Nucleic acid amplification testing for *Neisseria gonorrhoeae* – where are we going?

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N. gonorrhoeae NAAT background:

Key advantages:

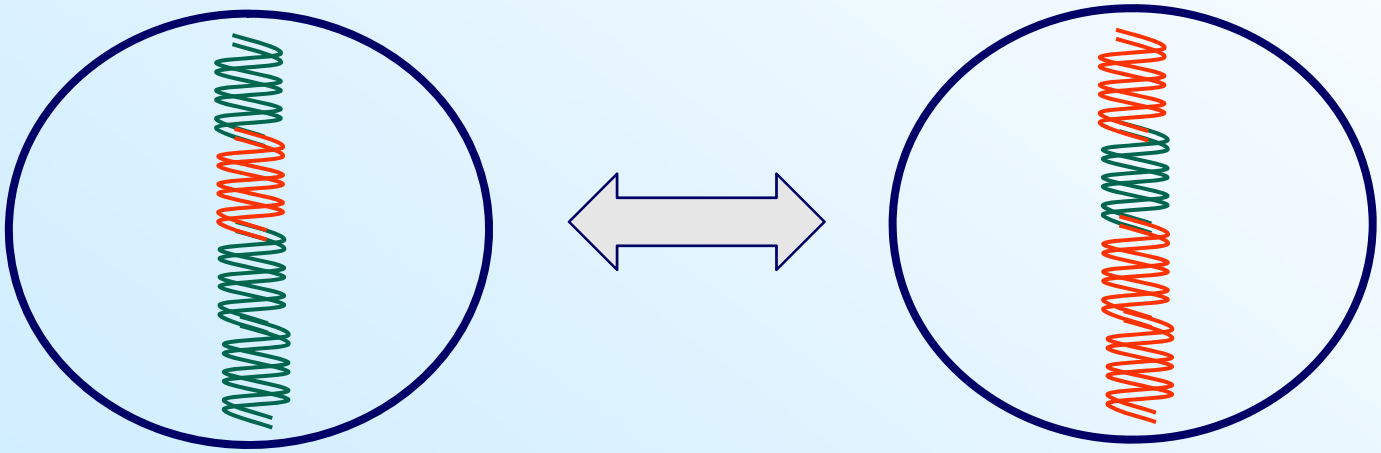
- Very sensitive
- Can be used on non-invasive specimens
- Do not require viable organism

Limitations:

1. **Specificity problems**
2. **Do not provide antimicrobial resistance data**

1. *N. gonorrhoeae* NAAT specificity problems:

- **A major concern for *N. gonorrhoeae* NAATs.**
  (Not a problem for most other STIs.)
- **Arises from frequent genetic exchange** between the *Neisseria* species, leading to acquisition of *N. gonorrhoeae* sequences by commensal *Neisseria* species



Neisseria gonorrhoeae

Commensal Neisseria

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Species	No. of isolates	No. positive
		COBAS Amplicor
Moraxella catarrhalis	**14**	**2**
Moraxella osloensis	**2**	**1**
Neisseria animalis	**1**	**1**
Neisseria caviae	**1**	**1**
Neisseria cinerea	**14**	**2**
Neisseria elongata	**1**	**0**
Neisseria flava	**1**	**0**
Neisseria flavescens	**7**	**2**
Neisseria kochii	**4**	**0**
Neisseria lactamica	**30**	**1**
Neisseria meningitidis	**75**	**13**
Neisseria mucosa	**11**	**0**
Neisseria pharyngis	**1**	**1**
Neisseria polysaccharеae	**5**	**1**
Neisseria sicca	**16**	**4**
Neisseria subflava	**41**	**3**
Neisseria weaver	**1**	**0**
Other *Neisseria* species	**9**	**1**

(Tabrizi et al. Evaluation of six commercial nucleic acid amplification tests for detection of Neisseria gonorrhoeae and other Neisseria species. J Clin Microbiol. 2011 Oct;49(10):3610-5.)

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Eg. Australian study – Victoria

Amplicor *N.gonorrhoeae* assay – positives confirmed by a second method.

Sample	Amplicor NG POS	Assay 2 POS (neg)	Confirm rate
Uro-genital	264	163 (101)	61.7%
Rectal	200	40 (160)	20.0%
Throat	447	25 (422)	5.6%

(Leslie et al. Commun Dis Intell. 2003;27:373-379)

Public Health Laboratory Network (PHLN) Meeting
Melbourne, March 2005:

- Brought together Australian experts of NG NAAT testing to create guidelines for use of NG NAATs in Australia. ***(Smith et al. Commun Dis Intell 2005; 29:358-365.)***

- Key recommendations (2-4):

    All NG NAAT positive results should be retested by a reliable supplementary NG NAAT.

However, things have changed…

Recent findings:

1. Later generation NG NAATs are more specific.

- Commercial companies have sought more specific sequence targets.

Species	No. of isolates	No. positive			
		COBAS Amplicor	Abbott CT/NG	APTIMA COMBO 2	COBAS 4800
Moraxella catarrhalis	14	2	0	0	0
Moraxella osloensis	2	1	0	0	0
Neisseria animalis	1	1	0	0	0
Neisseria caviae	1	1	0	0	0
Neisseria cinerea	14	2	0	0	0
Neisseria elongata	1	0	0	0	0
Neisseria flava	1	0	0	0	0
Neisseria flavescens	7	2	0	0	0
Neisseria kochii	4	0	0	0	0
Neisseria lactamica	30	1	0	0	1
Neisseria meningitidis	75	13	1	4	0
Neisseria mucosa	11	0	1	0	0
Neisseria pharyngis	1	1	0	0	0
N. polysacchareae	5	1	0	0	0
Neisseria sicca	16	4	0	1	0
Neisseria subflava	41	3	0	0	1
Neisseria weaver	1	0	0	0	0
Other *Neisseria* species	9	1	0	0	0

(Tabrizi et al. Evaluation of six commercial nucleic acid amplification tests for detection of Neisseria gonorrhoeae and other Neisseria species. J Clin Microbiol. 2011 Oct;49(10):3610-5.)

Recent findings:

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- Commercial companies have sought more specific sequence targets.

Eg. **Abbott RealTime NG PCR Assay.**

Sample	Abbott NG POS	Assay 2 POS (neg)	Confirm rate
Uro-genital	151	147 (4)	97.4%
Rectal	22	21 (1)	95.5%
Throat	62	59 (3)	95.2%

(Thanks to Todd Pryce, PathWest Laboratory Medicine, Perth, WA, Aust)

Low load NG-positive samples?

Eg. **Roche 4800 NG PCR Assay.**

Sample	4800 NG POS	Assay 2 POS (neg)	Confirm rate
Uro-genital	325	316 (9)	97.3%
Rectal	23	21 (2)	91.3%
Throat	21	17 (4)	81.0%

(Thanks to Fleur Francis, Pathology Queensland, Townsville, Qld, Aust)

Recent findings:

1. Later generation NG NAATs are more specific.

- Commercial companies have sought more specific sequence targets.

2. NG bacterial culture is particularly insensitive for extra-genital sites. Eg. throat swabs.

Specimen	***Bacterial culture (under optimal conditions)*** **Sensitivity**
Urethral swabs	**90-100%**
Throat swabs	**50%**

Why?:

- **NG generally at lower loads in throat, at or below detection limit of culture**
- **Throat sites polymicrobial.**

(Whiley et al. Diagn Microbiol Infect Dis. 2005 52:1-5)
(Bissessor et al. J Clin Microbiol. 2011 Dec;49(12):4304-6.)
(Schachter et al. J Sex Transm Dis. 2008 Jul;35(7):637-42.)

Summary: NG-NAAT specificity

- **Later generation *N.gonorrhoeae* NAATs are very specific / reliable.**

- **The argument for supplementary testing is no longer clear-cut.**

  - Proposed change: no need for supplementary PCR for uro-genital samples providing positive results in a later generation screening assays.

2. The antimicrobial resistance problem

The problem of N. gonorrhoeae resistance

N. gonorrhoeae has developed resistance to multiple classes of antimicrobials:

- tetracycline
- penicillin
- fluoroquinolones

} withdrawn from use in many parts of the world because of widespread resistance

Extended-spectrum cephalosporins (eg. ceftriaxone) are now the mainstay of treatment, **however:**

- Increasing numbers of gonococci with reduced-susceptibility to ceftriaxone observed in Asia, Europe, USA etc. over several years.
- Late 2010. the first ceftriaxone resistant-*N. gonorrhoeae* clinical isolate, now named **H041**, was observed in Japan.

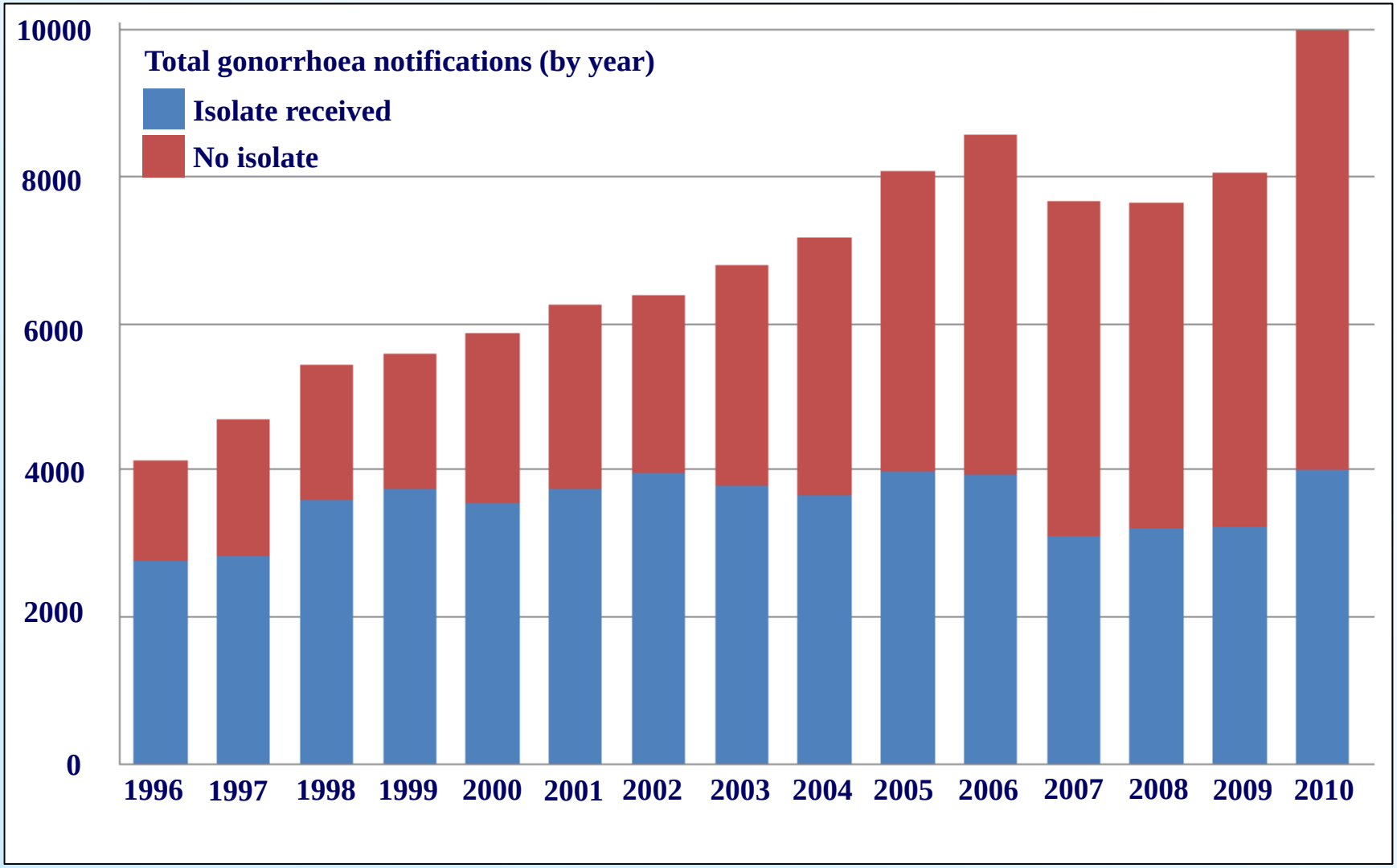
  (Ohnishi et al. Emerg Infect Dis. 2011 Jan;17(1):148-9.)
- 2011 - High-level cefixime- and ceftriaxone-resistant *N. gonorrhoeae* in France; now named **F89**.

  (Unemo et al. AAC. 2012 Mar;56(3):1273-80.)
  - **F89 subsequently found in Spain** *(Cámara J et al. JAC2012 May 7.)*

Surveillance is critical.

ISOLATES NEEDED!

Increased use of NAATs in Australia....

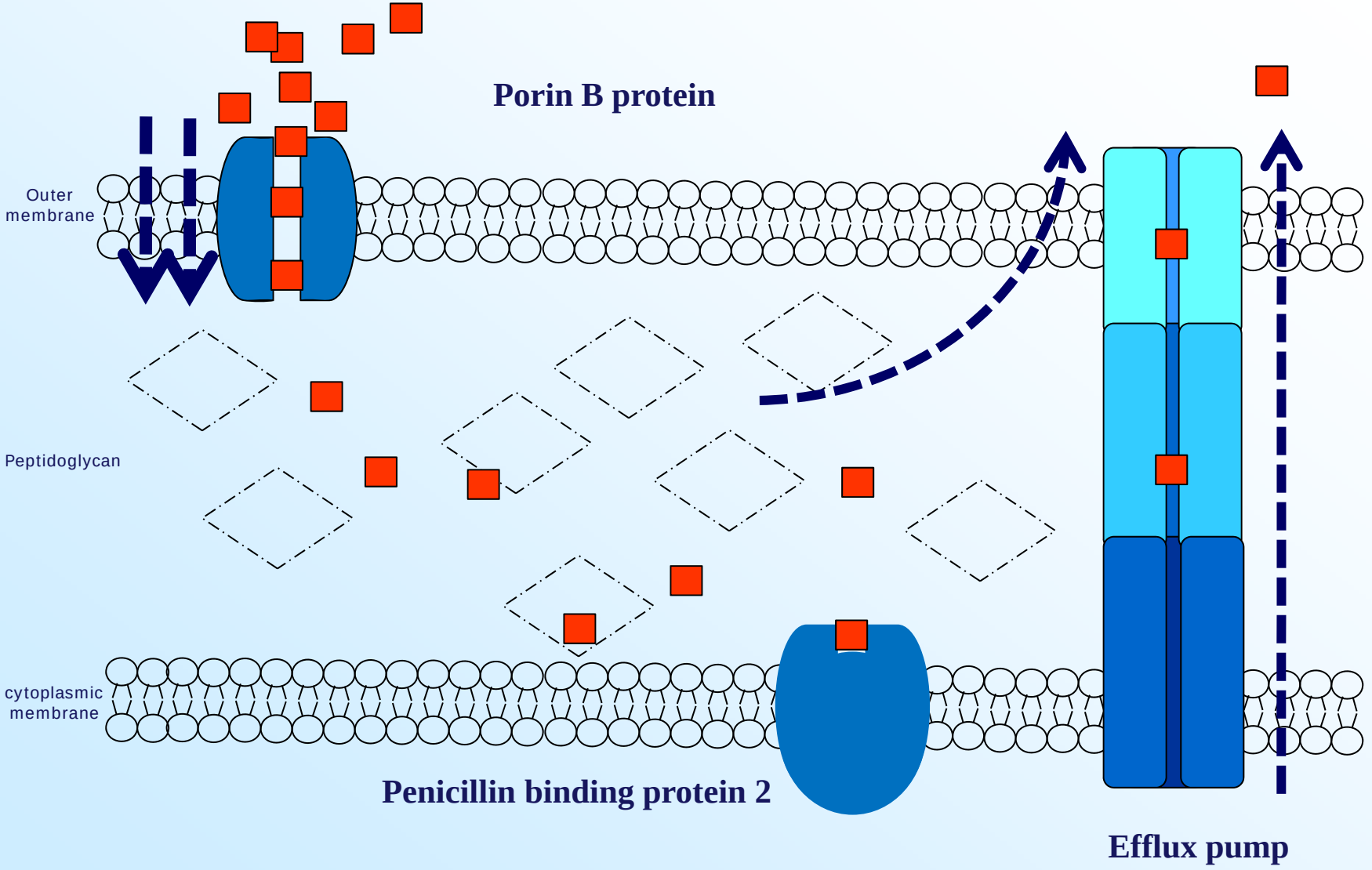


The solution:

Develop PCR methods for NG resistance surveillance.

But to this we must first understand the genetic basis of NG resistance...

NG resistance to ceftriaxone: HOW?



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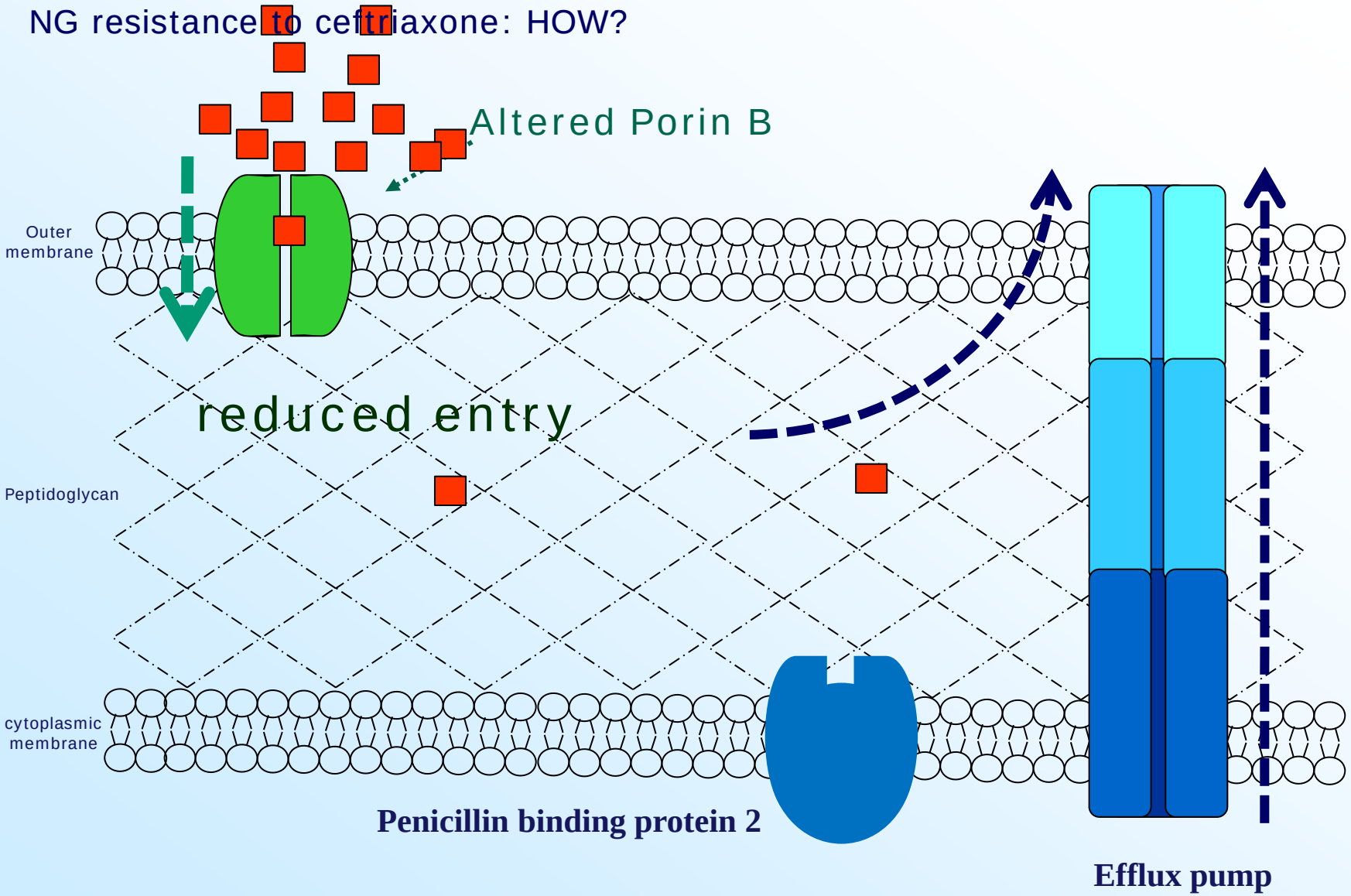

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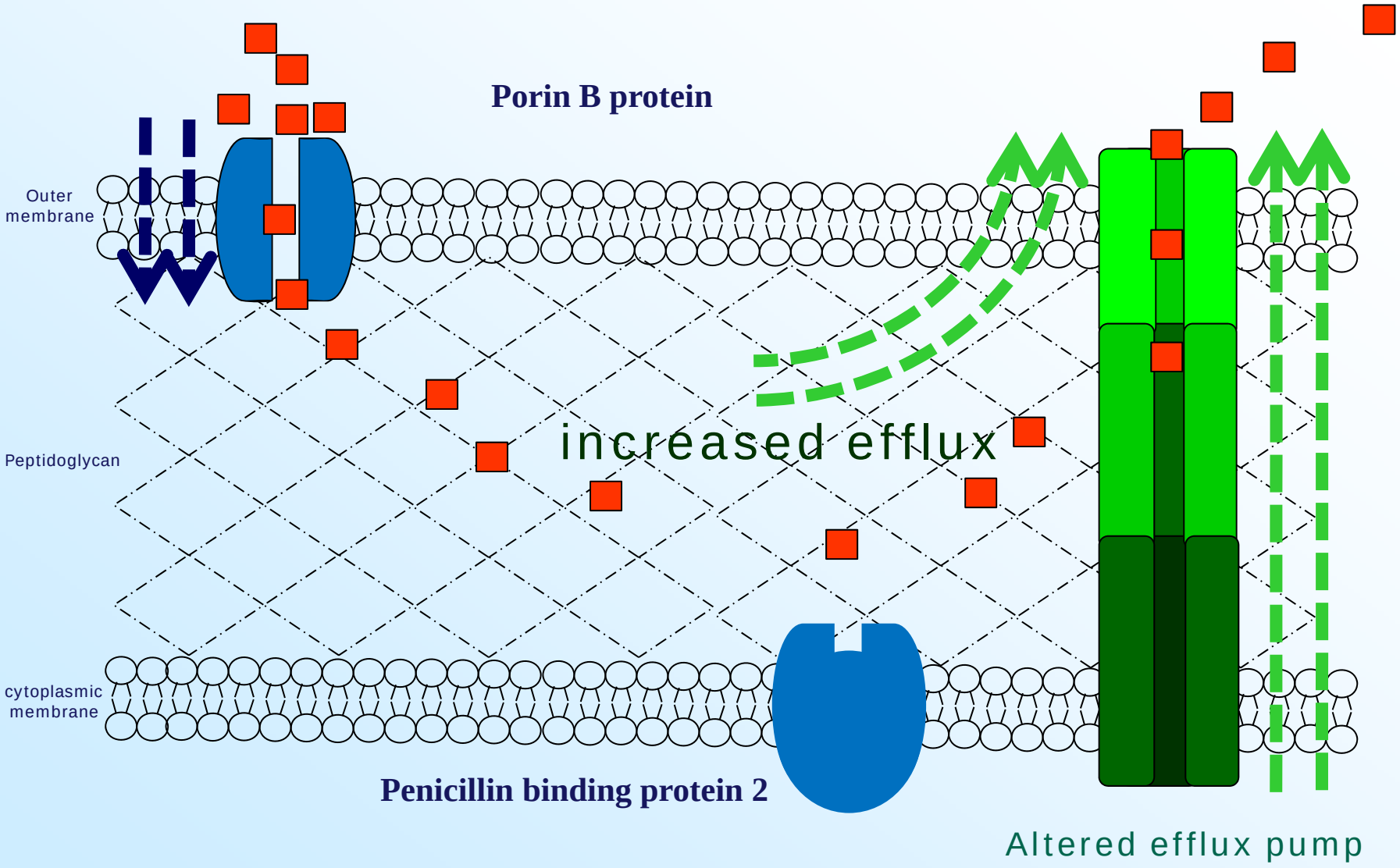

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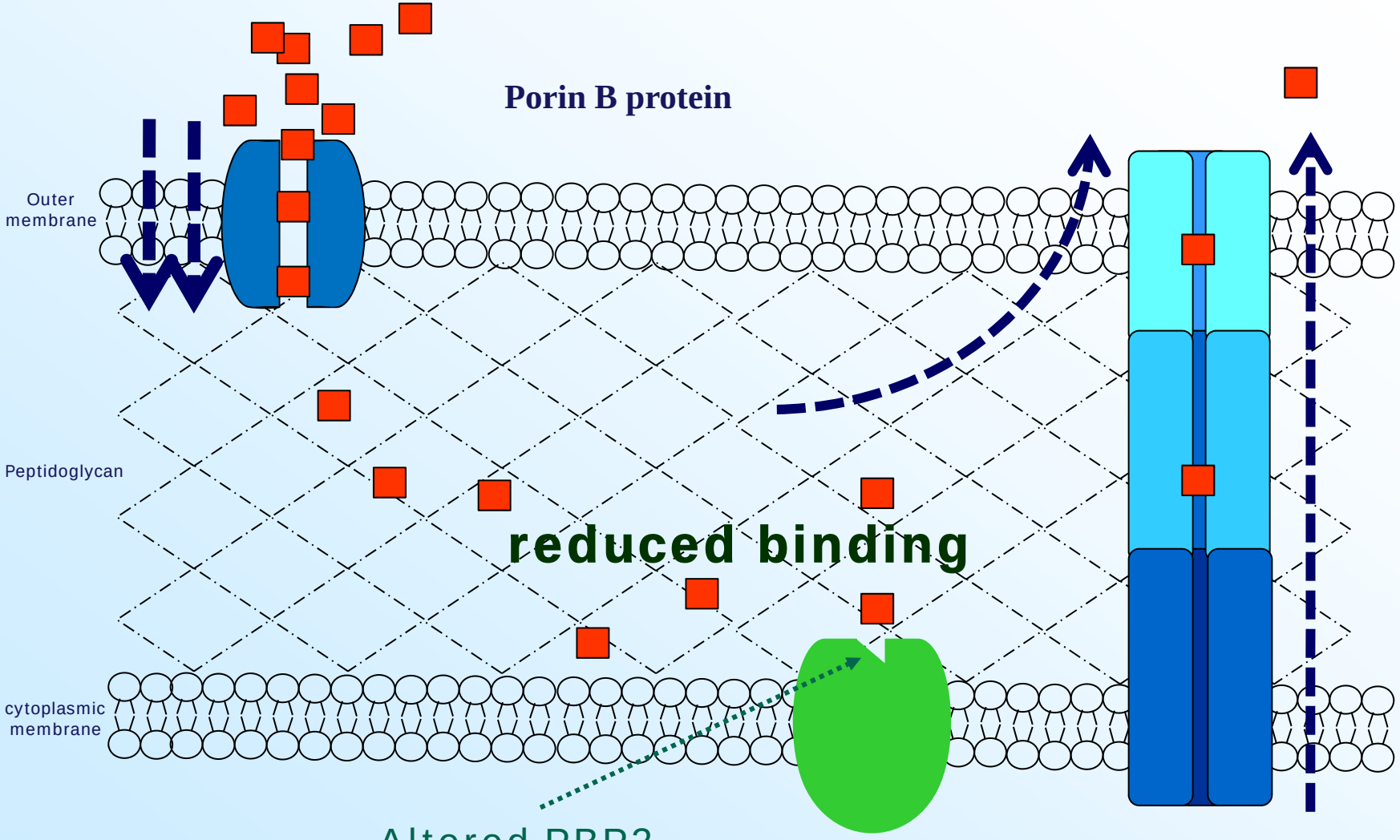
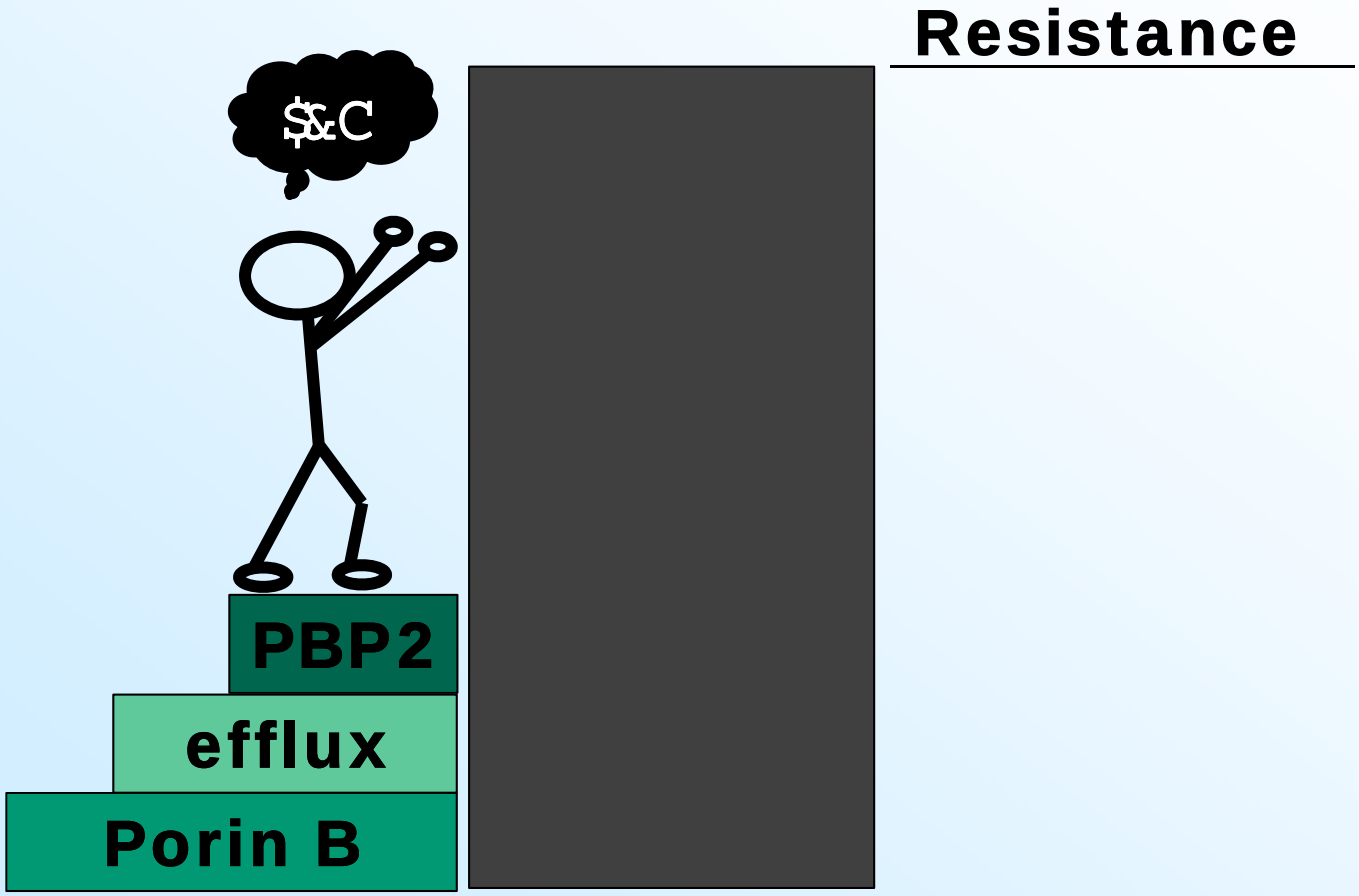


Altered PBP2
- Spontaneous mutation
- mosaic PBP2 – obtained from other species

NG resistance to ceftriaxone: HOW?



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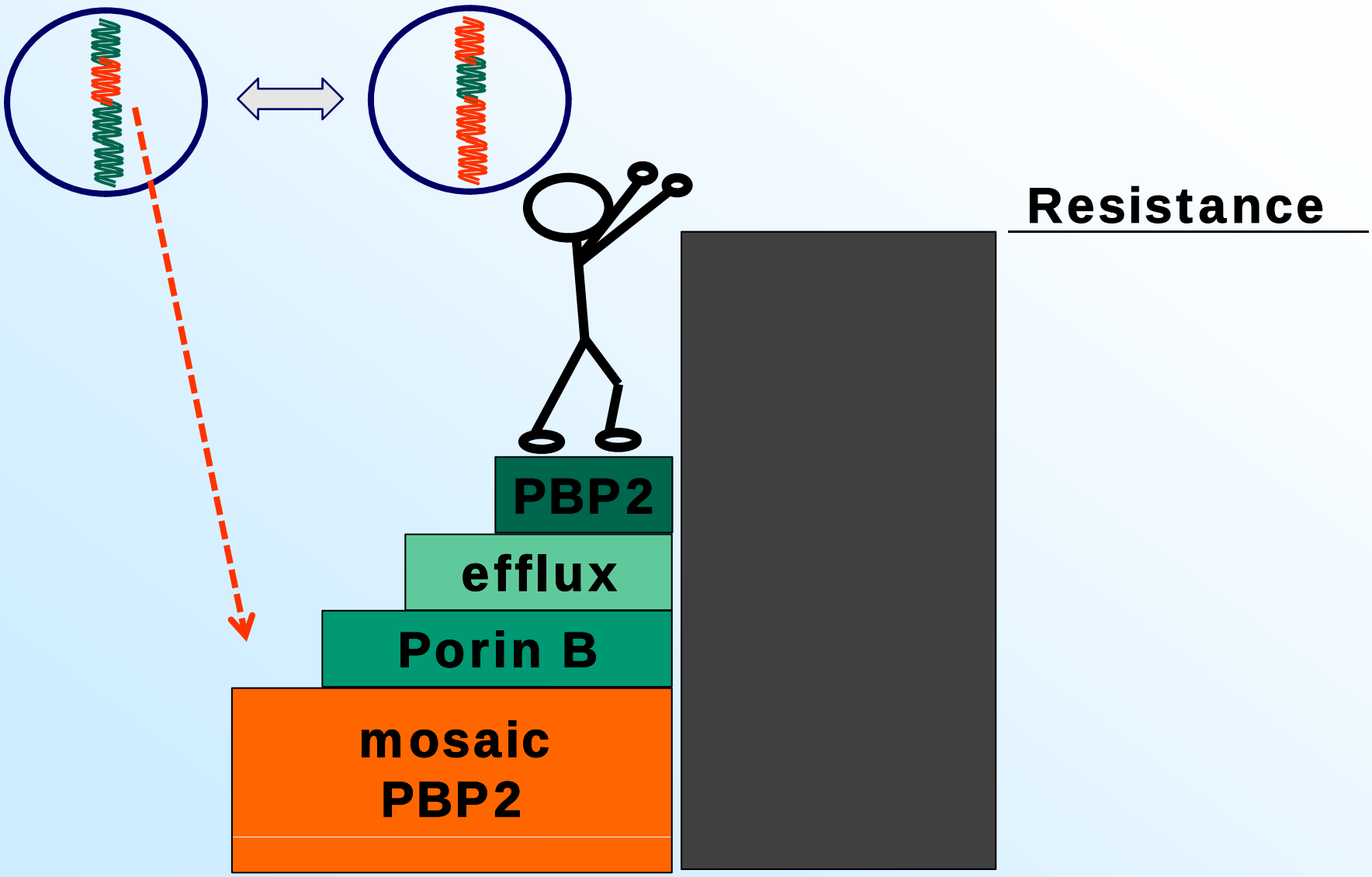
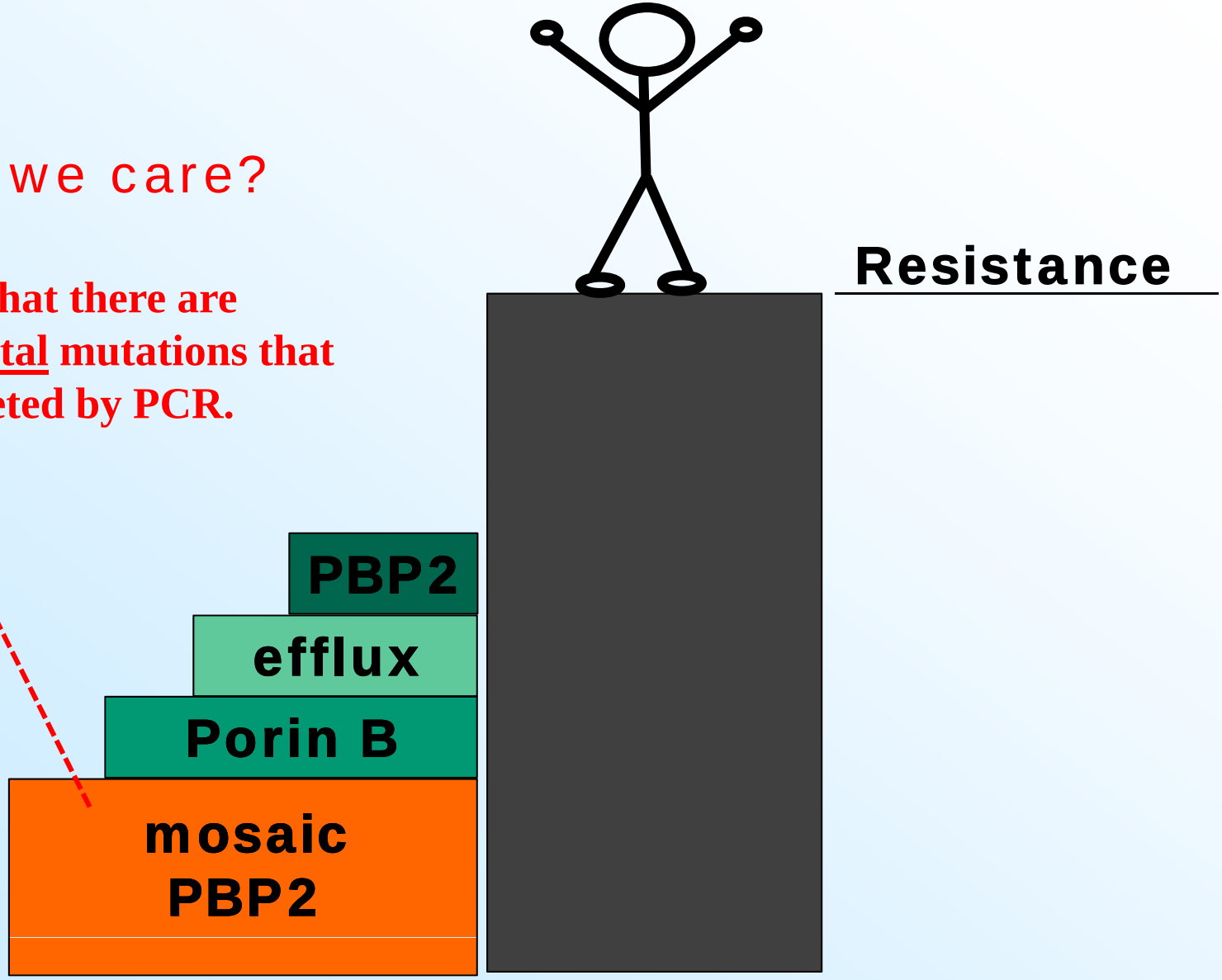


NG resistance to ceftriaxone: HOW?

Why do we care?

Highlights that there are certain pivotal mutations that can be targeted by PCR.



Eg. “Pivotal” NG mutations targeted by PCR to predict resistance:

- **ceftriaxone** resistance – H041 strain ***(Goire JAC 2012 Apr;67(4):902-5)***
- **ceftriaxone** resistance – F89 strain *(unpublished data).*
- **ciprofloxacin** resistance ***(Siedner JCM 2007 Apr;45(4):1250-4)***
- **penicillin** resistance ***(Goire JCM 2011 Feb;49(2):513-8)***

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  - Penicillin resistance: Northern Territory, Australia
    - PCR: **3.5%**
    - Culture based: 2.7%

  NOTE – suitable to examine low prevalence.

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- NHMRC (APP1025517) funded study:

➢ **Develop PCR methods for gonorrhoea resistance surveillance.**

- ❖ NG isolates obtained from throughout Aust. during first half of 2012.
- ❖ Isolates (approx 2,000) will be investigated for mutations that confer resistance to penicillin, ciprofloxacin, ceftriaxone, azithromycin etc

Conclusions:

- **PCR-based surveillance for NG resistance is feasible.**
  - Could be used in conjunction with (or in place of) current supplementary testing PCR methods. (**Eg. consider uro-genital specimens.)**

- **However, these methods can only be used to enhance bacterial-culture based surveillance, not replace it.**
  - Bacterial culture must be maintained to some extent:
    - identify new resistant strains
    - identify new resistance mechanisms

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