

Effects of Pneumococcal Conjugate Vaccine on Genotypic Penicillin Resistance and Serotype Changes, Japan, 2010–2017

Technical Appendix

Technical Appendix Table 1. Serotypes, penicillin-resistant and macrolide-resistant genotypes for all isolates (n = 2,849) from patients with invasive pneumococcal disease, Japan, April 2010–March 2017

Serotype (n)	Penicillin resistance genotype*	Macrolide resistance†			
		Non n = 258	<i>erm</i> (B) n = 1,704	<i>mef</i> (A) n = 557	<i>mef</i> (A) and <i>erm</i> (B) n = 330
4 (58)	gPISP (<i>pbp2x</i>)			1	
	gPSSP	7	2	48	
6B (237)	gPRSP		117	23	10
	gPISP (<i>pbp1a+2x</i>)	2	14	26	2
	gPISP (<i>pbp2x+2b</i>)		6	2	
	gPISP (<i>pbp2x</i>)		26	7	
9V (31)	gPSSP	2			
	gPRSP		1		
14 (111)	gPISP (<i>pbp2x</i>)		5	24	
	gPSSP		1		
	gPRSP	3	31	8	
	gPISP (<i>pbp1a+2x</i>)		54	6	1
18C (15)	gPISP (<i>pbp2x+2b</i>)		4	1	
	gPISP (<i>pbp2x</i>)		2		
	gPSSP	1			
	gPSSP	9	2	4	
19F (107)	gPRSP		10	80	4
	gPISP (<i>pbp1a+2x</i>)			3	2
	gPISP (<i>pbp1a+2b</i>)		1		
	gPISP (<i>pbp2x+2b</i>)			1	
	gPISP (<i>pbp2x</i>)		3	2	
23F (128)	gPSSP	1			
	gPRSP	2	41	22	57
	gPISP (<i>pbp1a+2x</i>)		3		
	gPISP (<i>pbp2x+2b</i>)		2		
1 (36)	gPISP (<i>pbp2x</i>)			1	
	gPSSP	11	9	16	
3 (314)	gPRSP		1		1
	gPISP (<i>pbp2x</i>)	15	279	2	
	gPISP (<i>pbp2b</i>)		1		
5 (1)	gPSSP	11	3	1	
	gPSSP		1		
6A (58)	gPRSP	3	25	6	7
	gPISP (<i>pbp1a+2x</i>)	2	3	1	
	gPISP (<i>pbp2x</i>)	1	6	2	
7F (60)	gPSSP	2			
	gPISP (<i>pbp2x</i>)		4		
19A (290)	gPSSP	15	28	13	
	gPRSP		1	1	89
	gPISP (<i>pbp1a+2x</i>)	1	7	1	28
	gPISP (<i>pbp2x+2b</i>)				3
8 (2)	gPISP (<i>pbp2x</i>)	4	3	51	85
	gPSSP	4	7	4	1
	gPSSP	2			
10A (123)	gPISP (<i>pbp1a+2x</i>)	8	3		1
	gPISP (<i>pbp2x+2b</i>)		1		
	gPISP (<i>pbp2x</i>)	9	86	4	
	gPSSP	2	9		

Serotype (n)	Penicillin resistance genotype*	Macrolide resistance†			
		Non n = 258	<i>erm(B)</i> n = 1,704	<i>mef(A)</i> n = 557	<i>mef(A)</i> and <i>erm(B)</i> n = 330
11A (67)	gPISP (<i>pbp1a+2x</i>)			2	
	gPISP (<i>pbp2x</i>)	10		3	26
	gPSSP	2	1	23	
12F (108)	gPISP (<i>pbp2x+2b</i>)		2		
	gPISP (<i>pbp2b</i>)		98		
	gPSSP	4	4		
15B (68)	gPRSP		1	1	
	gPISP (<i>pbp1a+2x</i>)		12		
	gPISP (<i>pbp2x</i>)		50	1	1
	gPSSP	1		1	
20 (23)	gPSSP	3	19	1	
22F (169)	gPISP (<i>pbp1a+2x</i>)			1	
	gPISP (<i>pbp2x</i>)	43	85	30	
	gPSSP	7	3		
33F (42)	gPISP (<i>pbp2x</i>)			5	
	gPSSP	2	34	1	
6C (154)	gPRSP		1	1	
	gPISP (<i>pbp1a+2x</i>)		1		
	gPISP (<i>pbp2x+2b</i>)	16	22	18	1
	gPISP (<i>pbp2x</i>)	4	62	25	
	gPSSP	2	1		
6D (2)	gPRSP		1	1	
7C (10)	gPSSP		10		
13 (1)	gPRSP		1		
15A (144)	gPRSP		77		
	gPISP (<i>pbp1a+2x</i>)		56		
	gPISP (<i>pbp1a+2b</i>)		7		
	gPISP (<i>pbp2x</i>)		2		1
	gPSSP			1	
15C (53)	gPRSP		2		
	gPISP (<i>pbp1a+2x</i>)		9	1	
	gPISP (<i>pbp2x</i>)		40	1	
16F (8)	gPRSP			4	
	gPISP (<i>pbp1a+2x</i>)			2	
	gPSSP	2			
18B (1)	gPSSP	1			
21 (2)	gPISP(<i>pbp2x</i>)	1		1	
23A (112)	gPRSP		1		
	gPISP (<i>pbp2x+2b</i>)	1	104		
	gPISP (<i>pbp2x</i>)		1		3
	gPSSP	2			
23B (8)	gPRSP	1			
	gPISP (<i>pbp2x+2b</i>)	2			
	gPISP (<i>pbp2x</i>)	1			
	gPSSP	2	1	1	
24F (100)	gPISP (<i>pbp1a+2x</i>)		1		
	gPISP (<i>pbp2x</i>)		2		
	gPSSP		97		
24B (17)	gPSSP		17		
28A (1)	gPSSP	1			
31 (5)	gPSSP	5			
34 (32)	gPRSP		1		
	gPISP(<i>pbp2x</i>)	3	1	9	
	gPSSP	5	13		
35B (106)	gPRSP	6	9	33	7
	gPISP (<i>pbp1a+2x</i>)		1		
	gPISP (<i>pbp2x</i>)		42	1	
	gPSSP		7		
37 (8)	gPSSP	5	2	1	
38 (37)	gPISP (<i>pbp2x+2b</i>)		1		
	gPISP (<i>pbp2x</i>)	1		22	
	gPSSP	8		5	

* 1a, 2x, and 2b in parenthesis indicate abnormal *pbp1a*, *pbp2x*, and *pbp2b* genes, respectively.

†Each gene mediates macrolide resistance such as clarithromycin (CLR) and azithromycin (AZM).

Technical Appendix Table 2. MIC₅₀, MIC₉₀, and MIC range of 6 intravenous antimicrobial agents for isolates from patients with invasive pneumococcal disease from April 2014 to March 2017 (n = 1229)

Antimicrobial agent	Concentration (µg/mL)		
	MIC ₅₀	MIC ₉₀	MIC range
Penicillin	0.063	2	0.016–4
Ampicillin	0.063	2	0.016–8
Cefotaxime	0.25	1	0.008–8
Meropenem	0.008	0.5	0.008–1
Levofloxacin	2	2	0.5–32
Vancomycin	0.5	1	0.5–1

Technical Appendix 3. Serotypes, resistance genotypes, and multilocus sequence types for all isolates (n = 2,849) from patients with invasive pneumococcal disease, Japan, April 2010 - March 2017

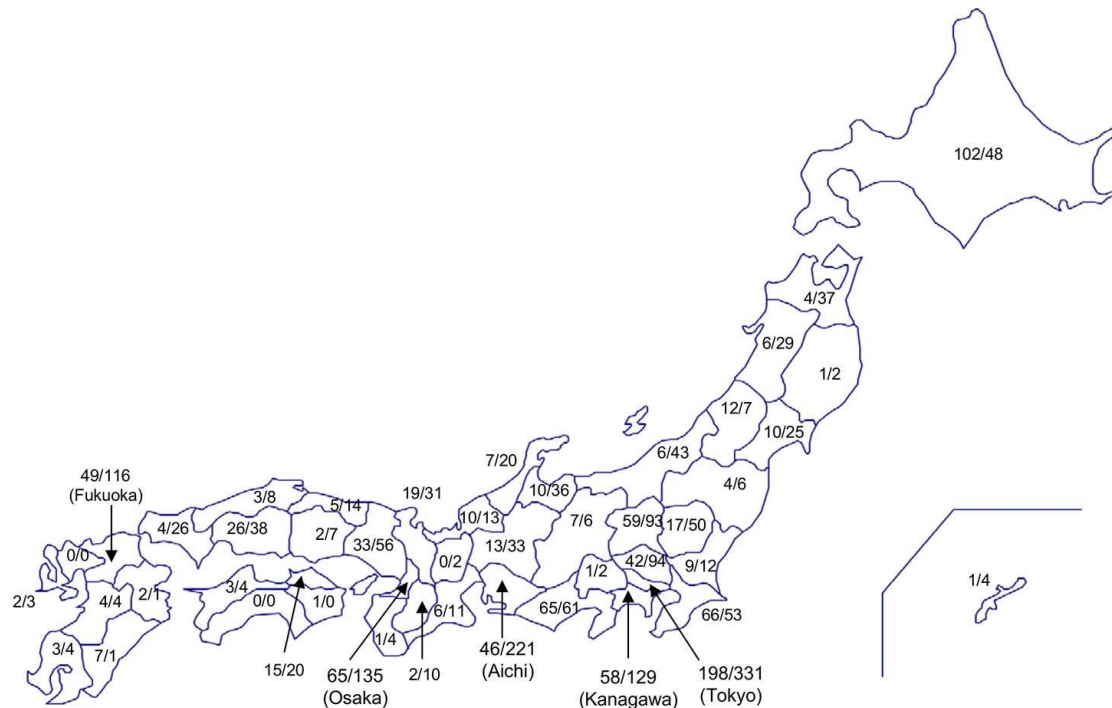
Serotype	Resistance genotype†	Clonal complex (n)	Sequence type (n)
PCV13			
4	gPISP(2x)	81 (1)	899 (1)
	gPSSP	81 (51); 3784 (1); 5902 (1); none (4)	246 (43) 695 (1) 899 (5) 7880 (1) 8649 (1); 7776 (1); 13576 (1)*; 5872 (4)
6B	gPRSP	156 (84); 81 (28); none (11); 9789 (9); 2224 (8); 2924 (5); Singleton (3); 242 (2)	90 (66) 95 (3) 273 (5) 5497 (3) 7417 (1) 7827 (1) 7831 (2) 7870 (1) 13591 (1)* 13593 (1)*; 81 (1) 282 (1) 902 (19) 2923 (1) 3849 (1) 7492 (2) 8345 (1) 8644 (1) 8648(1); 5232 (10) 7967 (1); 2756 (3) 3787 (6); 2224 (7) 7835 (1); 6413 (5); 5244 (3); 242 (2)
	gPISP(1a+2x)	156 (23); 2224 (14); 7834 (4); 2924 (2); Singleton (1)	2983 (16) 5497 (5) 7824 (2); 2224 (13) 7826 (1); 7834 (4); 6183 (2); 13046 (1)
	gPISP(2x+2b)	81 (5); 156 (1); 2924 (1); 7834 (1)	902 (3) 7965(1) 12044 (1); 5830 (1); 8348 (1); 7834 (1)
	gPISP(2x)	81 (15); 156 (12); 2924 (4); 7834 (1); Singleton (1)	902 (1) 2923 (11) 5245 (1) 6430 (2); 273 (1) 2983 (7) 7839 (2) 10194 (2); 2924 (4); 7834 (1); 13048 (1)
9V	gPSSP	2224 (1); 7585 (1)	2224(1); 6771(1)
	gPRSP	156 (1)	166 (1)
	gPISP(2x)	156 (29)	280 (18) 5231 (11)
14	gPSSP	156 (1)	280 (1)
	gPRSP	343 (30); 156 (5); 15 (2); 230 (1); 63 (1); 199 (1); 320 (1); Singleton (1)	343 (23) 554 (2) 3388 (1) 7837 (1) 7971 (2) 7974 (1); 156 (2) 5493 (3); 2922 (2); 230 (1); 782 (1); 876(1); 236 (1); 8642 (1)
18C	gPISP(1a+2x)	15 (41); 230 (19); 3111 (1)	13 (3) 2922 (38); 5240 (17) 7966 (1) 7973 (1); 13571 (1)*
	gPISP(2x+2b)	343 (4); 15 (1)	343 (2); 554(2); 15 (1)
	gPISP(2x)	156 (2)	124 (1) 7972 (1)
	gPSSP	156 (1)	124 (1)
19F	gPSSP	3594 (14); 870 (1)	3594 (13) 7829 (1); 870 (1)
	gPRSP	320 (88); 156 (3); 63 (1); 81 (1); 242 (1)	236 (77) 926 (1) 1421 (1) 1428 (1) 1464(1) 4472 (1) 7873 (2) 7991 (1) 8341 (1) 8344 (1) 13044 (1); 7993 (1) 8352 (2); 10211 (1); 81 (1); 8650 (1)
23F	gPISP(1a+2x)	156 (2); 320 (1); 3111 (1); Singleton (1)	1263 (1) 7993 (1); 926 (1); 3111 (1); 12001(1)
	gPISP(1a+2b)	156 (1)	7993 (1)
	gPISP(2x+2b)	320 (1)	236 (1)
	gPISP(2x)	320 (3); 177 (1); 2924 (1)	236 (2) 257 (1); 179 (1); 6183 (1)
	gPSSP	251 (1)	654 (1)
1	gPRSP	242 (59); 2924 (55); 156 (6); 81 (1); 5832 (1)	242 (48) 1435 (3) 1444 (1) 3589 (1) 7386 (1) 7832 (1) 7968 (1) 8343 (1) 10409 (1) 13575 (1)*; 1437 (51) 6434 (1) 7836 (1) 7872 (2); 338 (5) 2777 (1); 1623 (1); 7989 (1)
	gPISP(1a+2x)	63 (2); 242 (1)	63 (1) 8643 (1); 5844 (1)
	gPISP(2x+2b)	156 (2)	338 (1) 5242 (1)
	gPISP(2x)	242 (1)	242 (1)
3	gPSSP	306 (34); 217 (2)	306 (33) 5239 (1); 5002 (2)
	gPRSP	156 (1); 242 (1)	166 (1); 242 (1)
5	gPISP(2x)	180 (292); 99 (1); 113 (1); 156 (1); 242 (1)	180 (277) 5234 (5) 7875 (1) 7877 (1) 7878 (1) 8646 (1) 8652 (1) 10025 (1) 10420 (2) 11192 (1) 11997 (1); 99 (1); 13045 (1); 1263 (1); 7386 (1)
	gPISP(2b)	1527 (1)	13574 (1)*
	gPSSP	180 (13); Singleton (2)	180 (10) 505 (1) 2570 (1) 12002 (1); 10216 (1) 12005 (1)
6A	gPSSP	289 (1)	289 (1)
	gPRSP	9789 (25); 81 (12); 156 (1); 3115 (1); Singleton (2)	2756 (17) 3113 (1) 6432 (2) 6437 (2) 7825 (2) 8350 (1); 81 (6) 282 (5) 13572 (1)*; 90 (1); 3115 (1); 5244 (1) 7871 (1)
	gPISP(1a+2x)	9789 (3); 156 (1); 2924 (1); Singleton (1)	3113 (2) 3787 (1); 5833 (1); 6183 (1); 11998 (1)

Serotype	Resistance genotype†	Clonal complex (n)	Sequence type (n)
7F	gPISP(2x)	9789 (7); 81 (2)	3113 (5) 3787 (1) 7969 (1); 2923 (2)
	gPSSP	9789 (1); Singleton (1)	4271 (1); 8347 (1)
	gPISP(2x)	191 (4)	191 (4)
19A	gPSSP	191 (54); 218 (2)	191 (54); 405 (2)
	gPRSP	3111 (86); 156 (1); 320 (4)	3111 (85) 9045 (1); 156 (1); 320 (4)
	gPISP(1a+2x)	3111 (28); 2331 (9)	3111 (27) 7988 (1); 2331 (1) 5237 (8)
NVT	gPISP(2x+2b)	3111 (3)	3111 (3)
	gPISP(2x)	3111 (89); 2331 (54)	3111 (88) 7988 (1); 2331 (52) 13047 (2)
	gPSSP	2331 (13); 3111 (3)	2331 (9) 5842 (4); 3111 (2) 8339 (1)
8	gPSSP	156 (1); none (1)	11999 (1); 6022 (1)
10A	gPISP(1a+2x)	156 (9); 113 (2); Singleton (1)	1263 (8) 7773 (1); 5236 (2); 3078 (1)
	gPISP(2x+2b)	156 (1)	5246 (1)
11A	gPISP(2x)	113 (84); 156 (15)	5236 (84); 1263 (11) 3395 (1) 7773 (1) 7830 (2)
	gPSSP	113 (9); 156 (2)	5236 (9); 1263 (1) 12051 (1)
	gPISP(1a+2x)	99 (2)	99 (2)
12F	gPISP(2x)	99 (38); 62 (1)	99 (30) 7153 (7) 7811 (1); 1760 (1)
	gPSSP	99 (23); 62 (3)	99 (21) 7153 (1) 8651 (1); 62 (2) 1012 (1)
	gPISP(2x+2b)	1527 (2)	4846 (2)
15B	gPISP(2b)	1527 (98)	4846 (98)
	gPSSP	1527 (7); 989 (1)	6945 (6) 12003 (1); 989 (1)
	gPRSP	81 (1); 242 (1)	83 (1); 242 (1)
20	gPISP(1a+2x)	199 (12)	199 (12)
	gPISP(2x)	199 (52)	199 (51) 5609 (1)
	gPSSP	199 (2)	199 (2)
22F	gPSSP	4745 (20); 99 (1); 235 (1); 5349 (1)	4745 (16) 7828 (4); 99 (1); 235 (1); 5190 (1)
	gPISP(1a+2x)	433 (1)	4553 (1)
33F	gPISP(2x)	433 (147); 113 (11)	433 (145) 819 (1) 10023 (1); 5236 (2) 7158 (9)
	gPISP(2x)	433 (5); 113 (2); 2572 (1); 3594 (1); none (1)	433 (5); 7158 (1) 8353 (1); 5496 (1); 3594 (1); 3465 (1)
	gPSSP	100 (5)	100 (5)
6C	gPSSP	717 (34); 62 (2); 100 (1)	717 (34); 673 (2); 100 (1)
	gPRSP	156 (1); 5832 (1)	8352 (1); 5832 (1)
	gPISP(1a+2x)	2924 (1)	12080 (1)
6D	gPISP(2x+2b)	156 (30); 5832 (24); 315 (2); Singleton (1)	5241 (29) 12081 (1); 5025 (2) 5832 (20) 7384 (2); 386 (2); 9025 (1)
	gPISP(2x)	2924 (59); 81 (23); 7781 (4); 156 (2); 9789 (2); 242 (1)	2924 (37) 6183 (16) 7838 (1) 7879 (1) 9336 (2) 12080 (1) 12082 (1); 2923 (23); 7788 (2) 7876 (2); 4233 (1) 13587 (1)*; 3787 (2); 242 (1)
	gPSSP	156 (1); 473 (1); 7781 (1)	8645 (1); 473 (1); 2789 (1)
7C	gPRSP	81 (1); 156 (1)	282 (1); 90 (1)
13	gPSSP	2758 (10)	2758 (10)
15A	gPRSP	156 (1)	10303 (1)
	gPRSP	63 (77)	63 (73) 2105 (1) 2771 (1) 8354 (1) 12000 (1)
	gPISP(1a+2x)	63 (56)	63 (42) 7874 (13) 10211 (1)
15C	gPISP(1a+2b)	63 (6); Singleton (1)	63 (6); 8346 (1)
	gPISP(2x)	292 (2) 113 (1)	292 (2); 7992 (1)
	gPSSP	292 (1)	292 (1)
16F	gPRSP	81 (2)	83 (1) 6138 (1)
	gPISP(1a+2x)	199 (10)	199 (9) 1674 (1)
	gPISP(2x)	199 (41)	199 (39) 9222 (1) 12073 (1)
18B	gPRSP	3117 (4)	8351 (4)
	gPISP(1a+2x)	3117 (2)	3117 (2)
	gPSSP	3117 (2)	3117 (2)
21	gPSSP	3594 (1)	3594 (1)
23A	gPISP(2x)	1381 (2)	1233 (2)
	gPRSP	156 (1)	9619 (1)
23B	gPISP(2x+2b)	156 (105)	338 (57) 2612 (1) 3437 (1) 5242 (39) 5246 (5) 6685 (1) 8340 (1)
	gPISP(2x)	156 (4)	338 (1) 10000 (3)
	gPSSP	439 (2)	42 (1) 438 (1)
24	gPRSP	156 (1)	2372 (1)
	gPISP(2x+2b)	156 (2)	1373 (2)
	gPISP(2x)	439 (1)	439 (1)
24	gPSSP	439 (3); 63 (1)	439 (3); 63 (1)
	gPISP(1a+2x)	230 (1)	230 (1)
	gPISP(2x)	2572 (2)	2572 (2)
	gPSSP	2572 (112); 4982 (2)	2572 (62) 5496 (50); 4982 (2)

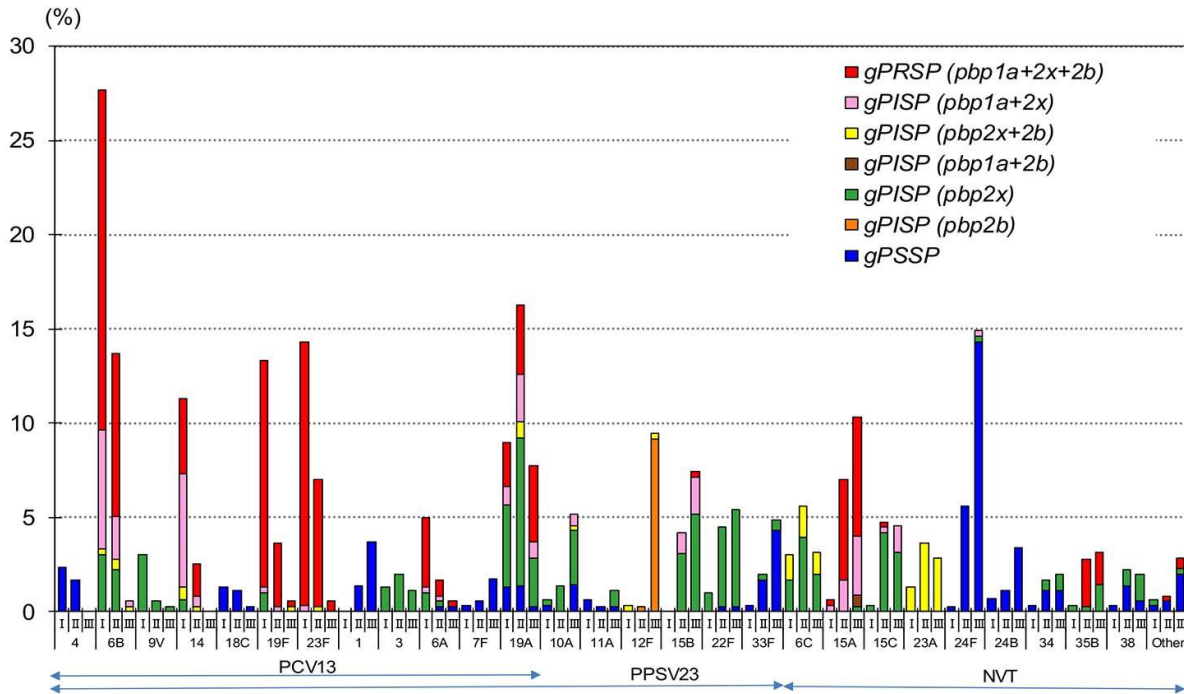
Serotype	Resistance genotype†	Clonal complex (n)	Sequence type (n)
28A	gPSSP	546 (1)	11601 (1)
31	gPSSP	7800 (5)	11184 (5)
34	gPRSP	15 (1)	9395 (1)
	gPISP(2x)	none (12); 15 (1)	3116 (12); 7388 (1)
	gPSSP	15 (13); none (5)	1439 (1) 7388 (12); 3116 (5)
35B	gPRSP	558 (53); 156 (1); Singleton (1)	558 (49) 1204 (2) 7809 (2); 156 (1); 7990 (1)
	gPISP(1a+2x)	1816 (1)	2755 (1)
	gPISP(2x)	1816 (43)	2755 (42) 12004 (1)
	gPSSP	1816 (7)	2755 (7)
37	gPSSP	447 (8)	447 (6) 7970 (2)
38	gPISP(2x+2b)	none (1)	7833 (1)
	gPISP(2x)	6429 (23)	6429 (23)
	gPSSP	393 (12); 6429 (1)	393 (12); 6429 (1)

*STs newly identified in this study.

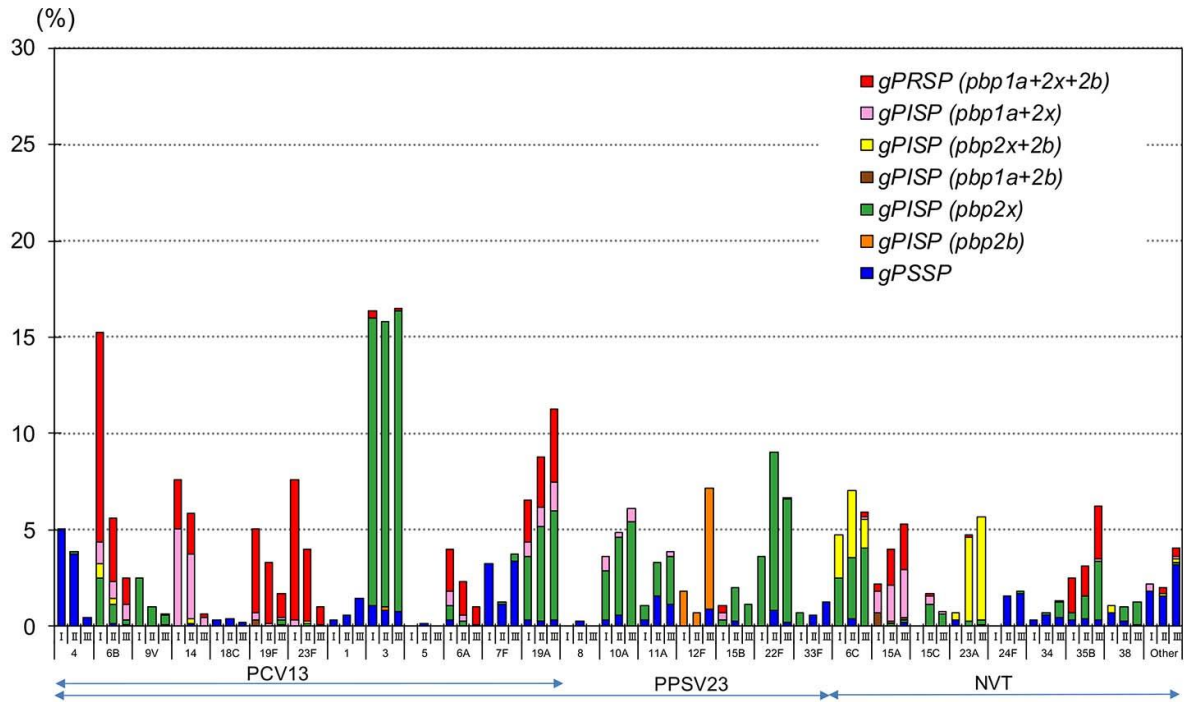
† 1a, 2x, and 2b in the parenthesis indicate *pbp1a*, *pbp2x* and *pbp2b* genes, respectively. PBPs (PBP1A, PBP2X, and PBP2B) involved in peptidoglycan synthesis are encoded by these genes. Amino acid substitutions within or near each PBP's conserved amino acid motifs were identified in resistance strains with various combinations of abnormal genes.



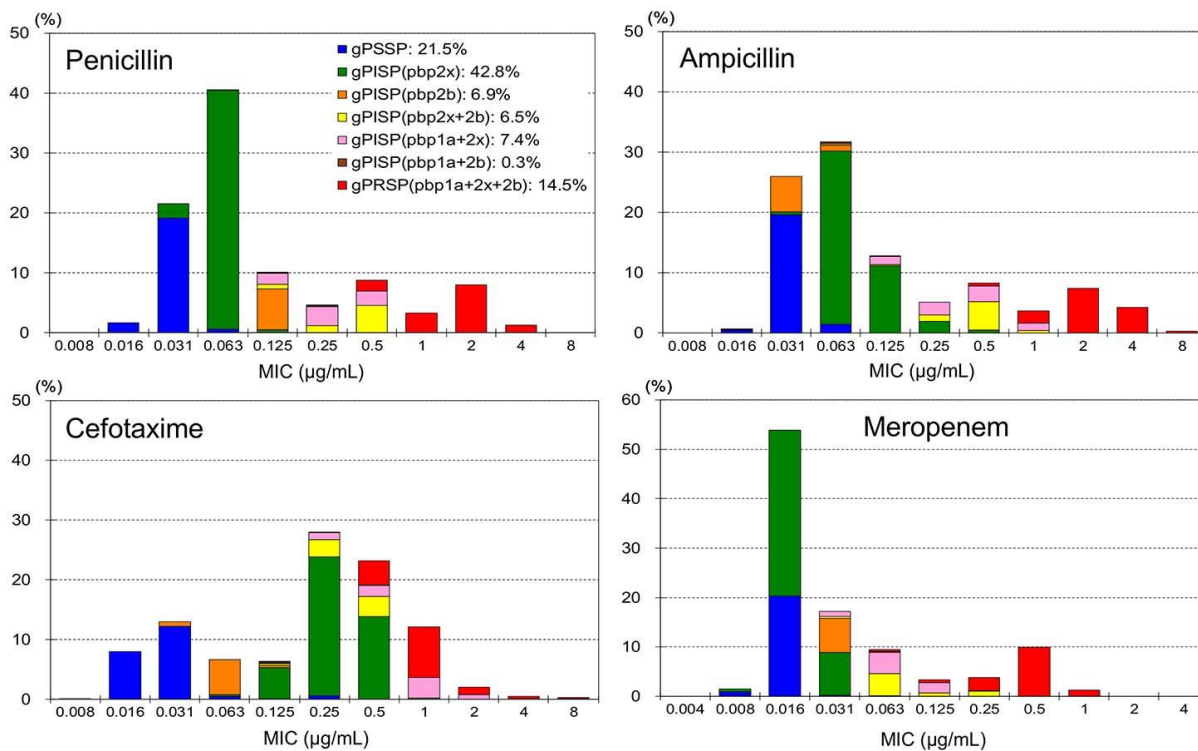
Technical Appendix Figure 1. Distribution of isolates collected by the surveillance for invasive pneumococcal disease in Japan from April 2010 to March 2017. Numbers of isolates obtained from children/adults are shown for each locality on the map.



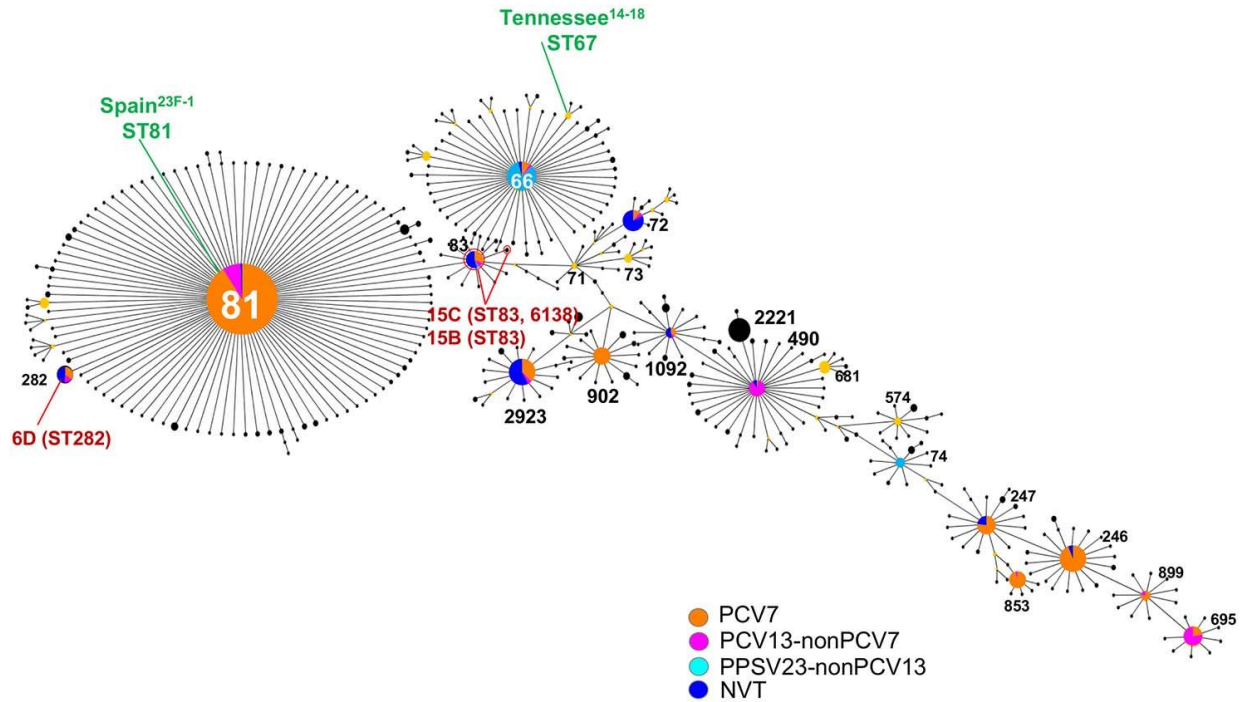
Technical Appendix Figure 2. Yearly changes of serotypes and penicillin-resistant genotypes among isolates from children with invasive pneumococcal disease in Japan from April 2010 to March 2017. I, II, and III on horizontal axis represent three surveillance periods as follows: pre-PCV7 period, from April 2010 through March 2011; PCV7 period, from April 2011 to March 2014; and PCV13 period, from April 2014 through March 2017. gPSSP, genotypic penicillin-susceptible *Streptococcus pneumoniae*; gPISP, genotypic penicillin-intermediate resistant *S. pneumoniae*; gPRSP, genotypic penicillin-resistant *S. pneumoniae*. Parentheses enclose mutated *pbp* genes, *pbp1a*, *pbp2x*, and/or *pbp2b*, that mediate penicillin resistance.



Technical Appendix Figure 3. Yearly changes of serotypes and penicillin-resistant genotypes among isolates from adults with invasive pneumococcal disease in Japan from April 2010 to March 2017. I, II, and III on horizontal axis represent three surveillance periods as follows: pre-PCV7 period, before vaccination to children, from April 2010 to March 2011; PCV7 period, after PCV7 vaccination to children, from April 2011 to March 2014; and PCV13 period, with PCV7 replaced by PCV13 vaccination in children, from April 2014 to March 2017. gPSSP, genotypic penicillin-susceptible *Streptococcus pneumoniae*; gPISP, genotypic penicillin-intermediate resistant *S. pneumoniae*; gPRSP, genotypic penicillin-resistant *S. pneumoniae*. Parentheses enclose mutated *pbp* genes, *pbp1a*, *pbp2x*, and/or *pbp2b*, that mediate penicillin resistance.



Technical Appendix Figure 4. Distribution of susceptibilities to 4 parenteral β -lactam antibiotics, penicillin, ampicillin, cefotaxime, and meropenem, by genotypes of isolates from patients with invasive pneumococcal disease in Japan from April 2014 to March 2017 ($n = 1229$). gPSSP indicates genotypic penicillin-susceptible *Streptococcus pneumoniae*; gPISP indicates genotypic penicillin-intermediate-resistant *S. pneumoniae*; and gPRSP indicates genotypic penicillin-resistant *S. pneumoniae*. Parentheses enclose mutated *pbp* genes *pbp1a*, *pbp2x*, and/or *pbp2b*, which mediate penicillin resistance.



Technical Appendix Figure 5. Details of clonal complex (CC) 81 (n = 1858) included 420 sequence types (STs) from the Multilocus Sequence Typing Web site (<https://pubmlst.org/spneumoniae/>) (download October/02/2017). Data include our own from the present study (n = 143). STs of 3 gPRSPs identified in serotypes 6D, 15B, and 15C (in red) were derived from ST81, belonging to CC81. The Pneumococcal Molecular Epidemiology Network (PMEN) clones identified in CC81 also are shown (in green).