

1953 DNA;  
James Watson and  
Francis Crick

# ***Neisseria gonorrhoeae* with reduced susceptibility to cefixime and ceftriaxone – association with genetic polymorphisms and a clinical concern?**

**Magnus Unemo, PhD, Assoc. Professor**

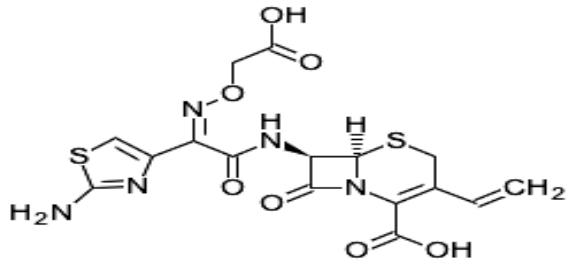
National Reference Laboratory for Pathogenic Neisseria  
Department of Clinical Microbiology



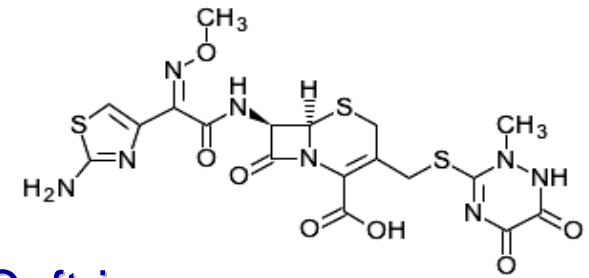
Örebro University Hospital  
Sweden

# **Reduced antimicrobial susceptibility or resistance of *N. gonorrhoeae* (GC) worldwide**

- Since decades, high prevalence of resistance to traditional antimicrobials used for gonorrhoea treatment (penicillin G, ampicillin, erythromycin, tetracycline) in most countries!
- Since mid-1990s, the prevalence of ciprofloxacin resistance has rapidly increased and is, at present, high in many countries!
- Resistance to azithromycin is still rather rare in many countries, however, has rapidly increased in several countries!
- Resistance to spectinomycin is rare but exists!
- Recently, emergence and transmission of rare strains with reduced susceptibility to cefixime and ceftriaxone have been identified!  
Occasional cefixime resistant isolates (conclusive results?)!



Cefixime



Ceftriaxone

***penA* mosaic alleles, encoding altered penicillin binding protein 2 (PBP2), are associated with reduced susceptibility to cefixime and, in less degree, ceftriaxone (cef<sup>I</sup> isolates)!**

- Ameyama S, et al. 2002. Antimicrob Agents Chemother; 46: 3744-3749.
- Ito M, et al. 2005. Antimicrob Agents Chemother; 49: 137-143.
- Tanaka M, et al. 2006. Int J Antimicrob Agents; 27: 20-26.
- Takahata S, et al. 2006. Antimicrob Agents Chemother; 50: 3638-3645.
- Ochiai S, et al. 2007. J Antimicrob Chemother; 60: 54-60.
- Lindberg R, et al. 2007. Antimicrob Agents Chemother; 51: 2117-2122.
- Whiley D, et al. 2007. Antimicrob Agents Chemother; June 25; [Epub].

# Wild type (WT) and mosaic PBP2 amino acid sequences of GC

*penA* WT  
*penA*  
mosaic  
alleles

M32091	MLIKSEYKPR	MLPKKEEQVKK	PMTSNGRISF	VLMAVAFLFA	CLIAARGLYLQ	TVTYNFLKEQ	GDNRIVRTQA	LPATRGTVSD	RNGAVLALSA	PTESLFAVPK	100
AB071984	.....	.....	.....	.....	.....	.....	.....	.....	..V.....	.....	100
30/02	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	100
59/03	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	100
35/02	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	100
158/04	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	100
19/04	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	100
188/03	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	100
201/03	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	100
273/04	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	100

M32091	DMKEMPSAAQ	LERLSELVDV	PVDVLRNKLE	QKGKSFIIWK	RQLDPKVAEE	VKALGLENFV	FEKELKRHYD	MGNLFAHVIG	FTDIDGKGQE	GLELSLEDSL	200
AB071984	E.....	.....	.....	.....	.....	.....	.....A.....	.....S.....	.....	.....	200
30/02	E.....	.....	.....	.....	.....	.....	.....A.....	.....S.....	.....	.....	200
59/03	E.....	.....	.....	.....	.....	.....	.....A.....	.....S.....	.....	.....	200
35/02	E.....	.....	.....	.....	.....	.....	.....A.....	.....	.....	.....	200
158/04	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	200
19/04	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	200
188/03	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	200
201/03	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	200
273/04	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	200

M32091	YGEDGAEVVL	RDRQGNIVDS	LDSPRNKAPQ	NGKDIILSLD	QRIQTLAYEE	LNKAVEYHQA	KAGTVVVLDA	RTGEILALAN	TPAYDPNRPD	RADSEQRNR	300
AB071984	HAGE.....	E.....	.....	.....	.....	.....	.....	.....V.....	.....E..K..	Q.....	300
30/02	HAGE.....	E.....	.....	.....	.....	.....	.....	.....V.....	.....E..K..	Q.....	300
59/03	HAGE.....	E.....	.....	.....	.....	.....	.....	.....V.....	.....E..K..	Q.....	300
35/02	HAGE.....	E.....	.....	.....	.....	.....	.....	.....V.....	.....E..K..	Q.....	300
158/04	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	300
19/04	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	300
188/03	.....	.....	.....	.....	.....	.....	.....T.....	.....	.....	.....	300
201/03	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	300
273/04	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	300

312 316

Asp(D)-345

M32091	AVTDMIEPGS	IPPIVIAKA	LI	.....	.....	.....	.....	.....	.....	.....	399
AB071984	.....	M..T.....	.....	.....	.....	.....	.....	.....	.....	.....	399
30/02	.....	M..T.....	.....	.....	.....	.....	.....	.....	.....	.....	399
59/03	.....	M..T.....	.....	.....	.....	.....	.....	.....	.....	.....	399
35/02	.....	M..T.....	.....	.....	.....	.....	.....	.....	.....	.....	399
158/04	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	100
19/04	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	100
188/03	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	100
201/03	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	100
273/04	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	100

Proposed to be responsible for the reduced susceptibility to cefixime (Takahata, et al. 2006)

M32091	TAGLLRNWRR	WRPIEQATMS	FGYGLQLSLL	QLARAYTALT	HDGVLPLPSF	EKQAVAPQK	RIFKESTARE	VRNLMVSVTE	PGGTGTAGAV	DGFDVGAKTG	499
AB071984	.....S.....	..QK.....	.....V..	.....E..V..	.....K..	..VI.A..KK	..E.....	A.....	.....	.....	499
30/02	.....S.....	..QK.....	.....V..	.....E..V..	.....K..	..VI.A..KK	..E.....	A.....	.....	.....	499
59/03	.....S.....	..QK.....	.....V..	.....E..V..	.....K..	..VI.A..KK	..E.....	A.....	.....	.....	499
35/02	.....S.....	..QK.....	.....V..	.....E..V..	.....K..	..VI.A..KK	..E.....	A.....	.....	.....	499
158/04	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	500
19/04	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	500
188/03	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	500
201/03	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	500
273/04	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	500

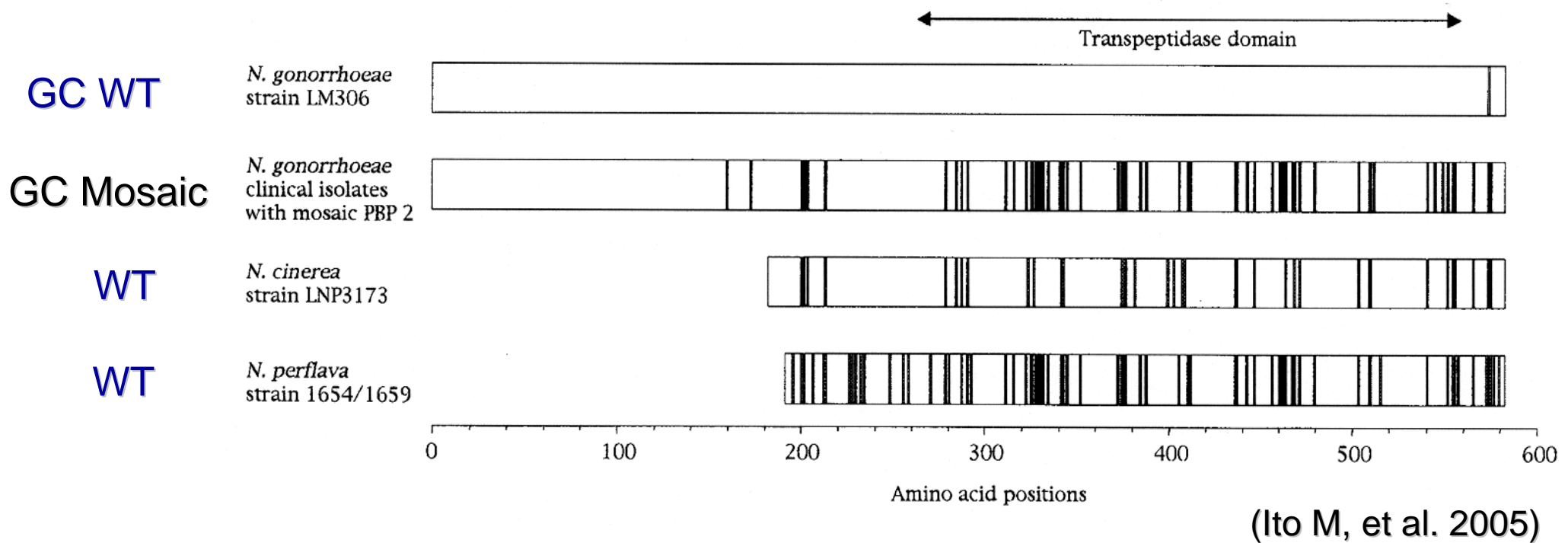
545

M32091	TARKFVNGRY	ADNKHVATFI	GFAPAKNPRV	IVAVTIDEPT	AHGYG	GVVA	GPPFKKIMGG	SLNILGISPT	KPLTA-AAVK	TPS*	582
AB071984	.....L.....	V.Y.....	.....	.....	..N..S..T..	..V..QV..	.....V..	.....NV.....	.....	.....	* 583
30/02	.....L.....	V.Y.....	.....	.....	..N..S..T..	..V..QV..	.....V..	.....NV.....	.....	.....	* 583
59/03	.....L.....	V.Y.....	.....	.....	..N..S..T..	..V..QV..	.....V..	.....NV.....	.....	.....	* 583
35/02	.....L.....	V.Y.....	.....	.....	..N..S..T..	..V..QV..	.....V..	.....NV.....	.....	.....	* 583
158/04	.....L.....	V.....	G.....	.....	..N..S..T..	..V..QV..	.....V..	.....NV.....	.....	.....	* 584
19/04	.....L.....	V.....	G.....	.....	.....	..L.....	.....	.....NV.....	.....	.....	* 583
188/03	.....V..L.....	V.....	G.....	.....	..S.....	.....	.....	.....NV.....	.....	.....	* 583
201/03	.....V..L.....	V.....	G.....	.....	..S.....	.....	.....	.....NV.....	.....	.....	* 583
273/04	.....V..L.....	V.....	G.....	.....	..S.....	.....	.....	.....NV.....	.....	.....	* 583

(Lindberg, et al. 2007)

# GC *penA* mosaic allele

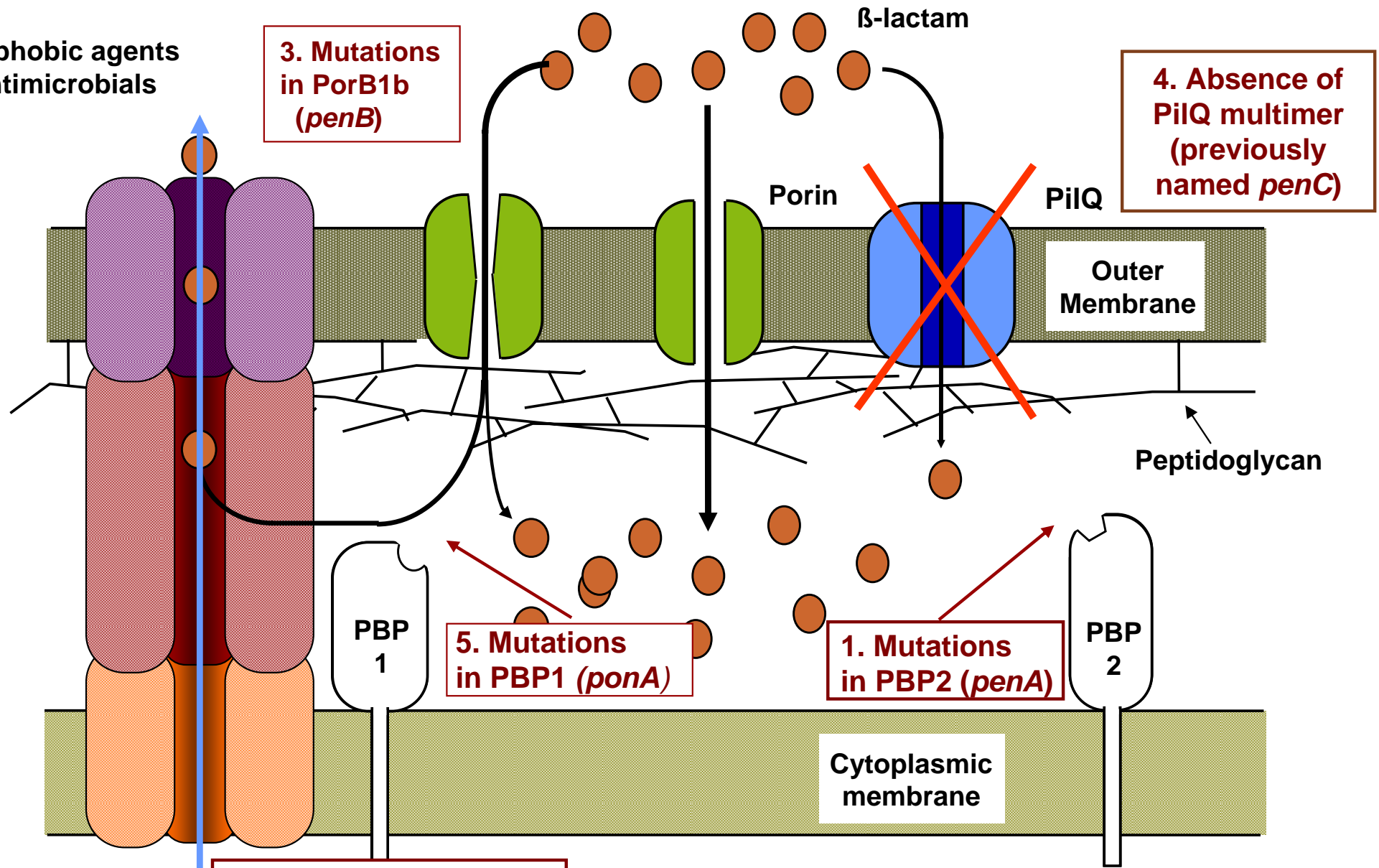
- Presumably evolved in vivo due to interspecies recombination of partial *penA* sequences from commensal *Neisseria* species such as *N. cinerea*, *N. perflava*, *N. sicca*, and *N. flavescens* (Ameyama, et al. 2002; Ito, et al. 2005; Takahata, et al. 2006; Lindberg, et al. 2007).



- GC *cef<sup>I</sup>* isolates may initially have emerged due to clonal expansion of rather few transformed strains (Ito, et al. 2005; Lindberg, et al. 2007) but the number of strains, i.e. different sequence types, have increased (Whiley, et al. 2007). Horizontal exchange of entire *penA* mosaic alleles between GC strains?

# Cell wall of *Neisseria gonorrhoeae* and determinants for chromosomally mediated resistance to penicillins

Hydrophobic agents and antimicrobials



Modified figure kindly provided by Rob Nicholas, University of North Carolina at Chapel Hill, USA

# *Neisseria gonorrhoeae* Isolates with Reduced Susceptibility to Cefixime and Ceftriaxone: Association with Genetic Polymorphisms in *penA*, *mtrR*, *porB1b*, and *ponA*<sup>∇</sup>

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National Reference Laboratory for Pathogenic *Neisseria*, Department of Clinical Microbiology, Örebro University Hospital, Örebro, Sweden,<sup>1</sup> and Department of Pharmacology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina<sup>2</sup>

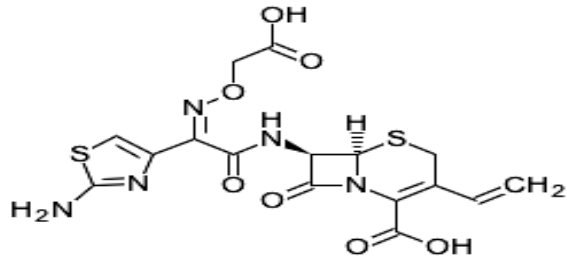
TABLE 1. Country of origin, serovar, antibiogram, NG-MAST, *porB* allele, and the polymorphisms in *penA*, *mtrR*, *porB1b*, and *ponA* alleles of *N. gonorrhoeae* isolates with reduced susceptibility to cefixime and ceftriaxone

Isolate (no./yr)	Origin	Serovar	<i>porB</i> allele	NG-MAST	MIC <sup>a</sup> (μg/ml)			Polymorphisms in:				
					CFM	CRO	PEN	<i>penA</i>		<i>mtrR</i> <sup>e</sup>	<i>porB1b</i> <sup>f</sup>	<i>ponA</i> <sup>g</sup>
								Mosaic allele	Insertion (GAC)			
119/04	Sweden	IB-2	3	ST5	0.032	0.064	2.0	No	D <sup>345a</sup>	Deletion of A	K <sup>101</sup> , D <sup>102</sup>	p <sup>421</sup>
158/04	Sweden	IB-3	4	ST1724 <sup>b</sup>	0.047	0.094	1.5	No	D <sup>345a</sup>	Deletion of A	K <sup>101</sup> , D <sup>102</sup>	WT
188/03	United Kingdom	IB-16	5	ST1619	0.064	0.064	1.0	No	D <sup>345a</sup>	WT <sup>c,d</sup>	K <sup>101</sup> , D <sup>102</sup>	p <sup>421</sup>
201/03	United Kingdom	IB-16	5	ST1619	0.064	0.064	1.0	No	D <sup>345a</sup>	WT <sup>d</sup>	K <sup>101</sup> , D <sup>102</sup>	p <sup>421</sup>
253/04	Sweden	IB-4	6	ST1723 <sup>b</sup>	0.064	0.064	1.5	No	D <sup>345a</sup>	WT <sup>d</sup>	D <sup>101</sup> , WT	p <sup>421</sup>
273/04	Sweden	IB-4	6	ST1723 <sup>b</sup>	0.064	0.094	1.5	No	D <sup>345a</sup>	WT <sup>d</sup>	D <sup>101</sup> , WT	p <sup>421</sup>
196/03	United Kingdom	IB-16	5	ST1619	0.094	0.094	1.0	No	D <sup>345a</sup>	WT <sup>d</sup>	K <sup>101</sup> , D <sup>102</sup>	p <sup>421</sup>
66/02	Sweden	IB-1	7	ST326	0.19	0.094	2.0	Yes		Deletion of A <sup>d</sup>	K <sup>101</sup> , D <sup>102</sup>	p <sup>421</sup>
30/02	Sweden	IB-1	7	ST326	0.25	0.094	3.0	Yes		Deletion of A <sup>d</sup>	K <sup>101</sup> , D <sup>102</sup>	p <sup>421</sup>
64/02	Sweden	IB-1	7	ST326	0.25	0.094	2.0	Yes		Deletion of A <sup>d</sup>	K <sup>101</sup> , D <sup>102</sup>	p <sup>421</sup>
65/02	Sweden	IB-1	7	ST326	0.25	0.094	1.5	Yes		Deletion of A <sup>d</sup>	K <sup>101</sup> , D <sup>102</sup>	p <sup>421</sup>
67/02	Sweden	IB-1	7	ST326	0.25	0.094	2.0	Yes		Deletion of A <sup>d</sup>	K <sup>101</sup> , D <sup>102</sup>	p <sup>421</sup>
59/03	United States	IB-1	8	ST925	0.25	0.094	6.0	Yes		Deletion of A	K <sup>101</sup> , D <sup>102</sup>	p <sup>421</sup>
66/03	United States	IB-1	8	ST925	0.25	0.125	4.0	Yes		Deletion of A	K <sup>101</sup> , D <sup>102</sup>	p <sup>421</sup>
35/02	Sweden	IB-1	7	ST326	0.38	0.094	2.0	Yes		Deletion of A <sup>d</sup>	K <sup>101</sup> , D <sup>102</sup>	p <sup>421</sup>
82/03	United States	IB-1	8	ST925	0.38	0.125	6.0	Yes		Deletion of A	K <sup>101</sup> , D <sup>102</sup>	p <sup>421</sup>
90/03	United States	IB-1	8	ST925	0.38	0.125	6.0	Yes		Deletion of A	K <sup>101</sup> , D <sup>102</sup>	p <sup>421</sup>
91/03	United States	IB-1	8	ST925	0.38	0.125	6.0	Yes		Deletion of A	K <sup>101</sup> , D <sup>102</sup>	p <sup>421</sup>
119/05 <sup>h</sup>	Sweden	IB-23	1	ST1722 <sup>b</sup>	<0.016	<0.002	0.012	WT		WT	WT, WT	WT
128/05 <sup>h</sup>	Sweden	IB-1	2	ST1580	<0.016	<0.002	0.008	WT		WT	WT, WT	WT

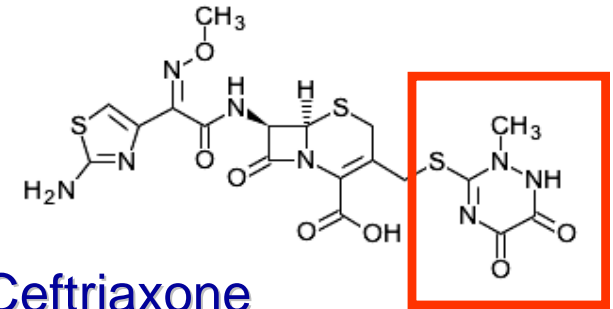
## Conclusions

- An unambiguous association between *penA* mosaic alleles, in conjunction with genetic polymorphisms in *mtrR*, *porB1b* (*penB*), and *ponA*, and greater reduced susceptibility to cefixime and ceftriaxone was identified.
- Maximum level of MIC attainable by solely these determinants as well as at least one resistance determinant are still unknown.
- Many of the Cef<sup>I</sup> isolates display a multiantimicrobial-resistant phenotype!
- Monitor the increasing MICs to expanded-spectrum cephalosporins and comprehensively elucidate the genetic basis for the reduced susceptibility are critical!

**An *unambiguous association between penA mosaic alleles*, in conjunction with genetic polymorphisms in *mtrR*, *porB1b (penB)*, and *ponA*, and greater *reduced susceptibility to cefixime and ceftriaxone?***



Cefixime



Ceftriaxone

- Still one/several unknown "resistance" determinant(s) exist and more knowledge is certainly needed!
- MICs of ceftriaxone are less affected of the *penA* mosaic alleles, which may be due to the longer side chain at the C-3 position of the cephem skeleton that might increase the affinity for the mosaic PBP2 (Takahata. 2006)
- Resistance to ceftriaxone, with subsequent treatment failure, based on the, at present, identified mechanisms will be developed?
- GC will even be able to adopt or develop some extended-spectrum  $\beta$ -lactamase (ESBL)???

*"It is difficult to make predictions – particularly about the future!"*  
Confucius (551-479 B.C.)



# Conclusions

- Emergence and increased transmission of **cefixime and ceftriaxone resistant GC** that commonly display a multiantimicrobial-resistant phenotype **is (or at least will become) a major clinical concern**, although hitherto treatment failures using ceftriaxone have not been observed!
- Given the proclivity of the GC to become resistant to all previously prescribed antimicrobials, it may be more a matter of **when and not if strains emerge that are resistant to also ceftriaxone!**
- Comprehensive knowledge regarding the **genetic basis of the reduced susceptibility** and, subsequently, maybe **development of objective genetic assays for resistance screening are crucial!**
- Continuous local, national and international **surveillance** of the antimicrobial susceptibility of *N. gonorrhoeae*, in order to **update treatment recommendations**, including dose regimens and follow-up, **is crucial!**
- **Culture and subsequent susceptibility testing** remain essential!
- Progress in development of **new or "rediscovery" of old antimicrobials?**