

Genomic Insight into the Spread of Meropenem-Resistant *Streptococcus pneumoniae* Spain^{23F}-ST81, Taiwan

Appendix 1

Appendix 1 Table 1. Primers used in this study

Primer name	Primer sequence (5'→3')	Purpose	Reference no.
<i>pbp1a</i> -F	CCAGCAACAGGTGAGAGTC	PCR	(1)
<i>pbp1a</i> -R	GTAACACAAGCCAAGACAC		
<i>pbp2b</i> -F	CCGTCTTAATCCCGATACC	PCR	(1)
<i>pbp2b</i> -R	ATTTTTGGGTGACTTGTTGAG		
<i>pbp2x</i> -F	GGAATTGGTGTCCCGTAAGC	PCR	(1)
<i>pbp2x</i> -R	CATCTGCTGGCCTGTAATTTG		
<i>pbp1a</i> -F2	CCWGTCCATACAGCCATWG	Sequencing	This study
<i>pbp1a</i> -R2	CTTCTCAGCAAGAAACCT		
<i>pbp2b</i> -F2	TATACTCTTTKGGWAYAA	Sequencing	This study
<i>pbp2b</i> -R2	CTGACTTATGTGAGCATC		
<i>pbp2x</i> -F2	ACGTGGGACTATTTATGAC	Sequencing	This study
<i>pbp2x</i> -R2	TTGGTAGAACCAACCAAG		
<i>ermB</i> -F	TGGTATTCCAAATGCGTAATG	PCR	(2)
<i>ermB</i> -R	CTGTGGTATGGCGGGTAAGT		
<i>mef(A/E)</i> -F	CAATATGGGCAGGGCAAG	PCR	(2)
<i>mef(A/E)</i> -R	AAGCTGTTCCAATGCTACGG		
<i>cat</i> -F	TTAGGYTATTGGGATAAGTTA	PCR	(3)
<i>cat</i> -R	CATGRTAACCATCACAWAC		
<i>tetM</i> -F	GTGGACAAAGGTACAACGAG	PCR	(2)
<i>tetM</i> -R	CGGTAAAGTTCGTACACAC		
<i>aphA3</i> -F	TAAAAGATACGGAAGGAATGTCTC	PCR	(2)
<i>aphA3</i> -R	TCGACCGGACGCAGAAGGCAATGT		
<i>int</i> -Tn916-F	GCGTGATTGTATCTCACT	PCR	(2)
<i>int</i> -Tn916-R	GACGCTCCTGTTGCTTCT		
<i>xis</i> -Tn916-F	AAGCAGACTGAGATTCCCTA	PCR	(2)
<i>xis</i> -Tn916-R	GCGTCCAATGTATCTATAA		
<i>tnpR</i> -F	CCAAGGAGCTAAAGAGGTCCC	PCR	(2)
<i>tnpR</i> -R	GTCCCAGTCCCATGGAAGC		
<i>tnpA</i> -F	GCTTCCATGGGACTCGGGAC	PCR	(2)
<i>tnpA</i> -R	GCTCCCAATTAATAGGAGA		
<i>folA</i> -F	TTGCCAGCAGAATTGCAGCA	PCR	(4)
<i>folA</i> -R	AAATAGGTATCTCCTTCCACC		
<i>folP</i> -F	GATGAATGCATCGTGTCCATC	PCR	(5)
<i>folP</i> -R	CCGTCCGGTAGTTAGCAATCC		

Appendix 1 Table 2. Sequence Read Archive accession numbers used in this study*

Isolate name	Serotype	ST	Year	Accession no.
B1136	15C	83	2013	SRR8867345
B999-49	15B	83	2013	SRR8867344
B1300936	15C	83	2013	SRR8867343
1018-71	15B	83	2014	SRR8867342
1025-08	15B	83	2014	SRR8867349
1054-32	15C	83	2014	SRR8867348
B1675	15B	83	2016	SRR8867347
B0236	15B	83	2016	SRR8867346
1148-64	15C	83	2016	SRR8867351
1217-52	15B	83	2016	SRR8867350
K54	15B	83	2016	SRR8867360
K55	15B	83	2016	SRR8867359
B2581	15B	83	2017	SRR8867362
B5939	15B	83	2017	SRR8867361
B4505	15C	83	2017	SRR8867364
B1757	15C	83	2017	SRR8867363
B3127	15C	83	2017	SRR8867366
B2404	15B	83	2017	SRR8867365
B8812	15B	83	2017	SRR8867368
1244-61	15B	83	2017	SRR8867367
1267-33	15B	83	2017	SRR8867355
1276-72	15C	83	2017	SRR8867356
K72	15B	83	2017	SRR8867357
K110	15B	83	2017	SRR8867358
SSP102	23F	81	2003	SRR8867352
SSP47	23F	81	2002	SRR8867353
B2362	23F	81	2009	SRR8867354

*ST, sequence type. Sequence Read Archive,
<http://www.ncbi.nlm.nih.gov/sra>.

Appendix 1 Table 3. Multilocus sequence type (MLST) analysis and penicillin binding-protein (PBP) allelic profile of 125 meropenem-nonsusceptible pneumococcal isolates

Serotype	ST no.	No. of isolates	MIC (mg/L)							PBP profile			
			Meropenem		Penicillin				PBP1a	PBP2b	PBP2x	no.	
			0.5	>1	<0.06	<2	4	>8					
14	63	1	1	0	0	1	0	0	13	new1	43	1	
	83	2	2	0	0	2	0	0	15	12	18	1	
									60	16	143-like	1	
	876	3	3	0	0	3	0	0	15	12	18	1	
									60	16	143-like	2	
	2652	3	3	0	0	3	0	0	15	31	7	1	
									15	31	18	2	
	3652	1	1	0	0	1	0	0	15	31	18	1	
	14312	1	1	0	0	1	0	0	60	16	143-like	1	
	14313	1	1	0	0	1	0	0	24	53	77	1	
	14314	1	1	0	0	1	0	0	60	16	143-like	1	
	14315	1	1	0	0	1	0	0	15	31	18	1	
15A	63	13	12	1	0	11	2	0	15	12	18	1	
									13	new1	43	10	
									13	new1	16	1	
									13	new1-like	43-like	1	
15B	83	16	8	8	0	15	1	0	15	12	18	16	
15C	83	8	6	2	0	8	0	0	15	12	18	7	
									15	12	36	1	
	4743	1	1	0	0	1	0	0	15	31	18	1	
	14359	1	0	1	0	1	0	0	15	12	18	1	
19A	320	30	12	18	0	25	5	0	13	11	16	27	
									13	11	16-like	2	
									13	11	16-like-2	1	
	876	1	1	0	0	1	0	0	60	16	143-like	1	
	7122	1	0	1	0	1	0	0	13	11	16	1	
19F	236	3	2	1	0	3	0	0	13	new1	47	1	
									13	16	8	1	
									60	16	20	1	
	271	2	1	1	0	2	0	0	13	11	33	1	
									13	16	47	1	
	320	3	3	0	0	3	0	0	13	11	33	1	
									13	11	16	1	
									13	49-like	16	1	
	1464	2	1	1	0	1	1	0	13	49-like	154-like	2	
	12444	3	0	3	0	0	3	0	13	37	112-like	3	
	14358	1	1	0	0	1	0	0	13	16	100-like	1	
	14357	1	0	1	0	1	0	0	13	11-like	112-like-2	1	
23A	166	3	2	1	0	3	0	0	15	11	36-like	3	
	242	1	1	0	0	1	0	0	13	31	73	1	
23F	81	1	1	0	0	1	0	0	15	12	18	1	
	166	1	1	0	0	1	0	0	15	11	36-like	1	
	242	2	2	0	0	2	0	0	13	31	73	1	
									13	31	20	1	
	1437	1	1	0	0	1	0	0	7	74-like	new2	1	
6A	76	1	1	0	0	1	0	0	25-like	46	36	1	
	81	3	3	0	0	3	0	0	15	12	18	3	
6B	720	1	1	0	0	1	0	0	13-like	31-like	73-like	1	
	8526	1	1	0	0	1	0	0	34	11	8-like	1	
Others													
1	63	1	1	0	0	1	0	0	13	new1	43	1	
15F	63	1	1	0	0	1	0	0	13	new1	43	1	
18F	81	1	0	1	0	1	0	0	15	12	18	1	
11A	166	2	1	1	0	2	0	0	15	12	18	1	
									15	12	8	1	
23B	242	1	1	0	0	1	0	0	13	31	73	1	
4	558	1	1	0	0	1	0	0	4	7	7	1	
35A/C	9395	1	1	0	0	1	0	0	4	7	7	1	
12	9395	1	1	0	0	1	0	0	4	7	7	1	
	12446	1	1	0	0	1	0	0	4	7	7	1	
Total		125	84	41	0	113	12	0					

Appendix 1 Table 4. Novel PBP1a transpeptidase domain sequences identified in this study

Sequences

>13-like
SMKPIDDYAPALEYGVYDSTASIVHDVPYNYPGTDTPLYNWDHVYFGNITIQYALQQSRNVTAVELTNKVGLDRAKTFNLGLGIDYP
SMHYANAISNTTESNKKYGASSEKMAAAYAAFANGGIYHKPMYINKIVFSDGSEKEFSDAGTRAMKETTAYMMTEMMKTVLTHGT
GRGAYLPWLPQAGKTGTSNYTDEEIEKYIKNTGYVAPDEMFGYTRKYSMVWVTGYSNRLTPIVGDGFLVAAKVYRSMMTYLSEG
SNPEDWNIPEGLYRNGEFV

>25-like
AMKPIDDYAPAIEYGVYDSTATMVDNIPYNYPGTSTPVYNDWDRAYFGNITLQYALQQSRNVTAVELTNKVGLDRAKTFNLGLGIDYP
SMHYANAISNTTESNKQYGASSEKMAAAYAAFANGGIYHKPMYINKVVFSDGSEKEFSDVGTRAMKETTAYMMTEMMKTVLAYG
TGRGAYLPWLAQAGKTGTSNYTDEEIEKHIKNTGYVAPDEMFGYTRKYSMVWVTGYSNRLTPIVGDGFLVAAKVYRSMITYLSED
THPEDWNIPEGLYRNGEFV

Appendix 1 Table 5. Novel PBP2b transpeptidase domain sequences identified in this study

Sequences

>31-like
TNVFPVGSVVKAAATISSGWENGVLSGNQTLTDQPIVFQGSAPIYSWYKLAYGSFPITAVEALEYSSNAYMVQ TALGIMGQTYQPNM
FVGTSNLETAMGKLRATFGEYGLGAATGIDLPESTGFVPKEYSFANYIANAFGQFDNYTPMQLAQYVATIANNVVRVAPRIVEGIY
GNNDKGGGLGDLIQQLPQTEMNKNVISDSMSILHQGFYQVAHGTSGLTTGRAFSNGALVSISGKTGTAESYVADGQQATNTNAVA
YAPSDNPQIAVAVVFPHTN

>49-like
TNVFIQGSVVKAAATISSGWENGVLSGNQTLTDQPIVFQGSAPIYSWYKLAYGSFPITAVEALEYSSNAYMVQ TALGIMGQTYQPNM
VGTSNLETAMGKLRATFGEYGLGAATGIDLPESTGFVPKEYSFANYITNAFGQFDNYTPMQLAQYVATIANDGVRVAPRIVEGIY
GNNDKGGGLGDLIQQLPQTEMNKNVISDSMSILHQGFYQVSHGTSPLTTGRAFSNGALVSISGKTGTAESYVADGQQATNTNAVA
APTENPQIAVAVVFPHTN

>new1-like
TNVFPVGSVVKAAATISSGWENGVLSGNQTLTDQPIVFQGSAPIYSWYKLAYGSFPITAVEALEYSSNAYMVQ TALGIMGQTYQPNM
FVGTSNLESAMEKLRSTFGEYGLGATGIDLPESTGFVPKEYSFANYITNAFGQFDNNTPMQLAQYVATIANNVVRVAPRIVEGIY
GNNDKGGGLGDLIQQLPQTEMNKNVISDSMSILHQGFYQVAHGTSGLTTGRAFSNGAAVSISGKTGTAESYVEGGQEAANTNAVA
YAPSDNPQIAVAVVFPHTN

Appendix 1 Table 6. Novel PBP2x transpeptidase domain sequences identified in this study

Sequences

>8-like
GKDGIIYEKDR LGNIVPGTEQVSQQTV DKG DVYTTLSSPLQSF METQMDAFLEKVKGKYMTATLVSAKTGEILATTQRPTFNADTK
EGITEDFVWRDILYQSNYEPGSAMKVM TLASSIDNNTFPSGEYFNSSEFKIADATTRDWDVNEGLTTGGMMTFLQGF AHSSNVGM
SLLEQKMGDATWLDYLKRFKFGVPTRFGLTDEYAGQLPADNIVSIAQSSFGQGISVTQTQMLRAFTAIANDGVMLEPKFISAIYDTNN
QSVRKSQKEIVGKPVSEEAASSTRNHMILVGTDP LYGTMYNHYTGKPIITVPGQNVAVKSGTAQIADEKNGGYLVGSTNYIFSAVTM
NPAENPDFILY

>16-like
GTDGIIYEKDRVGNIVPGTELVSQQTV DKG DVYTTLSSPLQSF METQMDAFLEKVKGKYMTATLVSAKTGEILATTQRPTFNADTK
EGITEDFVWRDILYQSNYEPGSAMKVM TLASSIDNNTFPSGEYFNSSEFKIADATTRDWDVNEGLTTGGMMTFLQGF AHSSNVGM
SLLEQKMGDATWLDYLKRFKFGVPTRFGLTDEYAGQLPADNIVSIAQSSFGQGISVTQTQMLRAFTAIANDGVMLEPKFISAIYDPNN
QSVRKSQKEIVGKPVSEDTASLTRTNMILVGTDP LYGTMYNHYTGKPIITVPGQNVAVKSGTAQIADEKNGGYLVGSTNYIFSAVTM
NPAENPDFILY

>16-like-2
GTDGIIYEKDRVGNIVPGTELVSQQTV DKG DVYTTLSSPLQSF METQMDAFLEKVKGKYMTATLVSAKTGEILATTQRPTFNADTK
EGITEDFVWRDILYQSNYEPGSAMKVM TLASSIDNNTFPSGEYFNSSEFKIADATTRDWDVNEGLTTGGMMTFLQGF AHSSNVGM
SLLEQKMGDATWLDYLKRFKFGVPTRFGLTDEYAGQLPADNIVSIAQSSFGQGISVTQTQMLRAFTAIANDGVMLEPKFISAIYDTNN
QSVRKSQKEIVGKPVSEDTASLTRTNMILVGTDP LYGTMYNHYTGKPIITVPGQNVAVKSGTAQIADEKNGGYLVGSTNYIFSAVTM
NPAENPDFILY

>36-like
GTDGIIYEKDRVGNIVPGTELVSQQTV DKG DVYTTLSSPLQSF METQMDAFLEKVKGKYMTATLVSAKTGEILATTQRPTFNADTK
EGITEDFVWRDILYQSNYEPGSAMKVM TLASSIDNNTFPSGEYFNSSEFKMADVTTTRDWDVNGGLTTGGMMTFLQGF AHSSNVGM
MSLLEQKMGDATWLDYLKRFKFGVPTRFGLTDEYAGQLPADNIVSIAQSSFGQGISVTQTQMLRAFTAIANDGVMLEPKFISAIYDT
NNQSVRKSQKEIVGNPVSKE TASTTRNHMILVGTDP LYGTMYNHYTGKPIITVPGQNVAVKSGTAQIADEKNGGYLVGSTNYIFSAV
TMNPAENPDFILY

>43-like
GTDGIIYEKDR LGNIVPGTEQVSQQTV DKG DVYTTISSTLQSF METQMDAFLEKVKGKYMTATLVSAKTGEILATTQRPTFNADTK
EGITEDFVWRDILYQSNYEPGSAMKVM TLASSIDNNTFPSGEYFNSSEFKIADATTRDWDVNEGLTTGGMMTFLQGF AHSSNVGMS

Sequences

LLEQKMGDATWLDYLKRFKFGVPTRFGLTDEYAGQLPADNIVSIAQSSFGQGISVTQTQMLRAFTAIANDGVMLEPKFISAIYDTNN
QSVRKSQKEIVGNPVSKEAASTTRNHMLLVGTDPLYGTMYNHYTGKPIITVPGQNVAVKSGTAQIADEKNGGYLVGSTNYIFSVAVTM
NPAENPDFILYV

>73-like

GTDGIITYEKDRLGNIVPGTEQVSQRTMDGKDVYTTISSPLQSFMETQMDFLEKVKGKYMTATLVSAKTGEILATTQRPTFNADTK
EGITEDFVWRDILYQSNYEPGSAMKVMTLASSIDNNTFPSGEYFNSSEFKIADATIRDWDVNDGLTTGGMMTFLQGFHSSNVGMS
LLEQKMGDATWLDYLKRFKFGVPTRFGLTDEYAGQLPADNIVSIAQSSFGQGISVTQTQMLRAFTAIANDGVMLEPKFISAIYDTNN
QSVRKSQKEIVGNPVSKEAASTTRNHMILVGTDPYGTMYNHYTGKPIITVPGQNVAVKSGTAQIADEKNGGYLVGSTNYIFSVAVTM
NPAENPDFILYV

>100-like

GTDGIITYEKDRVGNIVPGTELVSQQTVDGKDVYTTLSSPLQSFMETQMDFLEKVKGKYMTATLVSAKTGEILATTQRPTFNADTK
EGITEDFVWRDILYQSNYEPGSGMKVMTLASSIDNNTFPSGEYFNSSEFKIADATTRDWDVNEGLTTGGMMTFLQGFHSSNVGM
LLEQKMGDATWLDYLKRFKFGVPTRFGLTDEYAGQLPADNIVSISQSSFGQGISVTQTQMLRAFTAIANDGVMLEPKFISAIYDTNN
QSVRKSQKEIVGNPVSKEAASTTRNHMILVGTDPYGTMYNHYTGKPIITVPGQNVAVKSGTAQIADEKNGGYLVGSTNYIFSVAVTM
NPAENPDFILYV

>112-like

GKDGIIITYEKDRLGNIVPGTEQVSQQTVDGKDVYTTLSSPLQSFMETQMDFLEKVKGKYMTATLVSAKTGEILATTQRPTFNADTK
EGITEDFVWRDILYQSNYEPGSGMKVMTLASSIDNNTFPSGEYFNSSEFKIADATTRDWDVNEGLTTGGMMTFLQGFHSSNVGM
LLEQKMGDATWLDYLKRFKFGVPTRFGLTDEYAGQLPADNIVSISQSSFGQGISVTQTQMLRAFTAIANDGVMLEPKFISAIYDTNN
QSVRKSQKEIVGNPVSKEAASTTRNHMILVGTDPYGTMYNHYTGKPIITVPGQNVAVKSGTAQIADEKNGGYLVGSTNYIFSVAVTM
NPAENPDFILYV

>112-like-2

GKDGIIITYEKDRLGNIVPGTEQVSQQTVDGKDVYTTLSSPLQSFMETQMDFLEKVKGKYMTATLVSAKTGEILATTQRPTFNADTK
EGITEDFVWRDILYQSNYEPGSGMKVMTLASSIDNNTFPSGEYFNSSEFKIADATTRDWDVNEGLTTGGMMTFLQGFHSSNVGM
LLEQKMGDATWLDYLKRFKFGVPTRFGLTDEYAGQLPADNIVSIAQSSFGQGISVTQTQMLRAFTAIANDGVMLEPKFISAIYDTNN
QSVRKSQKEIVGNPVSKEAASTTRNHMILVGTDPYGTMYNHYTGKPIITVPGQNVAVKSGTAQIADEKNGGYLVGSTNYIFSVAVTM
NPAENPDFILYV

>143-like

GTDGIITYEKDRLGNIVPGTEQVSQQTVDGKDVYTTLSSPLQSFMETQMDFLEKVKGKYMTATLVSAKTGEILATTQRPTFNADTK
EGITEDFVWRDILYQSNYEPGSAMKVMTLASSIDNNTFPSGEYFNSSEFKIADATTRDWDVNDGLTTGGMMTFLQGFHSSNVGM
LLEQKMGDATTWLDYLNRFKFGVPTRFGLTDEYAGQLPADNIVSIAQSSFGQGISVTQTQMLRAFTAIANDGVMLEPKFISAIYDTNN
QSVRKSQKEIVGNPVSKEAASTTRNHMILVGTDPYGTMYNHYTGKPIITVPGQNVAVKSGTAQIADEKNGGYLVGSTNYIFSVAVTM
NPAENPDFILYV

>154-like

GKDGIIITYEKDRLGNIVPGTEQVSQQTVDGKDVYTTISSTLQSFMETQMNAFQEKVKGKYMTATLVSAKTGEILATTQRPTFNADTK
EGITEDFVWRDILYQSNYEPGSAMKVMTLASSIDNNTFPSGEYFNSSEFKIADATTRDWDVNEGLTTGGMMTFLQGFHSSNVGM
LLEQKMGDATWLDYLKRFKFGVPTRFGLTDEYAGQLPADNIVSIAQSSFGQGISVTQTQMLRAFTAIANDGVMLEPKFISAIYDTNN
QSVRKSQKEIVGKPVSEDTASTTRNHMILVGTDPYGTMYNHYTGKPIITVPGQNVAVKSGTAQIADEKNGGYLVGSTNYIFSVAVTM
NPAENPDFILYV

Appendix 1 Table 7. Results of PCR amplification of antimicrobial resistance genes and Tn916-like transposon gene in 24 meropenem-nonsusceptible 15B/C-ST83 clinical isolate strains*

Isolate name	<i>ermB</i>	<i>tetM</i>	<i>cat</i>	<i>mef(A/E)</i>	<i>aphA3</i>	<i>int-Tn916</i>	<i>xis-Tn916</i>	<i>tnpR-Tn916</i>	<i>tnpA-Tn916</i>	Tn of Tn916-like	<i>folA</i> mutation†	<i>folP</i> insertion‡
B1136	+	+	+	-	-	+	+	-	-	Tn6002	+	+
B999-49	+	+	+	-	-	+	+	-	-	Tn6002	+	+
B1300936	+	+	+	-	-	+	+	-	-	Tn6002	+	+
1018-71	+	+	+	-	-	+	+	-	-	Tn6002	+	+
1025-08	+	+	+	-	-	+	+	-	-	Tn6002	+	+
1054-32	+	+	+	-	-	+	+	-	-	Tn6002	+	+
B1675	+	+	+	-	-	+	+	-	-	Tn6002	+	+
B0236	+	+	+	-	-	+	+	-	-	Tn6002	+	+
1148-64	+	+	+	-	-	+	+	-	-	Tn6002	+	+
1217-52	+	+	+	-	-	+	+	-	-	Tn6002	+	+
K54	+	+	+	-	-	+	+	-	-	Tn6002	+	+
K55	+	+	+	-	-	+	+	-	-	Tn6002	+	+
B2581	+	+	+	-	-	+	+	-	-	Tn6002	+	+
B5939	+	+	+	-	-	+	+	-	-	Tn6002	+	+
B4505	+	+	+	-	-	+	+	-	-	Tn6002	+	+
B1757	+	+	+	-	-	+	+	-	-	Tn6002	+	+
B3127	+	+	+	-	-	+	+	-	-	Tn6002	+	+
B2404	+	+	+	-	-	+	+	-	-	Tn6002	+	+
B8812	+	+	+	-	-	+	+	-	-	Tn6002	+	+
1244-61	+	+	+	-	-	+	+	-	-	Tn6002	+	+
1267-33	+	+	+	-	-	+	+	-	-	Tn6002	+	+
1276-72	+	+	+	-	-	+	+	-	-	Tn6002	+	+
K72	+	+	+	-	-	+	+	-	-	Tn6002	+	+
K110	+	+	+	-	-	+	+	-	-	Tn6002	+	+

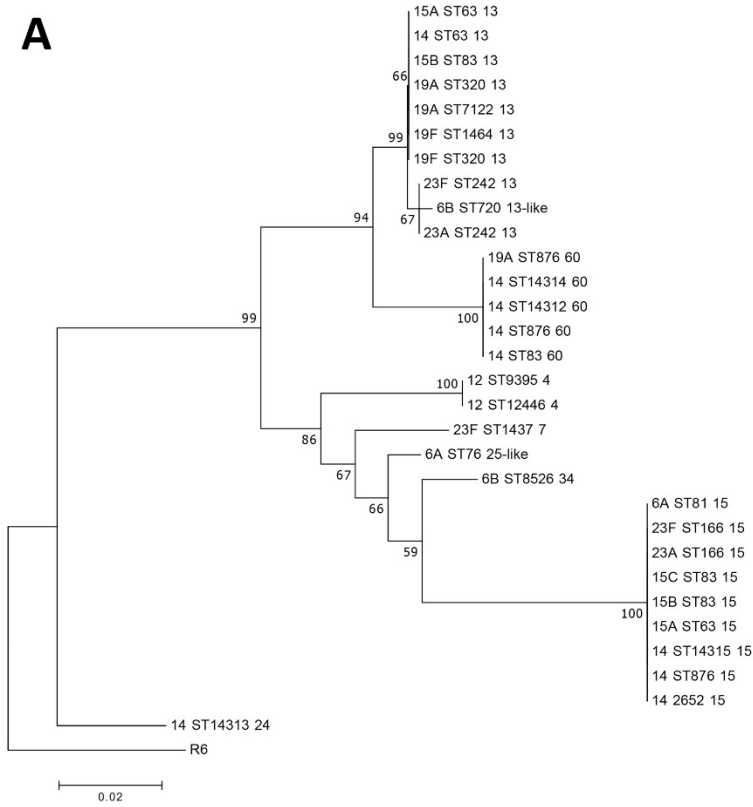
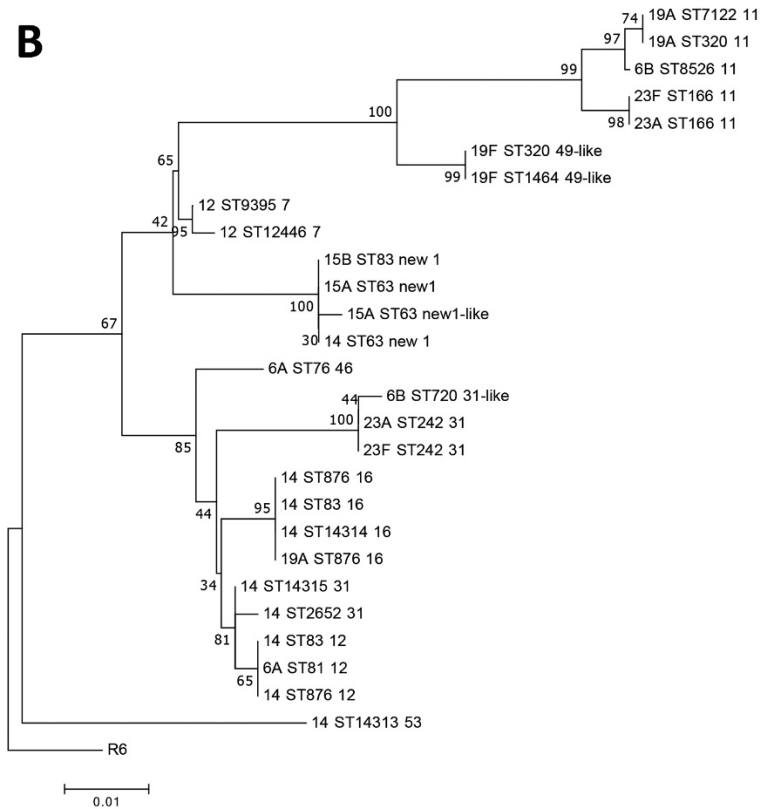
*+, PCR positive; -, PCR negative.

†DNA sequence analysis revealed I100L substitution in *folA*.

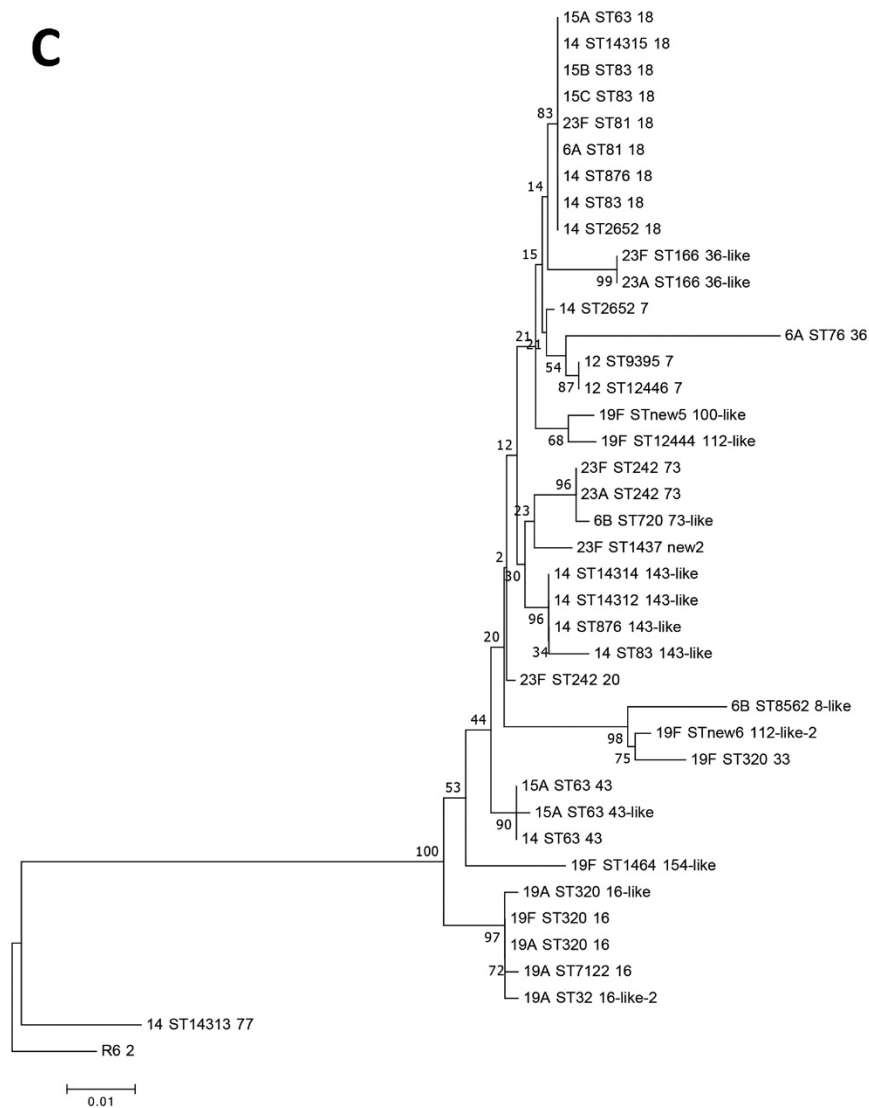
‡DNA sequence analysis revealed insertion of one codon between bases 168 and 201 in *folP*.

Appendix 1 Table 8. Sequence coordinates of recombination sites

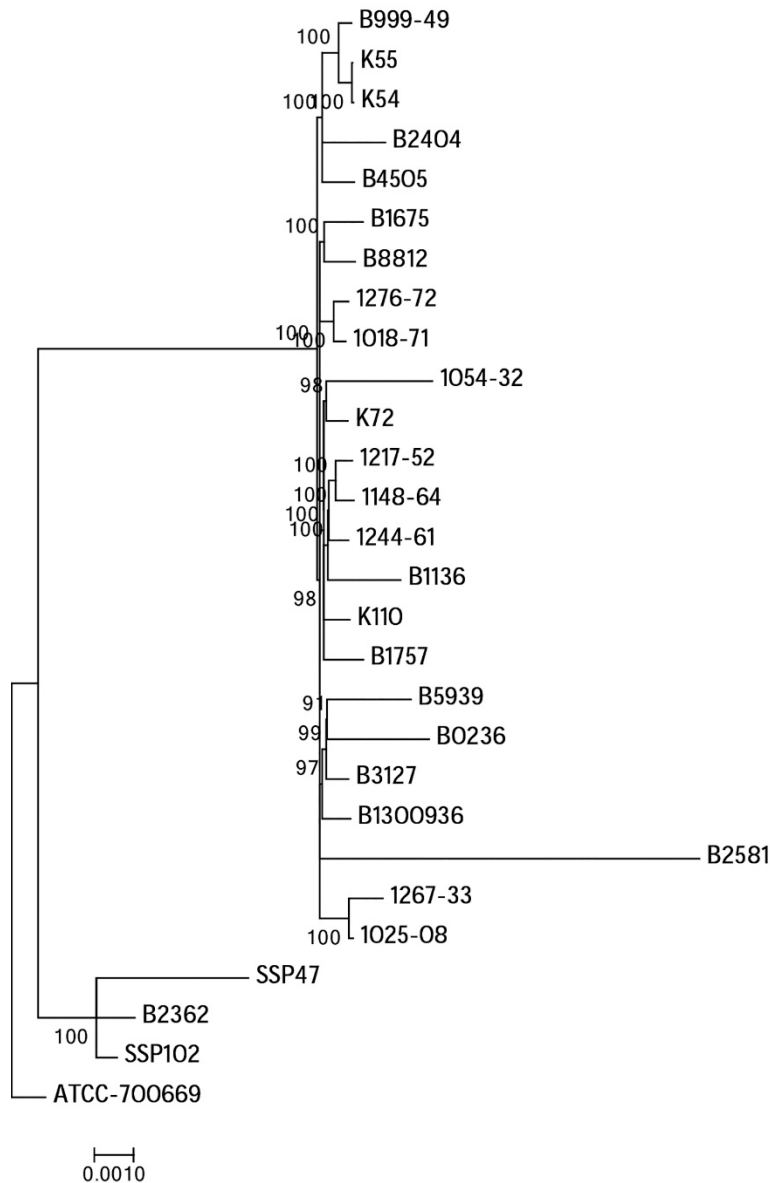
Number of recombination sites	Sequence coordinates	Length of recombination sites, bp	Genes or putative protein function	Recombination events
1	124732–124902	171	Part of <i>pspA</i> gene	15B/C-ST83
2	302151–329719	27,569	<i>cps</i> locus	15B/C-ST83
3	362492–389805	27,314	<i>tig</i> , <i>spi</i> , DNA helicase, <i>mutS2</i> , and <i>bacteriocin</i>	15B/C-ST83
4	750043–751380	1,338	IS861 of <i>orfA</i> and IS-Spn1 <i>orf8</i>	15B/C-ST83 and 23F-ST81
5	958399–961937	3,539	DNA primase, <i>ropD</i> and ABC transporter	15B/C-ST83 and 23F-ST81
6	1279909–1280068	160	Partial <i>tetM</i> in ICE element region	15B/C-ST83 and 23F-ST81
7	1487797–1526819	39,023	Autolysin, holing, putative phage protein cluster	15B/C-ST83 and 23F-ST81
8	1722313–1732499	10,187	Part of <i>psrP</i> gene	15B/C-ST83 and 23F-ST81
9	1831969–1839540	7,572	Siderophore uptake periplasmic binding protein, <i>rluB</i> , <i>scpB</i> , <i>scpA</i> , <i>xerD</i> , <i>glr</i>	15B/C-ST83 and 23F-ST81
10	1938458–1941716	3,259	<i>pflC</i> , <i>purR</i>	15B/C-ST83 and 23F-ST81
11	2094386–2095149	764	Putative DNA binding protein	15B/C-ST83
12	2171700–2171829	130	Part of <i>cbpA</i> gene	15B/C-ST83

A**B**

C



Appendix 1 Figure 1. Neighbor-joining trees showing the relatedness of the amino acid sequence of A) PBP1a; B) PBP2b; and C) PBP2x of *Streptococcus pneumoniae*, Taiwan, 2013–2017. The amino acid sequence of PBP in the *S. pneumoniae* R6 strain is included as a reference. The evolutionary history was inferred using the neighbor-joining method (6). The optimal trees had branch length sums of A) 0.29717399; B) 0.21161535; and C) 0.27634923. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) are shown next to the branches (7). The evolutionary distances were computed by using the Poisson correction method (8). Scale bars indicate the number of amino acid substitutions per site. PBP, penicillin binding protein.



Appendix 1 Figure 2. Recombination-free phylogenetic tree of draft genomes of *Streptococcus pneumoniae* isolates collected in Taiwan during 2013–2017. The tree was created by using the maximum likelihood method in RAxML (<https://github.com/stamatak/standard-RAxML>) and the GTRCAT model with 1,000 bootstrap values in Recombination Detection Program (RDP) version 4.89 (9). Draft genomes were generated from contigs by using a post-assembly genome-improvement toolkit (10), including 24 meropenem-nonsusceptible serotype 15B/C-ST83, and 3 23F-ST81 strains. The PMEN1 reference strain, accession no. ATCC-700669, was added as an outgroup. Parsnp version 1.2 of the Harvest Suite (11) was applied to perform a core alignment of these genomes before constructing the tree using RDP. Scale bar represents nucleotide substitutions per site.

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