

Penicillin and Cefotaxime Resistance of Quinolone-Resistant *Neisseria meningitidis* Clonal Complex 4821, Shanghai, China, 1965–2020

Appendix

Appendix 2 Table 1. Characteristics of 3 cefotaxime-resistant meningococcal isolates, Shanghai, China*

Isolate	Year	Source	Age	Vaccination (MPV)	Sero-group	Sequence type	cgMLST cluster	PorA	FetA-VR	Ciprofloxacin MIC	Cefotaxime MIC, µg/mL	Ceftriaxone MIC, µg/mL	<i>penA</i>	
													<i>penA</i> allele†	phylogenetic cluster
Nm462	2017	IMD	11 m	A (2 doses)	B	ST-7962	CC4821 cluster (L44.2)	P1.12-1,13-2	F4-21	0.5 (R)	0.5 (R)	0.064 (S)	777	<i>N. subflava</i>
Nm463	2017	carriage	3	A (2 doses), A+C (1 dose)	B	ST-13501	others	P1.21-2,28	F5-64	0.25 (R)	0.25 (R)	0.094 (S)	865	<i>N. subflava</i>
Nm507	2019	IMD	2	A (2 doses)	C	ST-4821	CC4821 cluster (L44.1)	P1.21-2,9	F3-3	0.5 (R)	0.25 (R)	0.125 (S)	795	none

* IMD, invasive meningococcal disease; MPV, meningococcal polysaccharide vaccine; ST, sequence type.

†These three alleles all possess 7 mutations in the penicillin binding-protein 2 (PBP2), including A311V, T483S, F504L, A510V, I515V, H541N, and I566V.

Appendix 2 Table 2. Crossover points of recombination events in *penA* alleles shared by *Neisseria meningitidis* and commensal *Neisseria* species

<i>penA</i> allele	Cluster	Donor species	Donor [isolate (PubMLST id, Country)]		Crossover point*		Fragment length (bp)†
			Recombinant, isolate (PubMLS id)	Upstream	Downstream		
<i>penA110</i>	None	<i>N. lactamica</i>	OX40308 (44473, UK)	Nm054 (19516)	718	2048	1331
		<i>N. cinerea</i>	LNP28127 (42057, France)	Nm054 (19516)	718	2357	1640
		<i>N. polysaccharea</i>	M15 240880 (41474, UK)	Nm054 (19516)	742	2150	1409
		<i>N. bergeri</i>	01_00007_XS1_1 (43428, Mali)	Nm054 (19516)	718	2153	1436
<i>penA405</i>	<i>N. lactamica</i>	<i>N. lactamica</i>	Nei171 (105709, China)	Nm075 (52223)	1000	1804	805
		<i>N. lactamica</i>	Nei157 (108165, China)	Nm395 (52373), Nm479 (72258)	873	3199	2327
<i>penA552</i>	<i>N. lactamica</i>	<i>N. lactamica</i>	Nei033 (84202, China)	Nm393 (52746)	913	1976	1064
		<i>N. lactamica</i>	Nei033 (84202, China)	Nm464 (58441)	859	1804	946
<i>penA795</i>	None	<i>N. lactamica</i>	Nei012 (84195, China)	Nm507 (72262)	691	1819	1132
		<i>N. cinerea</i>	Nei028 (84201, China)	Nm507 (72262)	892	1819	931
		<i>N. polysaccharea</i>	Nei367 (84258, China)	Nm507 (72262)	532	1819	1291
		<i>N. subflava</i>	Nei798 (105711, China)	Nm507 (72262)	532	1819	1291
		<i>N. gonorrhoeae</i>	FC428 (108261, Japan)	Nm507 (72262)	907	1819	916
<i>penA832</i>	None	<i>N. lactamica</i>	Nei386 (108166, China)	Nm424 (52183)	709	3199	2491
		<i>N. lactamica</i>	Nei341 (83066, China)	Nm512 (71401)	718	2094	1377

<i>penA</i> allele	Cluster	Donor species	Donor [isolate (PubMLST id, Country)]		Crossover point*		Fragment length (bp)†
				Recombinant, isolate (PubMLS id)	Upstream	Downstream	
<i>penA</i> B43	<i>N. lactamica</i>	<i>N. lactamica</i>	Nei264 (105710, China)	Nm419 (55186), Nm514 (106175)	233	1908	1679
		<i>N. cinerea</i>	Nei1004 (105712, China)	Nm419 (55186), Nm514 (106175)	922	1793	875

*Positions are given according to the first nucleotide of *penA* of *N. meningitidis* strain 053442.

†The length is determined according to the donor strain.

Appendix 2 Table 3. Characteristics of isolates in the genetic transformation into *Neisseria meningitidis* isolates*

Attribute of strain	Strain	Species	MIC, µg/mL		<i>penA</i> allele (mutation)†	Crossover point‡		Length of recombinant fragment (bp)§	PubMLST ID
			Penicillin	Cefo- taxime		Upstream	Down- stream		
Recipient	Nm040	<i>N. meningitidis</i>	0.032	0.008	1 (none)	NA	NA	NA	58130
Donor 1	Nei171	<i>N. lactamica</i>	0.125	0.06	405 (5 mutations)	NA	NA	NA	105709
Transformant 1.1	Nm040Nei171T1-1	<i>N. meningitidis</i>	0.125	0.06	405 (5 mutations)	742	3098	2357	111282
Transformant 1.2	Nm040Nei171T1-2	<i>N. meningitidis</i>	0.19	0.03	405 (5 mutations)	242	2697	2456	111283
Transformant 1.3	Nm040Nei171T1-3	<i>N. meningitidis</i>	0.19	0.06	405 (5 mutations)	343	2953	2611	111284
Donor 2	Nei033	<i>N. lactamica</i>	0.5	0.06	552 (5 mutations)	NA	NA	NA	84202
Transformant 2.1	Nm040Nei033T1-1	<i>N. meningitidis</i>	0.38	0.015	552 (5 mutations)	913	7393	5740	111279
Transformant 2.2	Nm040Nei033T1-2	<i>N. meningitidis</i>	0.125	0.015	552 (5 mutations)	913	5330	3765	111280
Transformant 2.3	Nm040Nei033T1-3	<i>N. meningitidis</i>	0.19	0.015	552 (5 mutations)	913	6877	5303	111281
Donor 3	Nei012	<i>N. lactamica</i>	1.5	1	795 (7 mutations)	NA	NA	NA	84195
Transformant 3.1	Nm040Nei012T1-6	<i>N. meningitidis</i>	0.25	0.5	795 (7 mutations)	718	2999	2285	111273
Transformant 3.2	Nm040Nei012T1n-2	<i>N. meningitidis</i>	0.19	0.5	795 (7 mutations)	817	9473	8628	111274
Transformant 3.3	Nm040Nei012T1n-3	<i>N. meningitidis</i>	0.25	0.5	795 (7 mutations)	262	7393	6443	111275
Donor 4	Nei028	<i>N. cinerea</i>	1	1	795 (7 mutations)	NA	NA	NA	84201

Attribute of strain	Strain	Species	MIC, µg/mL		<i>penA</i> allele (mutation)†	Crossover point‡		Length of recombinant fragment (bp)§	PubMLST ID
			Penicillin	Cefo-taxime		Upstream	Down-stream		
Transformant 4.1	Nm040Nei028T1n-1	<i>N. meningitidis</i>	0.38	0.5	795 (7 mutations)	-1489	6412	7215	111276
Transformant 4.2	Nm040Nei028T1n-2	<i>N. meningitidis</i>	0.25	0.5	795 (7 mutations)	643	1976	1337	111277
Transformant 4.3	Nm040Nei028T1n-3	<i>N. meningitidis</i>	0.25	0.5	795 (7 mutations)	864	2999	2139	111278
Donor 5	Nei367	<i>N. polysaccherae</i>	0.75	0.5	795 (7 mutations)	NA	NA	NA	84258
Transformant 5.1	Nm040Nei367T1-1	<i>N. meningitidis</i>	0.25	0.25	795 (7 mutations)	-1242	10501	10534	111288
Transformant 5.2	Nm040Nei367T1-2	<i>N. meningitidis</i>	0.38	0.25	795 (7 mutations)	85	596	512	111289
Transformant 5.3	Nm040Nei367T1-3	<i>N. meningitidis</i>	0.19	0.25	795 (7 mutations)	-285	3011	3299	111290
Donor 6	Nei798	<i>N. subflava</i>	0.25	0.25	795 (7 mutations)	NA	NA	NA	105711
Transformant 6.1	Nm040Nei798T1-1	<i>N. meningitidis</i>	0.19	0.25	795 (7 mutations)	532	2774	2246	111291
Transformant 6.2	Nm040Nei798T1-3	<i>N. meningitidis</i>	0.25	0.25	795 (7 mutations)	873	6294	3698	111292
Transformant 6.3	Nm040Nei798T1-4	<i>N. meningitidis</i>	0.25	0.25	795 (7 mutations)	418	2459	2045	111293
Donor 7	Nei341	<i>N. lactamica</i>	0.5	0.25	832 (5 mutations)	NA	NA	NA	83066
Transformant 7.1	Nm040Nei341T1-1	<i>N. meningitidis</i>	0.19	0.125	832 (5 mutations)	757	2048	1292	111265
Transformant 7.2	Nm040Nei341T1-2	<i>N. meningitidis</i>	0.19	0.125	832 (5 mutations)	328	8664	7805	111266
Transformant 7.3	Nm040Nei341T1-3	<i>N. meningitidis</i>	0.19	0.125	832 (5 mutations)	328	5534	4554	111267
Donor 8	Nei264	<i>N. lactamica</i>	0.75	0.015	843 (5 mutations)	NA	NA	NA	105710
Transformant 8.1	Nm040Nei264T1-1	<i>N. meningitidis</i>	0.25	0.015	843 (5 mutations)	475	3161	2690	111285
Transformant 8.2	Nm040Nei264T1-2	<i>N. meningitidis</i>	0.19	0.015	843 (5 mutations)	348	2693	2349	111286
Transformant 8.3	Nm040Nei264T1-3	<i>N. meningitidis</i>	0.125	0.015	843 (5 mutations)	348	2693	2349	111287
Donor 9	Nei1004	<i>N. cinerea</i>	0.5	0.015	843 (5 mutations)	NA	NA	NA	105712
Transformant 9.1	Nm040Nei1004T1-1	<i>N. meningitidis</i>	0.25	0.015	843 (5 mutations)	532	1976	1448	111294
Transformant 9.2	Nm040Nei1004T1-2	<i>N. meningitidis</i>	0.25	0.015	843 (5 mutations)	532	1976	1448	111295
Transformant 9.3	Nm040Nei1004T1-3	<i>N. meningitidis</i>	0.125	0.015	843 (5 mutations)	895	3199	2308	111296
Donor 10	Nm469	<i>N. meningitidis</i>	0.125	0.008	184 (A549T)	NA	NA	NA	105710
Transformant 10.1	Nm040Nm469T1-1	<i>N. meningitidis</i>	0.125	0.008	184 (A549T)	1645	1645	1	116982

Attribute of strain	Strain	Species	MIC, µg/mL		<i>penA</i> allele (mutation)†	Crossover point‡		Length of recombinant fragment (bp)§	PubMLST ID
			Penicillin	Cefo-taxime		Upstream	Down-stream		
Transformant 10.2	Nm040Nm469T1-3	<i>N. meningitidis</i>	0.125	0.008	184 (A549T)	1645	1645	1	116983
Transformant 10.3	Nm040Nm469T1-4	<i>N. meningitidis</i>	0.125	0.008	184 (A549T)	1645	1645	1	116984
Donor 11	Nm465	<i>N. meningitidis</i>	0.125	0.008	866 (F504L and A510V)	NA	NA	NA	105712
Transformant 11.1	Nm040Nm465T1-1	<i>N. meningitidis</i>	0.125	0.008	new1 (F504L and A510V) ¶	1467	1533	67	116985
Transformant 11.2	Nm040Nm465T1-5	<i>N. meningitidis</i>	0.125	0.008	new2 (F504L and A510V) #	1431	1533	103	116986

*NA, not applicable.

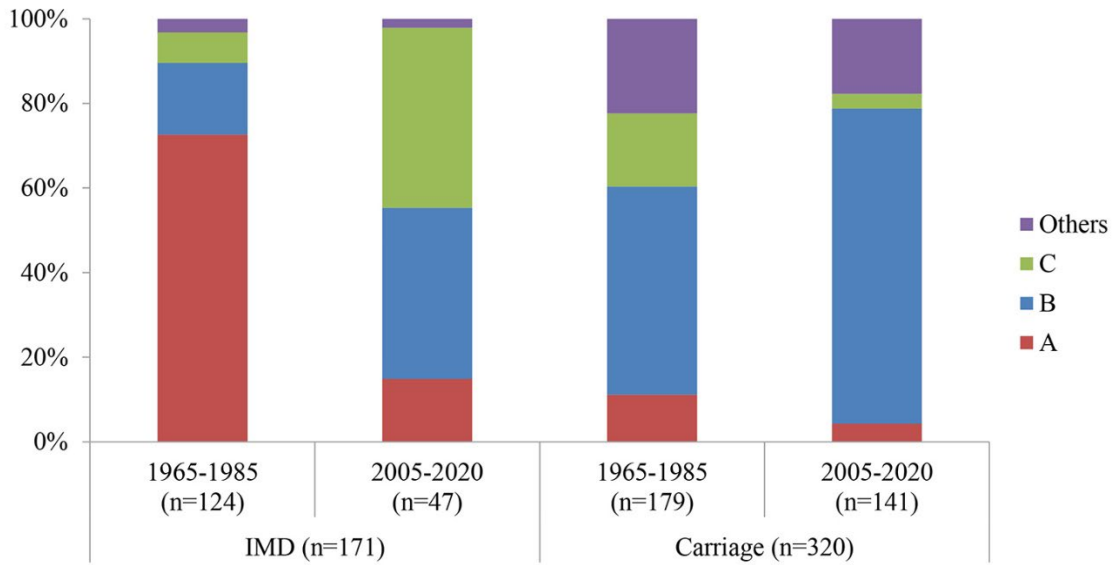
†The 5 mutations are F504L, A510V, I515V, H541N, and I566V; and the 7 mutations are A311V, T483S, F504L, A510V, I515V, H541N, and I566V.

‡Positions are given according to the first nucleotide of *penA* gene of the *N. meningitidis* strain Nm040. NA, not applicable.

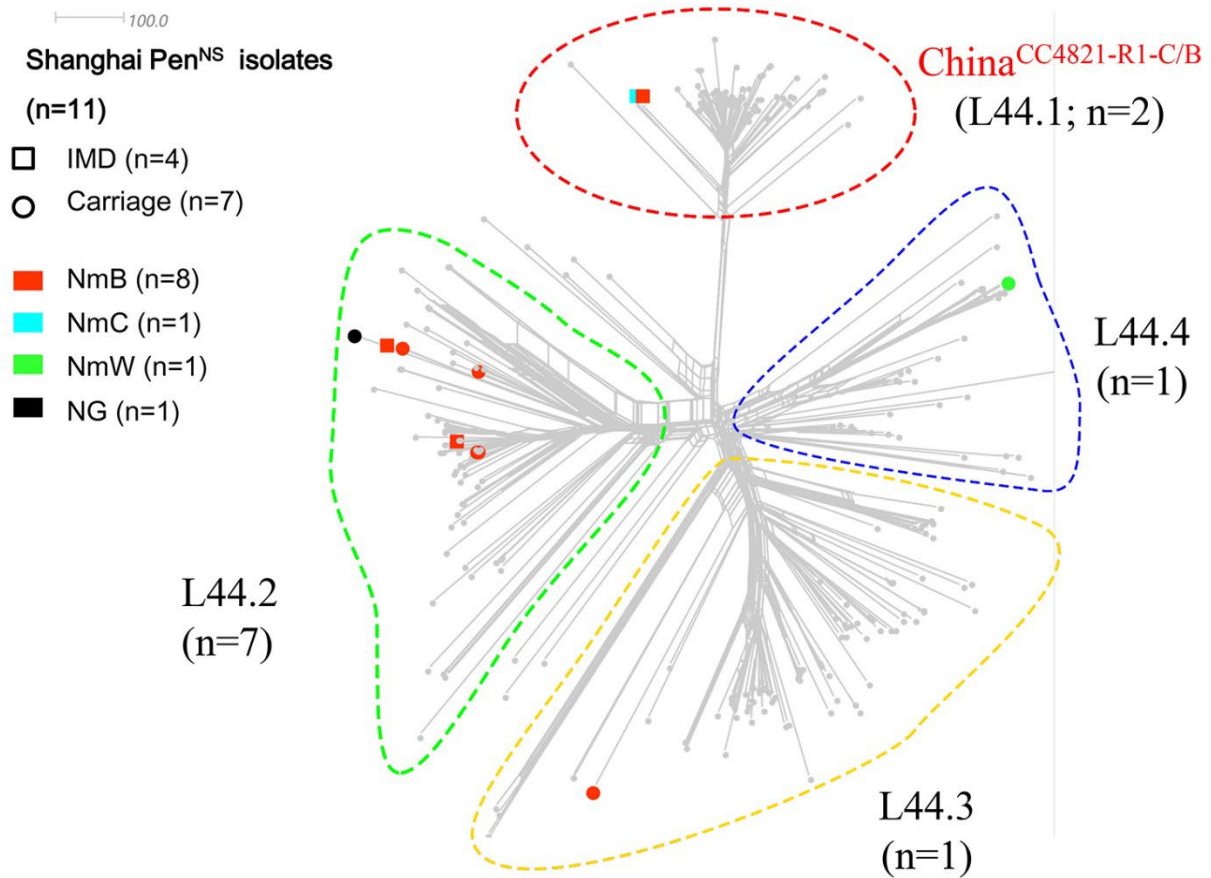
§The length is determined according to the donor strain.

¶The allele new1 and *penA866* differ in 2 nucleotides.

#The allele new2 and *penA866* differ in 1 nucleotide.

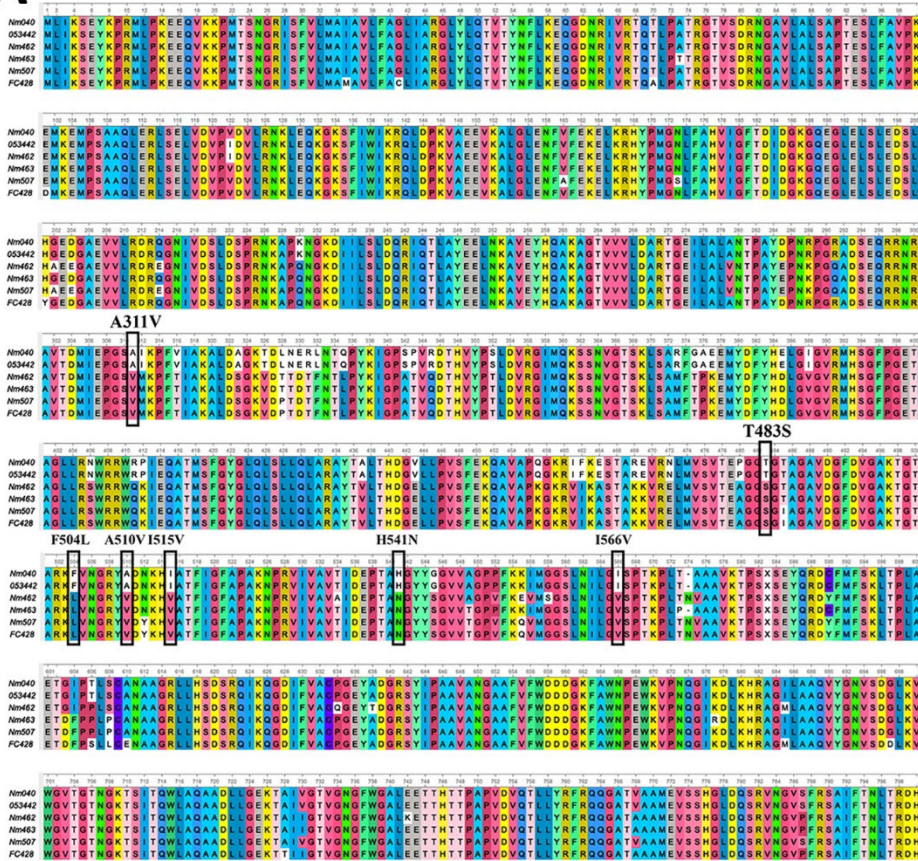


Appendix 2 Figure 1. Serogroup distribution of invasive and carriage meningococcal isolates in Shanghai during 1965-1985 and 2005-2020. IMD, invasive meningococcal disease. A total of 491 *N. meningitidis* isolates were collected from 1965 to 2020, including 234 NmB (47.7%), 123 NmA (25.1%), 65 NmC (13.2%), 31 nongroupable (6.3%), 13 NmE (2.6%), 11 NmY (2.2%), 7 NmW (1.4%), 4 NmZ (0.8%), and 3 NmX (0.6%).



Appendix 2 Figure 2. Distribution of CC4821 penicillin non-susceptible isolates in the four sublineages of CC4821 by genome analysis. Pen^{NS}, penicillin non-susceptible. IMD, invasive meningococcal disease. NmB, serogroup B. NmC, serogroup C. NmW, serogroup W. NG, nongroupable.

A



Appendix 2 Figure 3. Alignment of the *penA* full-length sequences of the three cefotaxime-resistant isolates, two wild-type *N. meningitidis* strains, and the *N. gonorrhoeae* FC428 isolate. A) Amino acid sequences. The two mutations associated with cefotaxime-resistance (A311V and T483S) and the five mutations associated with penicillin-resistance (F504L, A510V, I515V, H541N, and I566V) are indicated. B) Nucleotides. The 402bp-length sequences of isolates Nm507 and FC428 used to define *penA* allele are indicated by black box.