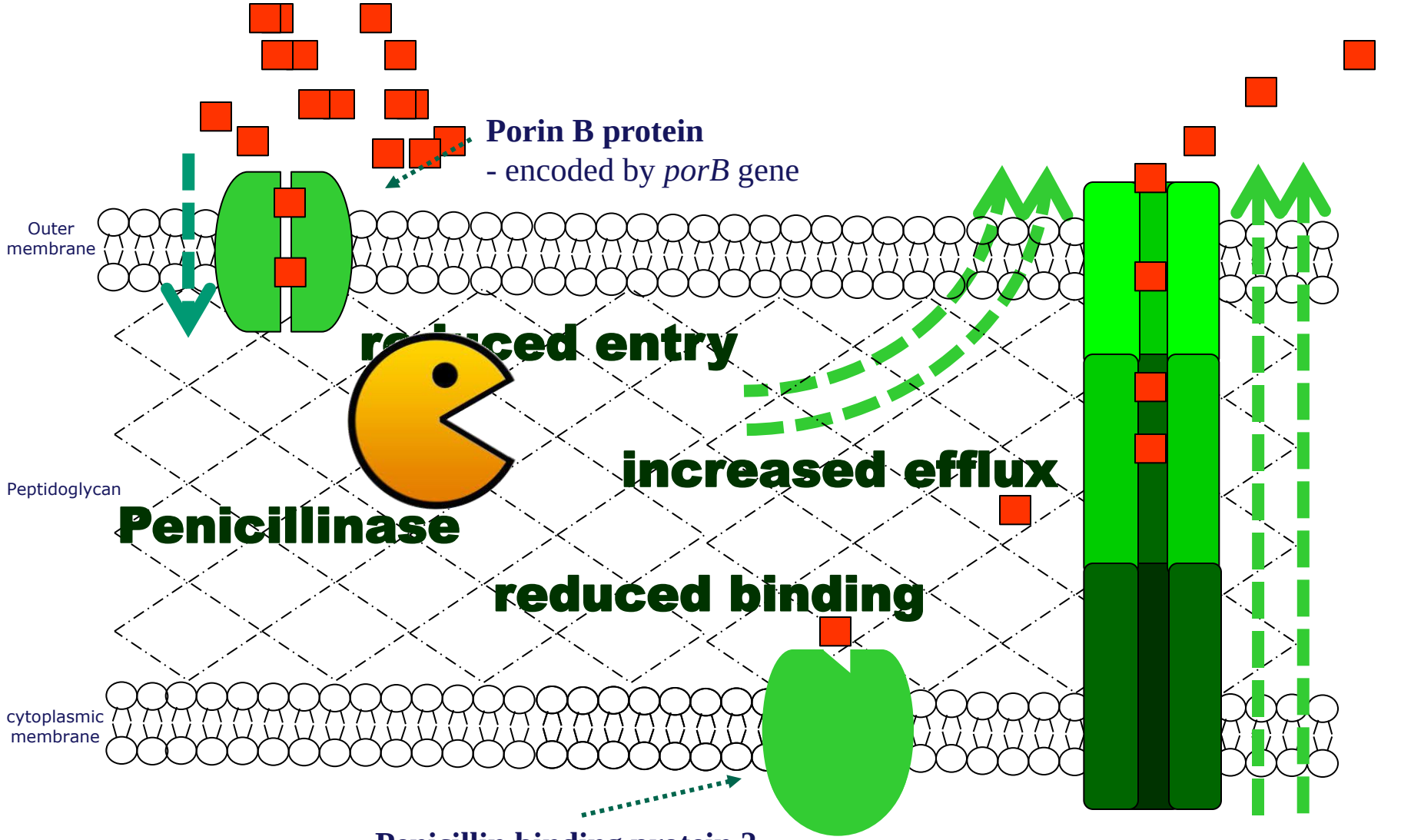

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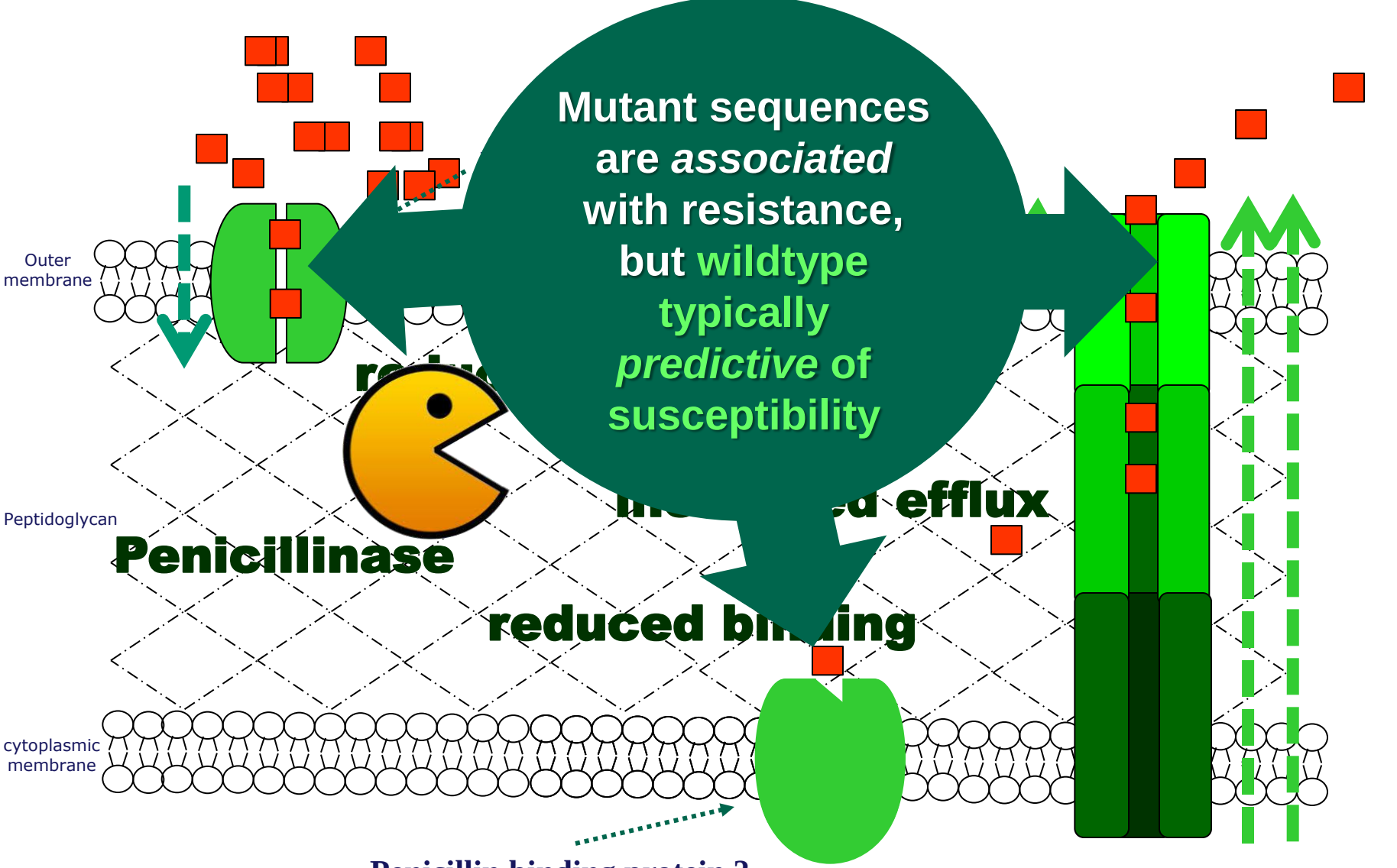

***N. gonorrhoeae* beta-lactam resistance**

Eg. Penicillin.

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wild-type porB / non-PPNG	**901**	**12**
Other porB / PPNG / not determined	**502**	**698**

	sensitivity	**specificity**
Prediction of susceptibility (wildtype):	**64.2%**	**98.7%**
Prediction of resistance (Other):	**98.3%**	**58.2%**

6)

Confident amoxicillin suitable for these patients

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ckley *et al.* JAC2016)

Miss approx. 1/3 of susceptible infections

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(Buckley *et al.* JAC2016)

Not good at predicting resistance

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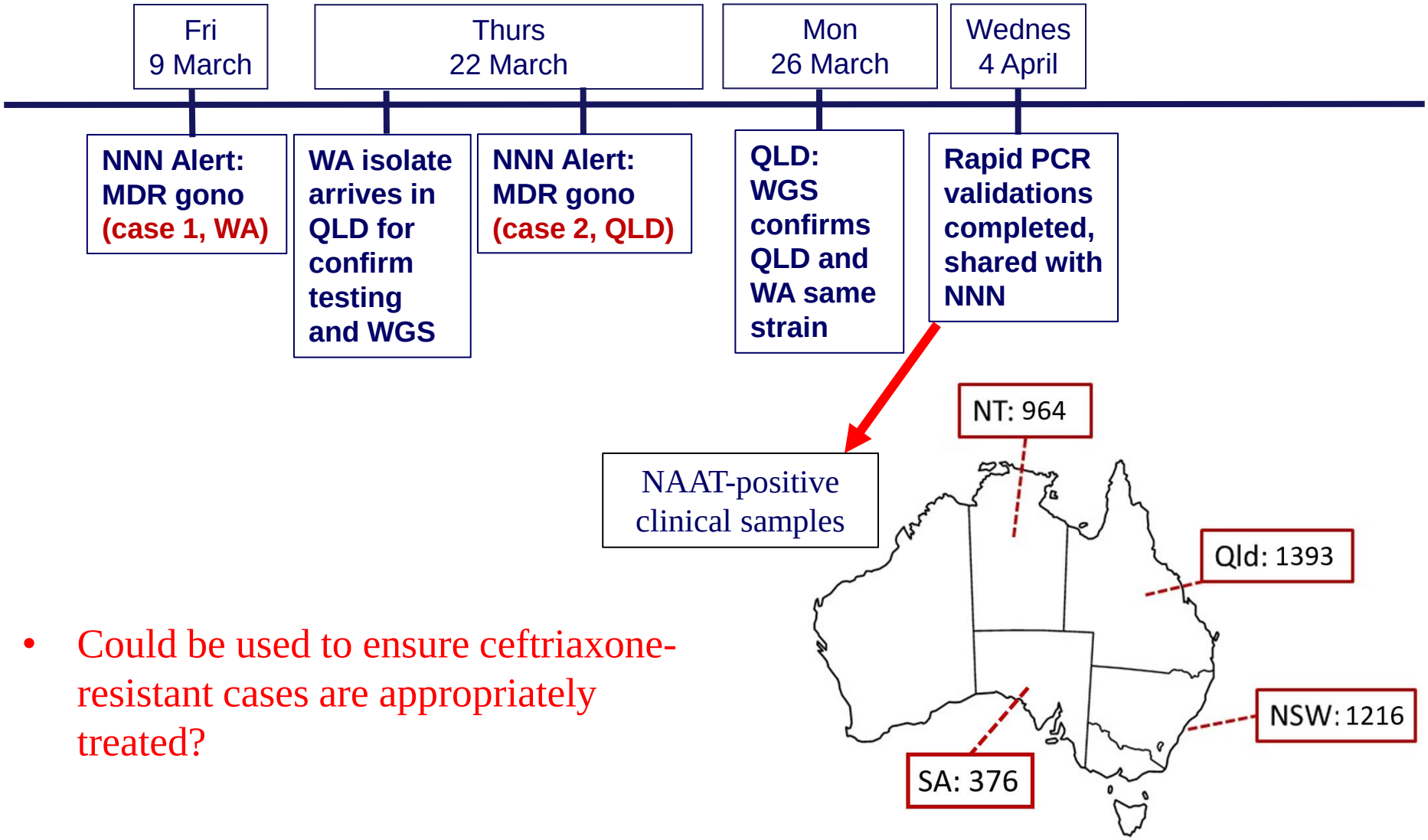
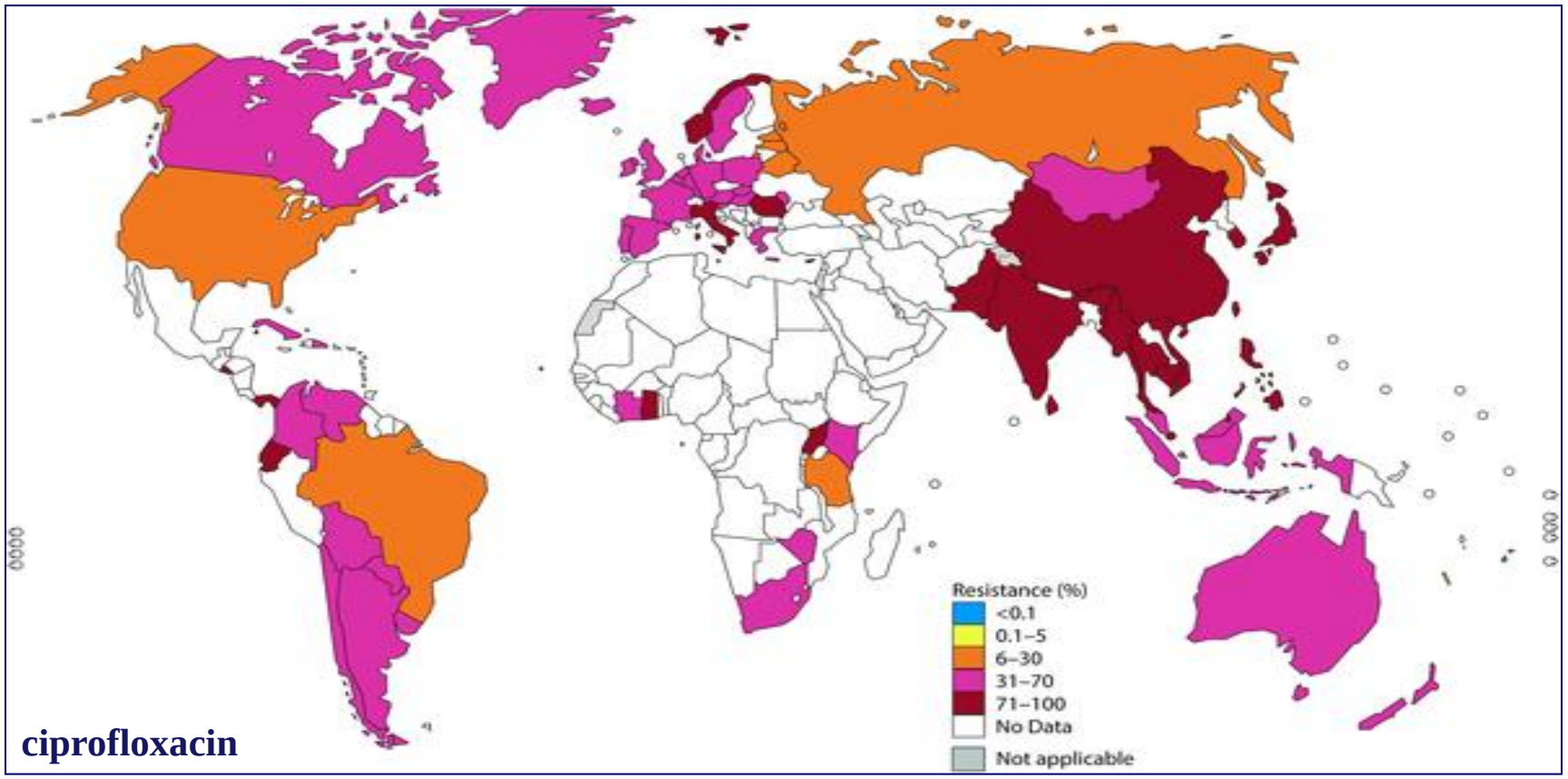
Eg. Ceftriaxone resistance and the mosaic penA-60 (FC428 and A2543 strains)

National Neisseria Network response (2018):



- Could be used to ensure ceftriaxone-resistant cases are appropriately treated?

Challenges for individualised treatment



ciprofloxacin

Wi et al. PLoS Med 2017

Challenges for individualised treatment

- Discordant results – different sites, same patient
  - Several cases involving multisite infection displayed either wild-type or mutant GyrA genotypes dependent on anatomical site. Pond *et al*. JAC. 2016; Allen-Blitz *et al*. Sex Transm Dis. 2019
- Predictive value of molecular markers need to be assessed in more settings

Conclusions

- Molecular tests for *N. gonorrhoeae* can be used to:
  - Enhance (not replace) culture-based NG AMR surveillance
  - Inform alternative treatment options
  - Rapidly assess prevalence of new markers (eg. penA-60)

Study investigators and collaborators, include:

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UQCCR	A/Prof David Whiley
	Dr Ella Trembizki
Prince of Wales Hospital, NSW	Prof Monica Lahra
	Athena Limnios
	Dr Tiffany Hogan
	Rodney Enriquez
	Dr Ratan Kundu
	Dr Namraj Goire
	Jasmin El-Nasser
Pathology Queensland	Prof Graeme Nimmo
	Dr Cheryl Bletchly
	Fleur Francis
Cairns Sexual Health Service	Dr Darren Russell
Princess Alexandra Sexual Health	Dr Cheryn Palmer
Townsville Sexual Health Services	Dr Arun Menon
Hunter New England Sexual Health	Dr Nathan Ryder
Kirby Institute, UNSW	A/Prof Rebecca Guy
	Prof John Kaldor
	Prof Basil Donovan
	Dr Handan Wand
	Dr Damian Conway
	Dr Lise Lafferty
	Dr David Regan
	Dr Stephen Bell
	Dr Marlene Kong
SAHMRI, SA	Dr James Ward
PathWest, WA	Dr David Speers
	Julie Pearson
Melbourne Sexual Health Centre	Prof Christopher Fairley
	A/Prof Marcus Chen
MDU, University of Melbourne	Prof Ben Howden
	Kerrie Stevens
Women's and Children's Hospital, SA	Andrew Lawrence
Royal Darwin Hospital	Dr Robert Baird
	Kevin Freeman
Western Diagnostic Pathology, WA	Dr Miles Beaman
	Mahdad Karimi
Forensic and Scientific Services, Qld	John Bates
	Helen Smith
	Dr Amy Jennisen
	Vicki Hicks
Westmead Clinical School	Prof David Lewis
School of Public Health, UCLA	Prof Jeff Klausner
Sydney Sexual Health Centre	A/Prof Anna McNulty
Royal Prince Alfred Sexual Health	A/Prof Catherine O'Connor
St Vincent's Hospital, Sydney	Philip Cunningham
Royal Prince Alfred Hospital	A/Prof Sebastian van Hal
CIDM Laboratory, Westmead Hospital	Ian Carter
Pathology North, Newcastle	Dr Rodney Givney
Clinic 34, NT	Dr Manoji Gunathilake
Menzies, NT	Dr Jiunn-Yih Su

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SpeeDx Pty Ltd

NNN AUSTRALIA
NATIONAL NEISSERIA NETWORK