Spread of Meropenem-Resistant *Streptococcus pneumoniae* Serotype 15A-ST63 Clone in Japan, 2012–2014

Satoshi Nakano, Takao Fujisawa, Yutaka Ito, Bin Chang, Yasufumi Matsumura, Masaki Yamamoto, Miki Nagao, Shigeru Suga, Makoto Ohnishi, Satoshi Ichiyama

After the introduction of pneumococcal conjugate vaccines, the incidence of pneumococcal infections due to meropenem-resistant serotype 15A-ST63 strains increased in Japan. By using whole-genome sequencing and comparing sequences with those of clones from the United Kingdom, the United States, and Canada, we clarified the traits of the serotype 15A-ST63 clone. Our analysis revealed that the meropenem-resistant serotype 15A-ST63 strains from Japan originated from meropenem-susceptible strains from Japan. Recombination site prediction analysis showed that the meropenem-resistant strain-specific recombination regions included the *pbp1a* and *pbp2b* regions. A detailed analysis of the composition of these genes indicated that resistance seems to be caused by *pbp1a* recombination. The *pbp1a* gene in meropenem-resistant isolates was identical to that in multidrug (including meropenem)–resistant serotype 19A-ST320 pneumococci, which have spread in the United States. The global spread of pneumococci of this lineage is noteworthy because serotype 15A is not included in the currently used 13-valent pneumococcal conjugate vaccine.

Streptococcus pneumoniae is a common pathogen that causes various types of bacterial infections, such as pneumonia, otitis media, occult bacteremia, and meningitis (*1*). *S. pneumoniae* is enclosed in a complex polysaccharide capsule that can be used to classify strains into serotypes. So far, at least 92 structurally and serologically distinct serotypes have been recognized (*2*). To prevent invasive pneumococcal diseases, vaccines currently in use in various regions of the world are 7-, 10,- and 13-valent pneumococcal conjugate vaccines (PCVs) that target a subset of the serotypes. Although these vaccines have decreased the total number of cases of invasive pneumococcal disease, they have also caused a shift in serotype (i.e., increased rate of identification of non-PCV serotype pneumococci) in areas where the vaccines have been introduced (*3–6*). Public health officials are concerned about the increased incidence of non-PCV serotype pneumococcal infections and the spread of resistant strains that are not covered by the currently used PCVs.

In Japan when PCV13 was in use (2012–2014), we conducted a nationwide pneumococcal infection surveillance study among children (*7*). We observed an increase in multidrug (penicillin, macrolide, and meropenem)–resistant pneumococcal isolates of serotype 15A and sequence type (ST) 63 (serotype 15A-ST63), which is not covered by PCV13. Similarly, several surveillance studies in other countries also reported an increase in serotype 15A-ST63 pneumococcal infections, including infections caused by penicillin-resistant strains (*8–16*) but not meropenem-resistant serotype 15A pneumococcal strains. A pneumococcal strain of serotype 15A-ST63 from Sweden was submitted as a resistant clone to the Pneumococcal Molecular Epidemiology Network (PMEN) collection (Sweden^{15A}-25, ATCC BAA-661) (*17*); however, the penicillin MIC for Sweden^{15A}-25 differs from that for serotype 15A-ST63 from Japan. Although the submitted PMEN strain is slightly resistant to penicillin (MIC 0.064), most serotype 15A-ST63 isolates from Japan are more resistant to penicillin. Although the introduction of PCVs is thought to have driven the increase in non-PCV pneumococcal infections, the mechanism by which the strains have become resistant remains unclear.

In general, pneumococcal resistance to penicillin and cephalosporins (including carbapenems) is caused by mutations in penicillin binding proteins (PBPs), especially PBPs 1a, 2b, and 2x (*18*). Although PBP genes of sensitive pneumococci are well conserved, PBPs of resistant

Author affiliations: Kyoto University Graduate School of Medicine, Kyoto, Japan (S. Nakano, Y. Matsumura, M. Yamamoto, M. Nagao, S. Ichiyama); National Hospital Organization Mie National Hospital, Tsu, Japan (T. Fujisawa, S. Suga); Nagoya City University Graduate School of Medical Science, Nagoya, Japan (Y. Ito); National Institute of Infectious Diseases, Tokyo, Japan (B. Chang, M. Ohnishi)

DOI: https://doi.org/10.3201/eid2402.171268

isolates are encoded by highly variable genes containing sequence blocks that are generally referred to as mosaic genes; these genes are generated by recombination events (*19–21*). Therefore, tracking the sequences of PBP genes is useful for predicting resistance to antimicrobial drugs and following pneumococcal epidemiologic trends. In various countries, meropenem is widely used to treat severe infectious diseases; however, infectious diseases caused by pathogens resistant to meropenem (carbapenems) have been increasing (*22*). Although carbapenem resistance has been noted mainly in *Enterobacteriaceae*, resistance in other pathogens, including *S. pneumoniae*, should be noted because such broad-spectrum antimicrobial drugs are often used empirically.

Our aim with this study was to clarify the genetic characteristics of meropenem-resistant serotype 15A-ST63 strains isolated in Japan. In addition, using whole-genome sequencing data, we mapped the evolution of the clone by revealing the genetic associations among drug-resistant serotype 15A-ST63 pneumococcus isolates from different areas of the world (Japan, United Kingdom, United States, and Canada).

Materials and Methods

Bacterial Isolates

From January 2012 through December 2014, we conducted a nationwide, prospective surveillance study among children in Japan with and without invasive pneumococcal disease (*7*). From 154 medical institutions, we collected isolates from patients with (343 isolates) and without (286 isolates) invasive pneumococcal disease. Among these 629 isolates, we obtained 52 serotype 15A-ST63 isolates, including 35 meropenem-nonsusceptible (MEM-NS) isolates and 17 meropenem-susceptible (MEM-S) isolates. With regard to MEM-NS non–serotype 15A isolates, we obtained 66 isolates comprising 8 serotypes (6A, 6B, 6D, 15B/C, 19A, 19F, 23F, and 35B) and nontypeable serotypes. Following the 2008 Clinical and Laboratory Standards Institute guidelines (*23*), we performed susceptibility testing for meropenem by using the broth microdilution method and defined the MEM-NS MIC as ≥0.5 mg/L and the MEM-S MIC as ≤0.25 mg/L.

Whole-Genome Sequencing

Of the isolates described above, we obtained read data for 24 MEM-NS serotype 15A-ST63 isolates, 10 MEM-S serotype 15A-ST63 isolates, and 32 MEM-NS non–serotype 15A (6A, 6B, 6D, 15B/C, 19A, 19F, 23F, 35B, and untypeable) isolates (online Technical Appendix Table 1, https://wwwnc.cdc.gov/EID/article/24/2/17-1268-Techapp1.pdf). To achieve a uniform distribution of regions and times where and when the isolates were identified, we randomly selected 24 MEM-NS serotype 15A-ST63, 10 MEM-S serotype 15A-ST63, and 32 MEM-NS non–serotype 15A isolates from the isolate repository (Table 1). In addition, we obtained read data for the PMEN 15A-25 strain (Sweden^{15A}-25, ATCC BAA-662). We used a QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany) to extract total genomic DNA from

Table 1. Pneumococcal isolates from Japan, 2012–2014, and MICs for penicillin, meropenem, and erythromycin

Serotype and sequence type	No. isolates	MIC, μg/mL							
		Penicillin			Meropenem		Erythromycin		
		≤0.06	0.12–1.0	≥2.0	≤0.06	≥0.5	≤0.25	0.5	≥1.0
15A									
63	34	0	13	21	10	24	0	0	34
15B/C									
83	2	0	0	2	0	2	0	0	2
3934	1	0	1	0	0	1	0	0	1
19A									
3111	7	0	4	3	0	7	0	0	7
320	4	0	0	4	0	4	0	0	4
19F									
236	2	0	0	2	0	2	0	0	2
115	1	0	0	1	0	1	0	0	1
23F									
242	1	0	0	1	0	1	0	0	1
35B									
558	8	0	5	3	0	8	2	0	6
6A									
2756	1	0	0	1	0	1	0	0	1
6B									
9335	1	0	0	1	0	1	0	0	1
6D									
282	1	0	0	1	0	1	0	0	1
Untypeable									
7502	1	0	0	1	0	1	0	0	1
4845	1	0	0	1	0	1	0	0	1
10253	1	0	0	1	0	1	0	0	1
Total	66	0	23	43	10	56	2	0	64

each bacterial isolate and the Nextera XT DNA Library Preparation Kit (Illumina, San Diego, CA, USA) to prepare libraries for sequencing. We multiplexed and sequenced the samples on an Illumina MiSeq for 600 cycles (2 × 300-bp paired-end).

Comparing Genomic Data

To compare the genomic characteristics of serotype 15A-ST63 isolates from Japan with those of isolates from the United Kingdom, the United States, and Canada, we downloaded whole-genome read data from the Sequence Read Archive database (http://www.ncbi.nlm.nih.gov/sra/) (online Technical Appendix Table 1) (*5*,*8*,*24*,*25*). To confirm that the data were from serotype 15A-ST63 isolates, we conducted a de novo assembly by using SPAdes (*26*). The contigs were analyzed by using BLAST+ (*27*) to verify the presence of the pneumococcal serotype 15A–specific region (online Technical Appendix Table 5) (*5*), followed by multilocus sequence typing (MLST) (http://pubmlst.org/spneumoniae/), which used the extracted subsequences of each allele from the contigs. We used the draft genome data that contained serotype 15A–specific genes from ST63 isolates for the subsequent analysis.

Phylogenomic Analyses

To create a phylogenic tree, we used Genealogies Unbiased By recomBinations In Nucleotide Sequences (Gubbins) (*28*), which identifies recombination sites and constructs a phylogenic tree based on the putative point mutations outside of the regions. First, we created 2 phylogenic trees. Although there were no ST63 isolates or isolates that were closely related to ST63 with non–serotype 15A, to prove that there were no serotype switch events from non–serotype 15A to serotype 15A, we created the first tree with isolates from Japan only. To reveal the genetic associations among serotype 15A-ST63 isolates from Japan and elsewhere around the world that are increasing in incidence, we created the second tree with only serotype 15A-ST63 isolates from Japan and other regions in the world. After obtaining the second phylogenic tree, to predict the recombination sites that caused meropenem resistance, we created an additional phylogenic tree by using the isolates that were clustered into the same clade that included MEM-NS and MEM-S serotype 15A-ST63 isolates.

PBP Profiles, Antimicrobial Resistance Genes, and Pilus Detection

To compare the sequences of the transpeptidase regions of *pbp1a*, *2b*, and *2x* of all isolates, we extracted each PBP transpeptidase region from all obtained contigs by using BLAST+. We allocated PBP transpeptidase type numbers to these contigs by using previous PBP sequence data from the United States (*5*,*29*–*31*). To predict a causal PBP transpeptidase type for meropenem resistance in serotype 15A-ST63 isolates from Japan, we identified recombination sites within these serotype 15A isolates by using Gubbins. In addition, we identified the presence of the *ermB*, *ermTR*, *mefA*, *mefE*, *tetM*, *tetO*, *rrgA-1* (pili1), and *pitB-1* (pili2) genes and searched for mutations within the *folA* and *folP* genes by using the assembled contigs (*5*). Details of the genomic analysis process are described in the online Technical Appendix.

Results

Whole-Genome Sequencing Statistics

The sequencing statistics are shown in online Technical Appendix Table 2. With use of the 2,078,953-bp *S. pneumoniae* G54 chromosome (reference sequence GenBank accession no. NC_011072.1), serotype 15A-ST63 and PMEN15A-25 isolate genomes were sequenced at an average depth (± SD) of 49.33 (± 9.65) and an average coverage of 95.60% (± 3.75%). The average numbers of contigs and N_{50} (bp) of isolates from Japan sequenced in this study were 109.2 (SD ± 42.2) and 70,819 (SD ± 10,701), respectively.

Phylogenomics

The phylogenic tree created by using all Japan and global isolates classified these isolates into 11 clusters (online Technical Appendix Figure 1). All of the Japan serotype 15A isolates were included in the same cluster, and none of the non–serotype 15A isolates were included in this cluster. This fact indicated that none of the non–serotype 15A isolates in this analysis seemed to be the origin of the MEM-NS serotype 15A-ST63 isolates.

The phylogenic tree created by using all Japan and global serotype 15A-ST63 isolates revealed the presence of 2 serotype 15A-ST63–specific clades (clades I and II) from Japan (Figure 1). Clade I included the subclade clade I-MNS, which consisted of all 24 MEM-NS serotype 15A-ST63 isolates. This result indicated that the Japan MEM-NS isolates originated from Japan MEM-S isolates. Four other Japan MEM-S serotype 15A-ST63 isolates were classified into clade II. With some exceptions, the global isolates were clustered according to the areas where the isolates were recovered.

We identified 52 genes that were specific to the subclade clade I-MNS. These genes did not exist in Japan MEM-S serotype 15A-ST63 isolates but were found in the Japan MEM-NS isolates (online Technical Appendix Methods, Table 4).

PBP Recombination Sites that Could Cause Meropenem Resistance

Using all of the clade I isolates, we predicted the recombination sites that caused meropenem resistance in the

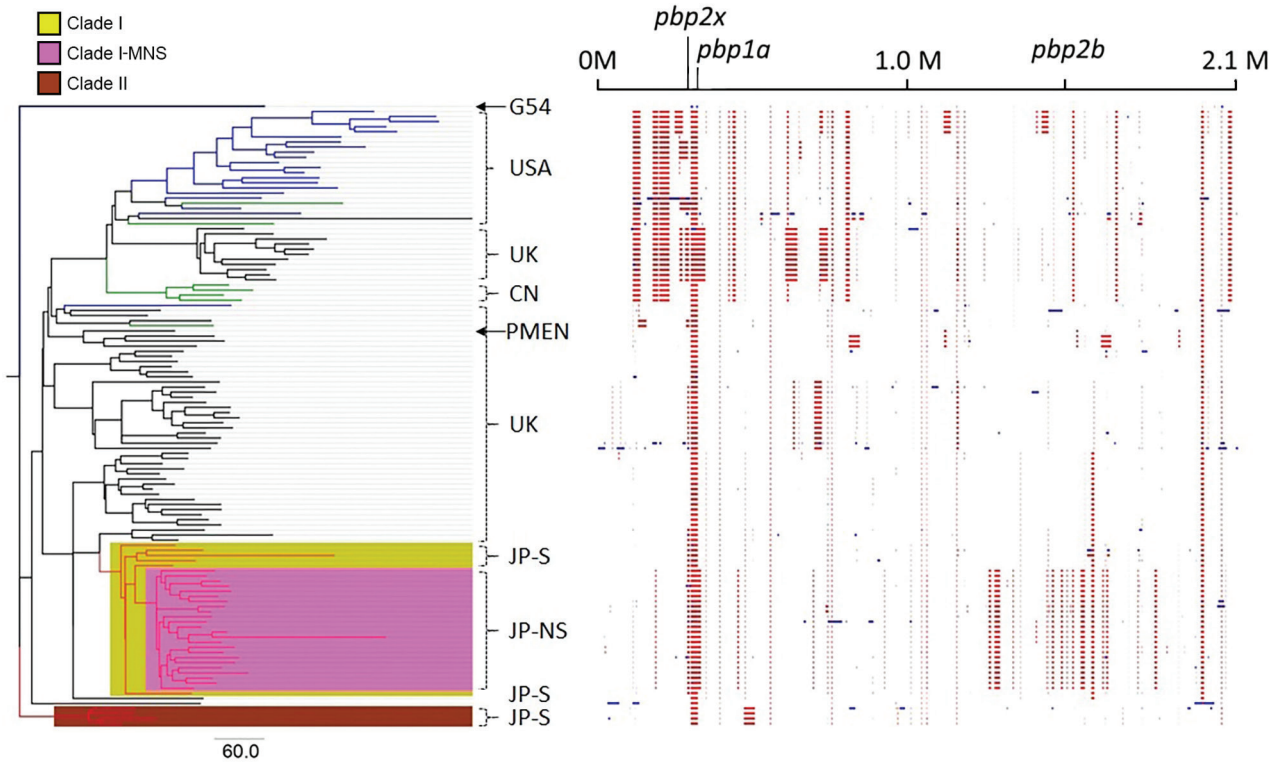


Figure 1. Phylogenic tree and predicted recombination sites created in Genealogies Unbiased By recomBinations In Nucleotide Sequences (*28*) by using all Japan and global serotype 15A-ST63 pneumococcal isolates. Branch colors in the tree indicate where the isolates were collected: red, Japan; black, United Kingdom; blue, United States; green, Canada. The column on the right of the tree indicates the main region from which the isolates were derived, meropenem susceptibility, and isolate names. The phylogenic tree was created by using *Streptococcus pneumoniae* G54 as an outgroup isolate. Clade I consists of only Japan serotype 15A-ST63 isolates; clade I-MNS consists of only Japan meropenem-nonsusceptible serotype 15A-ST63 isolates; clade II consists of the rest of the Japan meropenem-susceptible serotype 15A-ST63 isolates that are not included in clade I. The block chart on the right shows the predicted recombination sites in each isolate. Blue blocks are unique to a single isolate; red blocks are shared by multiple isolates. All isolates shaded in pink are meropenem nonsusceptible. Arrows indicate reference strains *S. pneumoniae* G54 and PMEN 15A-25. Scale bar indicates nucleotide substitutions per site; CN, Canada; G54, *S. pneumoniae* G54; M, million base pairs; JP-NS, Japan meropenem nonsusceptible; JP-S, Japan meropenem susceptible; PMEN, Pneumococcal Molecular Epidemiology Network; ST, sequence type; UK, United Kingdom; USA, United States.

MEM-NS serotype 15A-ST63 isolates from Japan. This analysis revealed 19 recombination sites that were specific to all MEM-NS isolates (Figure 2; online Technical Appendix Figure 2). One of these recombination sites included the whole *pbp1a* gene, and another overlapped with a part of the nucleotide sequence of *pbp2b*. The recombination site covering *pbp1a* included 8,384 bp (positions 326417–334800 of the reference strain *S. pneumoniae* G54) (online Technical Appendix Figure 3). All MEM-NS serotype 15A-ST63 isolates had the same nucleotide sequence of *pbp1a* as those of MEM-NS serotype 19A, 19F, 23F, 6A, 6B, and nontypeable isolates from Japan. The recombination site that overlapped with the *pbp2b* gene was a 1,970-bp region (positions 1523469–1525438 of the reference strain *S. pneumoniae* G54) (online Technical Appendix Figure 3). Because of this recombination, a portion of the nucleotide sequence of *pbp2b* was replaced with the current sequence, resulting in development of a novel MEM-NS serotype 15A-ST63 *pbp2b* gene that was not found in other isolates in this study or in the public database.

Comparison of the PBP Profiles and 3 Conserved Amino Acid Motifs

pbp1a

All Japan MEM-NS serotype 15A-ST63 isolates had type 13 *pbp1a*, which has been identified mainly in multidrug-resistant serotype 19A and 19F isolates from the United States (Table 2; online Technical Appendix Tables 1, 6) (*5*). Of 32 Japan MEM-NS non–serotype 15A isolates, 18 also had type 13 *pbp1a*. These 18 isolates included 10 serotype 19A isolates, 3 serotype 19F isolates, and isolates of 5 other serotypes. All Japan MEM-S serotype 15A-ST63 isolates had type 24 *pbp1a,* which was identified in the PMEN15A-25 isolate and in penicillin-intermediate-resistant and MEM-S serotype 15A isolates in the United States. Of

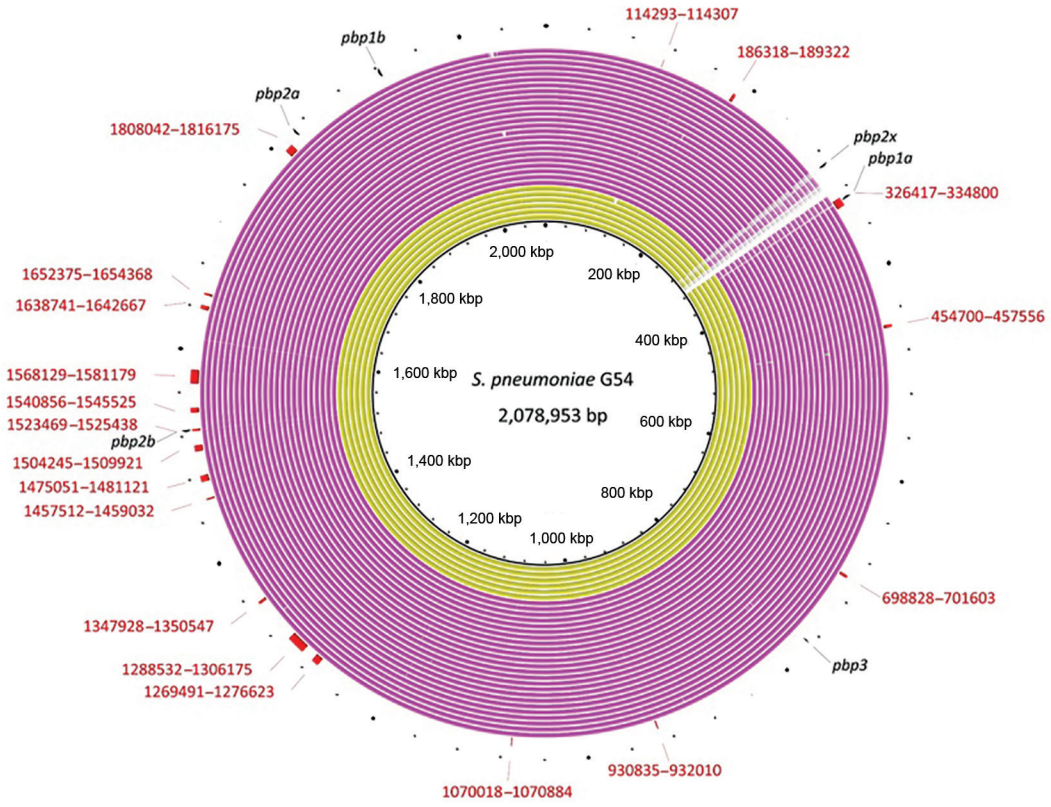


Figure 2. Genomic similarities to *Streptococcus pneumoniae* G54 (reference sequence GenBank accession no. NC_011072.11) and Japan meropenem-nonsusceptible serotype 15A-ST63 isolate-specific recombination sites that were obtained in Genealogies Unbiased By recomBinations In Nucleotide Sequences (*28*) by using all clade I and clade I-MNS isolates. Colored segments indicate >95% similarity; gray segments indicate >90% similarity by BLAST (*27*) comparison between each isolate genome and *S. pneumoniae* G54. The outside red bars indicate the recombination sites that were specific to meropenem-nonsusceptible serotype 15A-ST63 isolates and identified in all of these isolates. Red numbers indicate the sequence coordinates of the recombination sites when *S. pneumoniae* G54 was used. Outside short black lines indicate each penicillin binding protein region. MR, Japan meropenem nonsusceptible; MS, Japan meropenem susceptible; ST, sequence type.

86 global isolates, 74 also had type 24 *pbp1a* and the other 12 had novel *pbp1a* genes. All 24 Japan MEM-NS serotype 15A-ST63 isolates had the same SSMK motif (Table 3). All 10 Japan MEM-S serotype 15A-ST63 isolates had the STMK motif.

pbp2b

The Japan MEM-NS serotype 15A-ST63 isolates had a specific novel *pbp2b* type that was not found in the US *pbp2b* profile list (Table 2; online Technical Appendix Tables 1, 7) (*5*). In the reference *pbp2b* type database, type 74 *pbp2b* was the closest to the novel type from Japan, which possessed 5 aa sequence mutations. Japan MEM-NS non–serotype 15A isolates showed 8 *pbp2b* types, and the types differed from each other mainly by serotype. All Japan MEM-S serotype 15A-ST63 isolates showed type 27 *pbp2b,* which was identified in penicillin-intermediate-resistant and MEM-S serotype 15A isolates in the United States. Of 86 global isolates, 83 also had type 27 *pbp*2b. All sequenced isolates from Japan, including MEM-NS and MEM-S serotype 15A-ST63 isolates and MEM-NS non–serotype 15A isolates, had the same SVVK, SSN, and KTG motifs (Table 3).

pbp2x

In contrast to the *pbp1a* and *pbp2b* type profiles, the *pbp2x* type profile showed a more complicated distribution. The most prevalent *pbp2x* type in the Japan MEM-NS serotype 15A-ST63 isolates was type 43 (22/24 isolates), and 5 of 10 Japan MEM-S serotype 15A-ST63 isolates also had the type 43 *pbp*2x gene (Table 2; online Technical Appendix Tables 1, 8). This type was identified in a serotype 19F-CC177 isolate from the United States that was resistant to penicillin and susceptible to meropenem (*5*). The other 2 MEM-NS serotype 15A-ST63 isolates each had a novel *pbp*2x type. Among the global isolates, the most prevalent

Table 2. Penicillin binding protein profile of *Streptococcus pneumonaie* serotype 15A-ST63 isolates from Japan, 2012–2014*

Clone (no.)	Penicillin binding protein profile			
	pbp1a (no.)	*pbp2b* (no.)	*pbp2x* (no.)	*pbp1a:pbp2b:pbp2x* (no.)
MEM-S-15A-ST63 (10)	24 (10)	27 (10)	43 (5), 28 (3), 112 (1), new1 (1)	24:27:43 (5), 24:27:28 (3), 24:27:112 (1), 24:27:new1 (1)
MEM-NS-15A-ST63 (24)	13 (24)	new1 (24)	43 (22), new3 (1), new6 (1)	13:new1:43 (22), 13:new1:new3 (1), 13:new1:new6 (1)
MEM-NS-19A-ST320 (4)	13 (4)	11 (4)	16 (4)	13:11:16 (4)

*MEM-NS, meropenem-nonsusceptible; MEM-S, meropenem-susceptible; ST, sequence type.

*pbp*2x type was type 28 (55/86 isolates), followed by type 35 (12/86) and type 179 (12/86). Similar to the *pbp*2b type, the Japan MEM-NS non–serotype 15A isolates showed different *pbp2x* types on the basis of their serotypes. The most prevalent SXXK motif in the Japan MEM-NS serotype 15A-ST63 and MEM-S serotype 15A-ST63 isolates was SAMK (Table 3). All sequenced isolates from Japan had the same SSN and KSG motifs.

Antimicrobial Resistance Genes and Pilus Determinants

All serotype 15A-ST63 isolates from Japan had *tetM* and *ermB* genes and were negative for the *ermTR*, *tetO*, *mefA*, and *mefE* genes; *folA* mutation; and *folP* insertion (online Technical Appendix Table 3). Only 1 of 34 Japan serotype 15A-ST63 isolates had a deletion of 2 nt at codon 339 in *tetM*, generating a premature stop codon; resistance gene prevalence did not differ between MEM-S and MEM-NS serotype 15A-ST63 isolates from Japan. The same deletion was identified in 12 of the isolates from the United Kingdom and in PMEN15A-25. In addition, all Japan and global serotype 15A isolates lacked pilus determinants PI-1 and PI-2. With regard to global serotype 15A-ST63 isolates, the profiles of *tetM*, *ermB*, *tetO*, *mefA*, and *mefE* were the same as those of Japan serotype 15A-ST63 isolates: positive for *tetM* and *ermB* and negative for *ermTR*, *tetO*, *mefA*, and *mefE*. However, 16 isolates (5 from the United States and 11 from the United Kingdom) had a *folA* mutation (I100L substitution). All 16 of these isolates also had a *folP* insertion (1–2 codons between bases 168 and 201). An additional 8 isolates (7 from the United States and 1 from Canada) had only the *folP* insertion.

Discussion

After the introduction of PCVs, serotype 15A pneumococcal infections and colonization increased in many countries (*7*–*16*). According to previous molecular studies, most serotype 15A isolates belonged to ST63, which has been named Sweden^{15A}-25 (PMEN15A-25) in the PMEN and shows strong macrolide resistance. The PMEN database and previous studies regarding serotype 15A-ST63 strains indicate that most isolates that were closely related to PMEN15A-25 were susceptible (MIC ≤0.06 mg/L) or showed intermediate resistance (MIC 0.12–1.0 mg/L) to penicillin (*5*,*9*,*10*,*32*). Although data for meropenem susceptibility of these isolates are limited (*5*,*7*), no studies have demonstrated the meropenem resistance of this strain. Thus, the spread of this strain so far seems to be limited to Japan. However, this finding is of concern for several reasons. One reason is the fact that serotype 15A is not included in the currently used PCV13; therefore, the increased incidence would continue under the current vaccine pressure. In addition, penicillin, meropenem, and macrolide resistance may cause the strain to spread rapidly. In fact, after the introduction of PCV7, multidrug-resistant serotype 19A-CC320/271 spread rapidly and widely in the United States (*33*).

Several previous studies revealed the emergence of serotype-switched new strains that showed resistance to several antimicrobials (*33*–*35*). Most of the mechanisms underlying the emergence of new resistant strains are

Table 3. PBP 1a, 2b, and 2x transpeptidase conserved amino acid motif profile of *Streptococcus pneumonaie* isolates from Japan, 2012–2014*

Clone (no.)	Sequences of conserved amino acid motifs of PBPs								
	pbp1a			*pbp2b*			*pbp2x*		
	SXXK (no.)	SXN (no.)	KTG (no.)	SXXK (no.)	SXN (no.)	KSG (no.)	SXXK (no.)	SXN (no.)	KSG (no.)
MEM-S-15A-ST63 (10)	STMK (10)	SRN (10)	KTG (10)	SVVK (10)	SSNA (10)	KTG (10)	SPMK (1), STMK (3), SAMK (6)	HSSN (10)	VKSG (6), LKSG (4)
MEM-NS-15A-ST63 (24)	SSMK (24)	SRN (24)	KTG (24)	SVVK (24)	SSNA (24)	KTG (24)	SAMK (22), SAFK (2)	HSSN (24)	VKSG (24)
MEM-NS-non15A (32)	SSMK (29), SAMK (3)	SRN (32)	KTG (32)	SVVK (32)	SSNA (32)	KTG (32)	SAMK (32)	HSSN (32)	VKSG (32)
PMEN15A-25 (1)	STMK (1)	SRN (1)	KTG (1)	SVVK (1)	SSNA (1)	KTG (1)	STMK (1)	HSSN (1)	LKSG (1)

*MEM-NS, meropenem-nonsusceptible; MEM-S, meropenem-susceptible; PBP, penicillin binding protein; PMEN, Pneumococcal Molecular Epidemiology Network; ST, sequence type.

associated with the recombination of the *cps* region flanking *pbp1a* and *pbp2x*; resistant strains switched their serotypes via recombination of the *cps* region, or susceptible strains gained *pbp1a* and/or *pbp2x* resistance genes with the *cps* region. In the MEM-NS serotype 15A-ST63 isolates investigated in this study, the recombination sites that caused meropenem resistance included the *pbp1a* and *pbp2b* regions but did not include the *cps* region; thus, the serotype switch did not occur. The nucleotide sequence of *pbp1a,* including the transpeptidase region found in MEM-NS serotype 15A-ST63 isolates, was 100% identical to that of the meropenem-resistant serotype 19A-ST320 strain that is prevalent in the United States (type 13 *pbp1a*) (*5*). This serotype 19A-ST320 strain was also recovered in our previous surveillance study in Japan (*7*), and the transpeptidase region of the isolates was the same as that of MEM-NS serotype 15A-ST63 isolates. This finding suggests that the MEM-S serotype 15A-ST63 strain gained the meropenem resistance–related *pbp1a* gene to become the MEM-NS serotype 15A-ST63 strain. Of note, this *pbp1a* type was identified in several Japan MEM-NS serotype isolates, such as 19F, 23F, 6A, 6B, and nontypeable isolates. According to previous PBP profile data from the United States (*5*), type 13 *pbp1a* was found in serotype 19A-ST320 only, and there were no widely spread PBP types across many resistant lineages.

In Japan, broad-spectrum oral cephalosporin, fluoroquinolones, and macrolides have been frequently prescribed. The inappropriate use of antimicrobial drugs may provide selective pressure and cause the spread of meropenem-resistant strains by the transfer of the meropenem resistance–related *pbp1a* gene.

The Japan MEM-NS serotype 15A-ST63 isolates had a novel, specific *pbp2b* type. The contribution of this *pbp2b* type to meropenem resistance is not clear. One of the MEM-NS serotype 15A-ST63–specific recombination sites overlapped the *pbp2b* region; therefore, this recombination may have caused meropenem resistance. However, all of the MEM-NS and MEM-S serotype 15A-63 isolates had the same sequences in each of 3 conserved amino acid motifs of *pbp2b*. We believe that this result reduced the likelihood that *pbp2b* recombination is associated with meropenem resistance. In addition, we identified 17 other MEM-NS serotype 15A-ST63–specific recombination sites that occurred outside of *pbp1a* and *pbp2b*. It is possible that these recombination events could result in meropenem resistance by non-PBP mutations.

We note the usefulness of PBP typing for predicting drug resistance and tracing the geographic genetic trends in pneumococci. MLST has been widely used in epidemiologic studies of pneumococci to trace genetic trends and to predict serotype switch events that occasionally lead to the development of antimicrobial drug–resistant clones. However, as in our studies, MLST is unable to predict recombination events that occur without a serotype switch, even if the recombination leads to the development of resistance. These facts highlight the value of PBP typing, and these types of data would support future studies of pneumococci.

All analyzed isolates were positive for *ermB* and *tetM*; however, several isolates had a 2-nt deletion in *tetM,* which generated a premature stop codon. This deletion was mainly identified in the UK isolates, and only 1 Japan isolate showed this deletion. This deletion was also identified in PMEN15A-25, which was recovered in Portugal in 1998. Considering that *tetM* generally exists on a transposable element, these results may imply that the origins of *tetM* differ from those of isolates from Japan, the United States, and Europe; *tetM* may exist on different transposable elements in each region, which may have been imported from different sources. In addition, the complete conservation of *ermB* and *tetM* may indicate that these genes contribute to its global spread.

This study had limitations. First, we examined fewer Japan MEM-S than MEM-NS serotype 15A-ST63 isolates, which may have reduced the accuracy of the recombination site prediction. However, the 2 obtained phylogenic trees, one that was constructed by using all isolates of serotype 15A-ST63 and another that was constructed by using only Japan serotype 15A-ST63 isolates, resulted in similar clade Is. Therefore, we believe that the effect of the small number on the result was low. Second, we analyzed serotype 15A-ST63 isolates recovered from only 4 countries with a reference isolate. Future studies that include many isolates from other countries will provide additional insights into the spread and evolution of the serotype 15A-ST63 strains.

In conclusion, MEM-NS serotype 15A-ST63 pneumococci have spread in Japan after the introduction of PCV7 and PCV13. This strain originated from the MEM-S serotype 15A-ST63 strain that was prevalent in Japan; MEM-S serotype 15A-ST63 became MEM-NS serotype 15A-ST63 because of the recombination of the *pbp1a* region. The causative *pbp1a* fragment seemed to have been transferred from the MEM-NS serotype 19A-ST320 strain, and the fragment was identified in many meropenem-resistant serotype isolates in Japan. The Japan and North America serotype 15A-ST63 strains seemed to lack the original *tetM* gene that had a premature stop codon. The global spread of this lineage is noteworthy because serotype 15A is not included in the currently used PCV13.

Acknowledgments

We are grateful to Toshiaki Ihara for his substantial contribution to the Pneumocatch surveillance study and to the other members of the Pneumocatch surveillance study group.

B.C. was supported by research funding (16fk0108311j0403) from the Japan Agency for Medical Research and Development. T.F. was supported by a research grant through his institution from Pfizer Inc. Y.I. was supported by a research grant through his institution from Daiichi-Sankyo, Merck & Co., Inc., and the Japan Society for the Promotion of Science (17K10023). Y.I. and S.I. were supported by research funding from Pfizer for the surveillance study.

About the Author

Dr. Nakano is an assistant professor at the Department of Infection Control and Prevention, Kyoto University Hospital, and the Department of Clinical Laboratory Medicine, Kyoto University Graduate School of Medicine. His research interests focus on molecular microbiology and epidemiology.

References

1. O'Brien KL, Wolfson LJ, Watt JP, Henkle E, Deloria-Knoll M, McCall N, et al.; Hib and Pneumococcal Global Burden of Disease Study Team. Burden of disease caused by *Streptococcus pneumoniae* in children younger than 5 years: global estimates. Lancet. 2009;374:893–902. http://dx.doi.org/10.1016/S0140-6736(09)61204-6
2. Geno KA, Gilbert GL, Song JY, Skovsted IC, Klugman KP, Jones C, et al. Pneumococcal capsules and their types: past, present, and future. Clin Microbiol Rev. 2015;28:871–99. http://dx.doi.org/10.1128/CMR.00024-15
3. Waight PA, Andrews NJ, Ladhani NJ, Sheppard CL, Slack MP, Miller E. Effect of the 13-valent pneumococcal conjugate vaccine on invasive pneumococcal disease in England and Wales 4 years after its introduction: an observational cohort study. Lancet Infect Dis. 2015;15:629. http://dx.doi.org/10.1016/S1473-3099(15)70044-7
4. Chiba N, Morozumi M, Shouji M, Wajima T, Iwata S, Ubukata K; Invasive Pneumococcal Diseases Surveillance Study Group. Changes in capsule and drug resistance of pneumococci after introduction of PCV7, Japan, 2010–2013. Emerg Infect Dis. 2014;20:1132–9. http://dx.doi.org/10.3201/eid2007.131485
5. Metcalf BJ, Gertz RE Jr, Gladstone RA, Walker H, Sherwood LK, Jackson D, et al. Strain features and distributions in pneumococci from children with invasive disease before and after 13 valent conjugate vaccine implementation in the United States. Clin Microbiol Infect. 2015.p
6. Song JY, Nahm MH, Moseley MA. Clinical implications of pneumococcal serotypes: invasive disease potential, clinical presentations, and antibiotic resistance. J Korean Med Sci. 2013;28:4–15. http://dx.doi.org/10.3346/jkms.2013.28.1.4
7. Nakano S, Fujisawa T, Ito Y, Chang B, Suga S, Noguchi T, et al. Serotypes, antimicrobial susceptibility, and molecular epidemiology of invasive and non-invasive *Streptococcus pneumoniae* isolates in paediatric patients after the introduction of 13-valent conjugate vaccine in a nationwide surveillance study conducted in Japan in 2012-2014. Vaccine. 2016;34:67–76. http://dx.doi.org/10.1016/j.vaccine.2015.11.015
8. Duvvuri VR, Deng X, Teatero S, Memari N, Athey T, Fittipaldi N, et al. Population structure and drug resistance patterns of emerging non-PCV-13 *Streptococcus pneumoniae* serotypes 22F, 15A, and 8 isolated from adults in Ontario, Canada. Infect Genet Evol. 2016;42:1–8. http://dx.doi.org/10.1016/j.meegid.2016.04.007
9. van der Linden M, Perniciaro S, Imöhl M. Increase of serotypes 15A and 23B in IPD in Germany in the PCV13 vaccination era. BMC Infect Dis. 2015;15:207. http://dx.doi.org/10.1186/s12879-015-0941-9
10. Sheppard C, Fry NK, Mushtaq S, Woodford N, Reynolds R, Janes R, et al. Rise of multidrug-resistant non-vaccine serotype 15A *Streptococcus pneumoniae* in the United Kingdom, 2001 to 2014. Euro Surveill. 2016;21:30423. http://dx.doi.org/10.2807/1560-7917.ES.2016.21.50.30423
11. Chi HC, Hsieh YC, Tsai MH, Lee CH, Kuo KC, Huang CT, et al. Impact of pneumococcal conjugate vaccine in children on the serotypic epidemiology of adult invasive pneumococcal diseases in Taiwan. J Microbiol Immunol Infect. 2016;S1684-1182(16)30144-X.
12. Cilveti R, Olmo M, Pérez-Jove J, Picazo JJ, Arimany JL, Mora E, et al.; HERMES Study Group. Epidemiology of otitis media with spontaneous perforation of the tympanic membrane in young children and association with bacterial nasopharyngeal carriage, recurrences and pneumococcal vaccination in Catalonia, Spain—The Prospective HERMES Study. PLoS One. 2017;12:e0170316. http://dx.doi.org/10.1371/journal.pone.0170316
13. Devine VT, Cleary DW, Jefferies JM, Anderson R, Morris DE, Tuck AC, et al. The rise and fall of pneumococcal serotypes carried in the PCV era. Vaccine. 2017;35:1293–8. http://dx.doi.org/10.1016/j.vaccine.2017.01.035
14. Kaur R, Casey JR, Pichichero ME. Emerging *Streptococcus pneumoniae* strains colonizing the nasopharynx in children after 13-valent pneumococcal conjugate vaccination in comparison to the 7-valent era, 2006-2015. Pediatr Infect Dis J. 2016;35:901–6. http://dx.doi.org/10.1097/INF.0000000000001206
15. Horácio AN, Silva-Costa C, Lopes JP, Ramirez M, Melo-Cristino J; Portuguese Group for the Study of Streptococcal Infections. Serotype 3 remains the leading cause of invasive pneumococcal disease in adults in Portugal (2012–2014) despite continued reductions in other 13-valent conjugate vaccine serotypes. Front Microbiol. 2016;7:1616. http://dx.doi.org/10.3389/fmicb.2016.01616
16. Soysal A, Karabağ-Yılmaz E, Kepenekli E, Karaaslan A, Cagan E, Atıcı S, et al. The impact of a pneumococcal conjugate vaccination program on the nasopharyngeal carriage, serotype distribution and antimicrobial resistance of *Streptococcus pneumoniae* among healthy children in Turkey. Vaccine. 2016;34:3894–900. http://dx.doi.org/10.1016/j.vaccine.2016.05.043
17. Emory University. Pneumococcal Molecular Epidemiology Network (PMEN) [cited 2017 Nov 28]. http://web1.sph.emory.edu/PMEN/
18. Hakenbeck R, Brückner R, Denapaite D, Maurer P. Molecular mechanisms of β-lactam resistance in *Streptococcus pneumoniae*. Future Microbiol. 2012;7:395–410. http://dx.doi.org/10.2217/fmb.12.2
19. Laible G, Spratt BG, Hakenbeck R. Interspecies recombinational events during the evolution of altered PBP 2x genes in penicillin-resistant clinical isolates of *Streptococcus pneumoniae*. Mol Microbiol. 1991;5:1993–2002. http://dx.doi.org/10.1111/j.1365-2958.1991.tb00821.x
20. Dowson CG, Hutchison A, Brannigan JA, George RC, Hansman D, Liñares J, et al. Horizontal transfer of penicillin-binding protein genes in penicillin-resistant clinical isolates of *Streptococcus pneumoniae*. Proc Natl Acad Sci U S A. 1989;86:8842–6. http://dx.doi.org/10.1073/pnas.86.22.8842
21. Martin C, Sibold C, Hakenbeck R. Relatedness of penicillin-binding protein 1a genes from different clones of penicillin-resistant *Streptococcus pneumoniae* isolated in South Africa and Spain. EMBO J. 1992;11:3831–6.
22. Tzouvelekis LS, Markogiannakis A, Psichogiou M, Tassios PT, Daikos GL. Carbapenemases in *Klebsiella pneumoniae* and other *Enterobacteriaceae*: an evolving crisis of global dimensions. Clin Microbiol Rev. 2012;25:682–707. http://dx.doi.org/10.1128/CMR.05035-11

23. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; twenty-fifth informational supplement (M100–S25). Wayne (PA): The Institute; 2015.
24. Croucher NJ, Finkelstein JA, Pelton SI, Parkhill J, Bentley SD, Lipsitch M, et al. Population genomic datasets describing the post-vaccine evolutionary epidemiology of *Streptococcus pneumoniae*. Sci Data. 2015;2:150058. http://dx.doi.org/10.1038/sdata.2015.58
25. Kapatai G, Sheppard CL, Al-Shahib A, Litt DJ, Underwood AP, Harrison TG, et al. Whole genome sequencing of *Streptococcus pneumoniae*: development, evaluation and verification of targets for serogroup and serotype prediction using an automated pipeline. PeerJ. 2016;4:e2477. http://dx.doi.org/10.7717/peerj.2477
26. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, et al. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol. 2012;19:455–77. http://dx.doi.org/10.1089/cmb.2012.0021
27. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol. 1990;215:403–10. http://dx.doi.org/10.1016/S0022-2836(05)80360-2
28. Croucher NJ, Page AJ, Connor TR, Delaney AJ, Keane JA, Bentley SD, et al. Rapid phylogenetic analysis of large samples of recombinant bacterial whole genome sequences using Gubbins. Nucleic Acids Res. 2015;43:e15. http://dx.doi.org/10.1093/nar/gku1196
29. Metcalf BJ, Chochua S, Gertz RE, Jr., Li Z, Walker H, Tran T, et al. Using whole genome sequencing to identify resistance determinants and predict antimicrobial resistance phenotypes for year 2015 invasive pneumococcal disease isolates recovered in the United States. Clin Microbiol Infect. 2016;22:1002.e1–8. http://dx.doi.org/10.1016/j.cmi.2016.08.001
30. Li Y, Metcalf BJ, Chochua S, Li Z, Gertz RE Jr, Walker H, et al. Penicillin-binding protein transpeptidase signatures for tracking and predicting β-lactam resistance levels in *Streptococcus pneumoniae*. MBio. 2016;7:e00756-16. http://dx.doi.org/10.1128/mBio.00756-16
31. Centers for Disease Control and Prevention. Minimum inhibitory concentrations predicted by the penicillin binding protein type [cited 2017 Nov 28]. https://www.cdc.gov/streplab/mic-tables.html
32. Gertz RE Jr, Li Z, Pimenta FC, Jackson D, Juni BA, Lynfield R, et al.; Active Bacterial Core Surveillance Team. Increased penicillin nonsusceptibility of nonvaccine-serotype invasive pneumococci other than serotypes 19A and 6A in post–7-valent conjugate vaccine era. J Infect Dis. 2010;201:770–5. http://dx.doi.org/10.1086/650496
33. Beall BW, Gertz RE, Hulkower RL, Whitney CG, Moore MR, Brueggemann AB. Shifting genetic structure of invasive serotype 19A pneumococci in the United States. J Infect Dis. 2011;203:1360–8. http://dx.doi.org/10.1093/infdis/jir052
34. Ardanuy C, de la Campa AG, García E, Fenoll A, Calatayud L, Cercenado E, et al. Spread of *Streptococcus pneumoniae* serotype 8-ST63 multidrug-resistant recombinant clone, Spain. Emerg Infect Dis. 2014;20:1848–56. http://dx.doi.org/10.3201/eid2011.131215
35. Chochua S, Metcalf BJ, Li Z, Walker H, Tran T, McGee L, et al. Invasive serotype 35B pneumococci including an expanding serotype switch lineage, United States, 2015–2016. Emerg Infect Dis. 2017;23:922–30. http://dx.doi.org/10.3201/eid2306.170071

Address for correspondence: Satoshi Nakano, Kyoto Daigaku Igakubu Fuzoku Byoin, 54 Kawahara-cho, Syogoin, Sakyo-ku, Kyoto, Kyoto 606-8507, Japan; email: snakano@kuhp.kyoto-u.ac.jp

Article DOI: http://dx.doi.org/10.3201/eid2402.171268

Spread of Meropenem-Resistant *Streptococcus pneumoniae* Serotype 15A-ST63 Clone in Japan, 2012–2014

Technical Appendix

Supplementary Methods

We used a core genome single-nucleotide polymorphism (SNP)-based approach to create a phylogenetic tree using the current standard procedure (*1*). To perform this approach, we used Genealogies Unbiased By recomBinations In Nucleotide Sequences (Gubbins) (*2*), which identifies recombination events using an algorithm that iteratively identifies loci containing elevated densities of base substitutions while concurrently constructing a phylogeny based on the putative point mutations outside of these regions. To create input files for Gubbins, we performed raw read mapping followed by duplicate read removal, indel removal, and realignment (*3*).

Core Genome Analysis using Gubbins

Reads from 67 isolates sequenced in this study and reads from 86 global isolates downloaded from Sequence Read Archive (SRA) database (http://www.ncbi.nlm.nih.gov/sra/) underwent quality trimming using Trimmomatic (*4*). Trimmed reads were aligned against a reference genome of *Streptococcus pneumoniae* G54 (NCBI Reference Sequence: NC_011072.11) using the Burrows-Wheeler Aligner (*5*). After the removal of duplicate reads and indels using the GATK Best Practices workflow (*6*), consensus sequences fasta files were created using VCFtools (*7*). Gubbins was run with standard parameters. We created a total of three phylogenic trees using Gubbins. First, we created a tree using all of the isolates (all of the 67 isolates sequenced in this study and 86 global serotype 15A isolates) and *Streptococcus pneumoniae* G54 without any outgroup to find the ancestral strain of the Japanese meropenem-non-susceptible serotype 15A-ST63 strain. We found that there was no candidate for the ancestor

among isolates of any serotype except for 15A. We then created a second phylogenic tree using 35 serotype 15A isolates sequenced in this study and 86 global serotype 15A isolates using *Streptococcus pneumoniae* G54 as an outgroup. This tree generated a clade that included all 24 Japanese meropenem-non-susceptible serotype 15A isolates and six Japanese meropenem-susceptible serotype 15A isolates. Finally, we created a phylogenic tree using these 30 serotype 15A isolates with the PMEN15A-25 isolate used as the outgroup.

Identification of SNPs Specific to Clade-I-MNS

To identify the core genome changes that separated clade-I-MNS from the rest of clade-I, we extracted the core genomes of all of the clade-I isolates and searched for SNPs in Japanese MEPM-NS isolates that were identified in all of the clade-I-MNS isolates and not identified in any of the rest of clade-I. We obtained the core genomes using GET_HOMOLOGS (*8*) and aligned the clustered genes. Then, we identified the SNPs manually. We obtained a total of 1869 core genomes and 550,762 substrates of amino-acid sequences. These SNPs were distributed in 52 genes that are listed in Technical Appendix Table 4.

Genome Assembly

Trimmed reads sequenced in this study were assembled using SPAdes (*9*) with k-mer values ranging from 29 to 101 and in careful mode. Trimmed reads from the downloaded global isolates were assembled using SPAdes with standard parameters and in careful mode. The quality of the assemblies was evaluated using QUAST (*10*).

Comparative Genome Analysis

To define the presence of genes and their alleles, we extracted the target gene regions from the assembled contigs using BLAST+ (*11*). With regard to *pbp1a, 2b*, and *2x*, we used the corresponding gene sequences from *Streptococcus pneumoniae* G54 as reference sequences (NCBI Reference Sequence: NC_011072.11). The reference sequences used to identify *mefA* (*12*), *mefE* (*13*), *folA* (*14*), *folP* (*14*), *tetO* (*14*), *tetM* (*14*), PI-1 (*rrgA-1*) (*14*), and PI-2 (*pitB-1*) (*14*) are listed in Technical Appendix Table 5.

Estimation of the Date when Meropenem-Non-Susceptible Serotype 15A-ST63 Originated

The result of core genome analysis using Gubbins indicated that the Japanese meropenem-non-susceptible (MEPM-NS) serotype 15A-ST63 strain was derived from the Japanese meropenem-susceptible (MEPM-S) strain. We estimated the date of the most recent common ancestor (MRCA) of each of the two groups using BEAST (*15*). The program was used to analyze the final maximum likelihood tree, the topology of which was fixed, and the alignment of base substitutions occurring outside of putative recombination events using a strict clock model. The ages of the isolates (month and year) were used as input data. Exponential growth was used as the tree prior. The length of chain value was set so that all output values had an effective sample size greater than 200. The analysis estimated that the lineage originated around 1970 (95% credibility interval 1672–2006); the small number of tested isolates may explain the broad credibility interval. In addition, the tree generated in this analysis was slightly different from that in core genome analysis using Gubbins. In this analysis, MEPM-NS isolates were divided into two clades even though MEPM-NS and –S isolates were clearly separated (Technical Appendix Figure 4).

References

1. Robinson ER, Walker TM, Pallen MJ. Genomics and outbreak investigation: from sequence to consequence. Genome Med. 2013;5:36. PubMed http://dx.doi.org/10.1186/gm440

2. Croucher NJ, Page AJ, Connor TR, Delaney AJ, Keane JA, Bentley SD, et al. Rapid phylogenetic analysis of large samples of recombinant bacterial whole genome sequences using Gubbins. Nucleic Acids Res. 2015;43:e15. PubMed http://dx.doi.org/10.1093/nar/gku1196

3. Olson ND, Lund SP, Colman RE, Foster JT, Sahl JW, Schupp JM, et al. Best practices for evaluating single nucleotide variant calling methods for microbial genomics. Front Genet. 2015;6:235. PubMed http://dx.doi.org/10.3389/fgene.2015.00235

4. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics. 2014;30:2114–20. PubMed http://dx.doi.org/10.1093/bioinformatics/btu170

5. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics. 2009;25:1754–60. PubMed http://dx.doi.org/10.1093/bioinformatics/btp324

6. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res. 2010;20:1297–303. PubMed http://dx.doi.org/10.1101/gr.107524.110

7. Danecek P, Auton A, Abecasis G, Albers CA, Banks E, DePristo MA, et al.; 1000 Genomes Project Analysis Group. The variant call format and VCFtools. Bioinformatics. 2011;27:2156–8. PubMed http://dx.doi.org/10.1093/bioinformatics/btr330

8. Contreras-Moreira B, Vinuesa P. GET_HOMOLOGUES, a versatile software package for scalable and robust microbial pangenome analysis. Appl Environ Microbiol. 2013;79:7696–701. PubMed http://dx.doi.org/10.1128/AEM.02411-13

9. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, et al. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol. 2012;19:455–77. PubMed http://dx.doi.org/10.1089/cmb.2012.0021

10. Gurevich A, Saveliev V, Vyahhi N, Tesler G. QUAST: quality assessment tool for genome assemblies. Bioinformatics. 2013;29:1072–5. PubMed http://dx.doi.org/10.1093/bioinformatics/btt086

11. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol. 1990;215:403–10. PubMed http://dx.doi.org/10.1016/S0022-2836(05)80360-2

12. Clancy J, Petitpas J, Dib-Hajj F, Yuan W, Cronan M, Kamath AV, et al. Molecular cloning and functional analysis of a novel macrolide-resistance determinant, mefA, from Streptococcus pyogenes. Mol Microbiol. 1996;22:867–79. PubMed http://dx.doi.org/10.1046/j.1365-2958.1996.01521.x

13. Tait-Kamradt A, Clancy J, Cronan M, Dib-Hajj F, Wondrack L, Yuan W, et al. mefE is necessary for the erythromycin-resistant M phenotype in Streptococcus pneumoniae. Antimicrob Agents Chemother. 1997;41:2251–5. PubMed

14. Metcalf BJ, Gertz RE Jr, Gladstone RA, Walker H, Sherwood LK, Jackson D, et al. Strain features and distributions in pneumococci from children with invasive disease before and after 13 valent conjugate vaccine implementation in the United States. Clin Microbiol Infect. 2015. PubMed

15. Drummond AJ, Rambaut A. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol Biol. 2007;7:214. PubMed http://dx.doi.org/10.1186/1471-2148-7-214

Technical Appendix Table 1. Strain information and penicillin binding protein profile

Isolate name	Accession no.	Serotype	ST*	Year	Region†	MIC (mg/L) ‡				*pbp1a*:*pbp2b*: *pbp2x*
						PCG	CTX	MEPM	EM	
USA15A-16	ERR065297	15A	63	2004	USA	0.03	NA	NA	0.5	24:27:28
USA15A-10	ERR065320	15A	63	2004	USA	0.12	NA	NA	0.5	24:27:28
USA15A-5	ERR065332	15A	63	2004	USA	0.12	NA	NA	0.5	24:27:28
USA15A-13	ERR068026	15A	63	2004	USA	0.25	NA	NA	0.5	24:27:28
USA15A-14	ERR068028	15A	63	2004	USA	0.25	NA	NA	0.5	24:27:28
USA15A-17	ERR068032	15A	63	2004	USA	0.25	NA	NA	0.5	24:27:28
USA15A-11	ERR068049	15A	63	2004	USA	0.25	NA	NA	0.5	24:27:28
USA15A-18	ERR069724	15A	63	2004	USA	0.25	NA	NA	0.5	24:27:28
USA15A-12	ERR069725	15A	63	2004	USA	0.25	NA	NA	0.5	24:27:28
USA15A-21	ERR124239	15A	63	2007	USA	0.38	NA	NA	256	24:27:28
USA15A-19	ERR124249	15A	63	2007	USA	0.19	NA	NA	256	24:27:28
USA15A-9	ERR124283	15A	63	2007	USA	0.25	NA	NA	256	24:27:28
USA15A-20	ERR124300	15A	63	2007	USA	0.25	NA	NA	32	24:27:28
USA15A-6	ERR129026	15A	63	2007	USA	0.19	NA	NA	256	24:27:28
USA15A-8	ERR129060	15A	63	2007	USA	0.064	NA	NA	256	24:27:28
USA15A-15	ERR129061	15A	63	2007	USA	0.125	NA	NA	256	24:27:28
USA15A-7	ERR129198	15A	63	2007	USA	0.38	NA	NA	256	24:27:28
UK15A-1	ERR1439011	15A	63	2013	UK	NA	NA	NA	NA	24:27:28
UK15A-2	ERR1439047	15A	63	2013	UK	NA	NA	NA	NA	24:27:28
UK15A-3	ERR1439048	15A	63	2013	UK	NA	NA	NA	NA	67:27:35
UK15A-4	ERR1439052	15A	63	2013	UK	NA	NA	NA	NA	24:27:43
UK15A-5	ERR1439054	15A	63	2013	UK	NA	NA	NA	NA	24:27:28
UK15A-6	ERR1439056	15A	63	2013	UK	NA	NA	NA	NA	24:27:28
UK15A-7	ERR1439057	15A	63	2013	UK	NA	NA	NA	NA	24:27:179
UK15A-8	ERR1439069	15A	63	2013	UK	NA	NA	NA	NA	24:27:43
UK15A-9	ERR1439074	15A	63	2013	UK	NA	NA	NA	NA	24:27:179
UK15A-10	ERR1439082	15A	63	2013	UK	NA	NA	NA	NA	67:new3:35
UK15A-11	ERR1439083	15A	63	2013	UK	NA	NA	NA	NA	24:27:8
UK15A-12	ERR1439097	15A	63	2013	UK	NA	NA	NA	NA	24:27:28
UK15A-13	ERR1439101	15A	63	2013	UK	NA	NA	NA	NA	24:27:28
UK15A-14	ERR1439103	15A	63	2013	UK	NA	NA	NA	NA	24:27:179
UK15A-15	ERR1439104	15A	63	2013	UK	NA	NA	NA	NA	24:27:179
UK15A-16	ERR1439112	15A	63	2013	UK	NA	NA	NA	NA	24:27:43
UK15A-17	ERR1439117	15A	63	2013	UK	NA	NA	NA	NA	24:27:28
UK15A-18	ERR1439120	15A	63	2014	UK	NA	NA	NA	NA	24:27:28
UK15A-19	ERR1439131	15A	63	2014	UK	NA	NA	NA	NA	24:27:28
UK15A-20	ERR1439141	15A	63	2014	UK	NA	NA	NA	NA	24:27:28
UK15A-21	ERR1439151	15A	63	2014	UK	NA	NA	NA	NA	new4:27:28
UK15A-22	ERR1439155	15A	63	2014	UK	NA	NA	NA	NA	24:27:28
UK15A-23	ERR1439162	15A	63	2014	UK	NA	NA	NA	NA	24:27:28
UK15A-24	ERR1439167	15A	63	2014	UK	NA	NA	NA	NA	24:27:179
UK15A-25	ERR1439172	15A	63	2014	UK	NA	NA	NA	NA	24:27:28
UK15A-26	ERR1439190	15A	63	2014	UK	NA	NA	NA	NA	67:27:35
UK15A-27	ERR1439193	15A	63	2014	UK	NA	NA	NA	NA	24:27:28
UK15A-28	ERR1439207	15A	63	2014	UK	NA	NA	NA	NA	24:27:28
UK15A-29	ERR1439211	15A	63	2014	UK	NA	NA	NA	NA	24:new4:28
UK15A-30	ERR1439215	15A	63	2014	UK	NA	NA	NA	NA	24:27:28

Isolate name	Accession no.	Serotype	ST*	Year	Region†	MIC (mg/L) ‡				*pbp1a*:*pbp2b*: *pbp2x*
						PCG	CTX	MEPM	EM	
UK15A-31	ERR1439225	15A	63	2014	UK	NA	NA	NA	NA	24:27:28
UK15A-32	ERR1439240	15A	63	2014	UK	NA	NA	NA	NA	24:27:179
UK15A-33	ERR1439256	15A	63	2014	UK	NA	NA	NA	NA	24:27:28
UK15A-34	ERR1439260	15A	63	2014	UK	NA	NA	NA	NA	24:27:28
UK15A-35	ERR1439264	15A	63	2014	UK	NA	NA	NA	NA	24:27:28
UK15A-36	ERR1439269	15A	63	2014	UK	NA	NA	NA	NA	24:27:179
UK15A-37	ERR1439272	15A	63	2014	UK	NA	NA	NA	NA	24:27:179
UK15A-38	ERR1439276	15A	63	2014	UK	NA	NA	NA	NA	24:27:179
UK15A-39	ERR1439301	15A	63	2014	UK	NA	NA	NA	NA	24:27:28
UK15A-40	ERR1439303	15A	63	2014	UK	NA	NA	NA	NA	67:27:35
UK15A-41	ERR1439306	15A	63	2014	UK	NA	NA	NA	NA	24:27:179
UK15A-42	ERR1439311	15A	63	2014	UK	NA	NA	NA	NA	67:27:35
UK15A-43	ERR1439312	15A	63	2014	UK	NA	NA	NA	NA	24:27:28
UK15A-44	ERR1439320	15A	63	2014	UK	NA	NA	NA	NA	new5:27:28
UK15A-45	ERR1439325	15A	63	2014	UK	NA	NA	NA	NA	new5:27:28
UK15A-46	ERR1439332	15A	63	2014	UK	NA	NA	NA	NA	24:27:179
UK15A-47	ERR1439335	15A	63	2014	UK	NA	NA	NA	NA	67:27:35
UK15A-48	ERR1439338	15A	63	2014	UK	NA	NA	NA	NA	24:27:28
UK15A-49	ERR1439348	15A	63	2014	UK	NA	NA	NA	NA	67:27:35
UK15A-50	ERR1439365	15A	63	2014	UK	NA	NA	NA	NA	67:27:35
UK15A-51	ERR1439384	15A	63	2014	UK	NA	NA	NA	NA	24:27:179
UK15A-52	ERR1439400	15A	63	2014	UK	NA	NA	NA	NA	67:27:35
UK15A-53	ERR1439403	15A	63	2014	UK	NA	NA	NA	NA	67:27:35
UK15A-54	ERR1439412	15A	63	2014	UK	NA	NA	NA	NA	24:27:28
UK15A-55	ERR1439442	15A	63	2014	UK	NA	NA	NA	NA	24:27:28
UK15A-56	ERR1439469	15A	63	2014	UK	NA	NA	NA	NA	24:27:28
UK15A-57	ERR1439551	15A	63	2014	UK	NA	NA	NA	NA	67:27:35
UK15A-58	ERR1439560	15A	63	2014	UK	NA	NA	NA	NA	24:27:28
USA15A-1	ERR505735	15A	63	NA	USA	NA	NA	NA	NA	24:27:35
USA15A-2	ERR600092	15A	63	NA	USA	NA	NA	NA	NA	24:27:28
USA15A-3	ERR600173	15A	63	NA	USA	NA	NA	NA	NA	24:27:28
USA15A-4	ERR600180	15A	63	NA	USA	NA	NA	NA	NA	24:73:114
MR15A-2_PC118	DRR098620	15A	63	2012	JP(Chiba)	4	0.5	0.5	>128	13:new1:43
MR15A-3_PC126	DRR098621	15A	63	2012	JP(Osaka)	2	1	0.5	>128	13:new1:43
MR15A-1_PC13	DRR098624	15A	63	2012	JP(Chiba)	2	0.5	0.5	>128	13:new1:43
MR15A-4_PC131	DRR098623	15A	63	2012	JP(Chiba)	2	0.5	0.5	>128	13:new1:43
MS15A-6_PC283	DRR098637	15A	63	2013	JP(Hokkaido)	0.25	≦0.06	≦0.06	>128	24:27:28
MR15A-5_PC206	DRR098628	15A	63	2013	JP(Yamaguchi)	4	1	0.5	>128	13:new1:43
MS15A-8_PC358	DRR098648	15A	63	2013	JP(Hokkaido)	0.25	0.12	≦0.06	>128	24:27:28
MS15A-11_PC723	DRR098683	15A	63	2014	JP(Yamagata)	0.25	≦0.06	≦0.06	>128	24:27:28
MS15A-1_PC16	DRR098626	15A	63	2012	JP(Kyoto)	0.25	0.5	≦0.06	>128	24:27:43
MR15A-6_PC282	DRR098636	15A	63	2013	JP(Saga)	2	0.5	0.5	>128	13:new1:43
MS15A-4_PC239	DRR098632	15A	63	2013	JP(Gifu)	0.25	0.5	≦0.06	>128	24:27:43
MS15A-5_PC273	DRR098635	15A	63	2012	JP(Okayama)	0.25	0.25	≦0.06	>128	24:27:43
MR15A-20_PC313	DRR098642	15A	63	2013	JP(Yamaguchi)	4	4	1	>128	13:new1:new3
MR15A-7_PC324	DRR098643	15A	63	2013	JP(Shizuoka)	2	0.5	0.5	>128	13:new1:43
MR15A-21_PC342	DRR098645	15A	63	2013	JP(Osaka)	2	1	1	>128	13:new1:43
MS15A-7_PC307	DRR098641	15A	63	2013	JP(Tokyo)	0.5	0.5	≦0.06	>128	24:27:43

Isolate name	Accession no.	Serotype	ST*	Year	Region†	MIC (mg/L) ‡				*pbp1a*:*pbp2b*: *pbp2x*
						PCG	CTX	MEPM	EM	
MR15A-8_PC376	DRR098649	15A	63	2013	JP(Tokushima)	2	0.5	0.5	>128	13:new1:43
MR15A-9_PC418	DRR098654	15A	63	2013	JP(Okayama)	2	0.5	0.5	>128	13:new1:43
MR15A-10_PC441	DRR098655	15A	63	2013	JP(Yamaguchi)	2	0.5	0.5	>128	13:new1:43
MS15A-9_PC443	DRR098656	15A	63	2013	JP(Hokkaido)	0.25	0.25	≦0.06	>128	24:27:43
MR15A-11_PC495	DRR098659	15A	63	2013	JP(Osaka)	2	0.5	0.5	>128	13:new1:43
MR15A-22_PC518	DRR098663	15A	63	2014	JP(Yamaguchi)	4	1	1	>128	13:new1:43
MR15A-12_PC572	DRR098666	15A	63	2014	JP(Gifu)	2	0.5	0.5	>128	13:new1:43
MR15A-13_PC618	DRR098670	15A	63	2014	JP(Saga)	2	0.5	0.5	>128	13:new1:43
MR15A-14_PC620	DRR098671	15A	63	2014	JP(Chiba)	1	0.5	0.5	>128	13:new1:43
MR15A-23_PC624	DRR098672	15A	63	2014	JP(Chiba)	2	0.5	1	>128	13:new1:43
MR15A-15_PC638	DRR098673	15A	63	2014	JP(Wakayama)	2	0.5	0.5	>128	13:new1:43
MR15A-16_PC646	DRR098676	15A	63	2014	JP(Ibaraki)	1	0.25	0.5	>128	13:new1:43
MR15A-17_PC654	DRR098677	15A	63	2013	JP(Yamaguchi)	2	4	0.5	>128	13:new1:new6
MR15A-24_PC662	DRR098678	15A	63	2014	JP(Ohita)	2	0.5	1	>128	13:new1:43
MR15A-18_PC686	DRR098680	15A	63	2014	JP(Wakayama)	1	0.5	0.5	>128	13:new1:43
MS15A-3_PC228	DRR098631	15A	63	2013	JP(Yamagata)	0.5	1	≦0.06	>128	24:27:112
MR15A-19_PC718	DRR098682	15A	63	2014	JP(Kanagawa)	2	0.5	0.5	>128	13:new1:43
MS15A-10_PC702	DRR098681	15A	63	2014	JP(Miyagi)	0.25	0.25	≦0.06	>128	24:27:new1
MR15BC-2_PC267	DRR098634	15B/C	83	2012	JP(Okayama)	2	1	0.5	>128	15:12:18
MR15BC-3_PC516	DRR098662	15B/C	83	2014	JP(Kumamoto)	2	1	1	>128	15:12:18
MR15BC-1_PC227	DRR098630	15B/C	3934	2013	JP(Kyoto)	1	1	0.5	>128	new1:31:new2
MR19A-8_PC297	DRR098639	19A	320	2013	JP(Kumamoto)	4	2	1	>128	13:11:16
MR19A-9_PC396	DRR098652	19A	320	2013	JP(Gifu)	4	1	1	>128	13:11:16
MR19A-10_PC576	DRR098668	19A	320	2014	JP(Osaka)	4	2	1	>128	13:11:16
MR19A-11_PC641	DRR098675	19A	320	2014	JP(Miyazaki)	4	2	1	>128	13:11:16
MR19A-2_PC345	DRR098646	19A	3111	2013	JP(Yamaguchi)	2	1	0.5	>128	13:24:112
MR19A-3_PC381	DRR098650	19A	3111	2013	JP(Saga)	1	0.5	0.5	>128	13:24:112
MR19A-4_PC475	DRR098658	19A	3111	2014	JP(Kanagawa)	2	1	0.5	>128	13:24:112
MR19A-5_PC505	DRR098661	19A	3111	2014	JP(Yamaguchi)	2	1	0.5	>128	13:24:112
MR19A-6_PC543	DRR098665	19A	3111	2014	JP(Gifu)	1	1	0.5	>128	13:24:112
MR19A-7_PC583	DRR098669	19A	3111	2014	JP(Yamaguchi)	1	0.5	0.5	>128	13:24:112
MR19A-1_PC93	DRR098685	19A	3111	2012	JP(Tokyo)	1	1	0.5	>128	new2:16:112
MR19F-2_PC306	DRR098640	19F	115	2013	JP(Shizuoka)	2	1	0.5	16	13:31:47
MR19F-3_PC463	DRR098657	19F	236	2013	JP(Shizuoka)	2	1	0.5	4	13:7:8
MR19F-1_PC49	DRR098660	19F	236	2012	JP(Yamaguchi)	2	1	0.5	4	13:16:47
MR23F-1_PC329	DRR098644	23F	242	2013	JP(Kanagawa)	2	1	0.5	>128	13:31:73
MR35B-1_PC129	DRR098622	35B	558	2012	JP(Chiba)	2	1	0.5	>128	4:7:7
MR35B-2_PC216	DRR098629	35B	558	2013	JP(Yamaguchi)	1	1	0.5	16	4:7:7
MR35B-3_PC291	DRR098638	35B	558	2013	JP(Yamaguchi)	1	1	0.5	2	4:7:7
MR35B-4_PC357	DRR098647	35B	558	2013	JP(Ohita)	1	0.5	0.5	4	4:7:7
MR35B-5_PC393	DRR098651	35B	558	2013	JP(Osaka)	1	0.5	0.5	≦0.06	4:7:7
MR35B-8_PC540	DRR098664	35B	558	2014	JP(Yamaguchi)	2	1	1	8	4:7:7
MR35B-6_PC574	DRR098667	35B	558	2014	JP(Yamaguchi)	1	0.5	0.5	8	4:7:7
MR35B-7_PC640	DRR098674	35B	558	2014	JP(Yamaguchi)	2	1	0.5	0.12	4:7:7
MR6A-1_PC240	DRR098633	6A	2756	2013	JP(Chiba)	2	1	0.5	>128	13:31:73
MR6B-1_PC140	DRR098625	6B	9335	2013	JP(Kumamoto)	4	4	2	>128	13:49:new7
MR6D-1_PC80	DRR098684	6D	282	2012	JP(Gifu)	2	1	0.5	4	15:12:18
MRUT-3_PC676	DRR098679	NT	4845	2014	JP(Chiba)	2	1	0.5	4	13:16:new4

Isolate name	Accession no.	Serotype	ST*	Year	Region†	MIC (mg/L) ‡				*pbp1a*:*pbp2b*: *pbp2x*
						PCG	CTX	MEPM	EM	
MRUT-1_PC192	DRR098627	NT	7502	2013	JP(Chiba)	2	1	0.5	>128	new3:16:new5
MRUT-2_PC400	DRR098653	NT	10253	2013	JP(Chiba)	2	1	1	16	13:new2:new4
PMEN15A-25	DRR098686	15A	63	1998	PTG	0.064	0.047	NA	>256	24:27:28
CN15A-1	SRR3211689	15A	63	2012	CN	NA	NA	NA	NA	24:27:28
CN15A-2	SRR3211690	15A	63	2011	CN	NA	NA	NA	NA	24:27:28
CN15A-3	SRR3211691	15A	63	2010	CN	NA	NA	NA	NA	24:27:28
CN15A-4	SRR3211692	15A	63	2010	CN	NA	NA	NA	NA	24:27:8
CN15A-5	SRR3211693	15A	63	2009	CN	NA	NA	NA	NA	24:27:28
CN15A-6	SRR3211694	15A	63	2013	CN	NA	NA	NA	NA	24:27:11
CN15A-7	SRR3211695	15A	63	2013	CN	NA	NA	NA	NA	24:27:28

*ST, sequence type.
†JP, Japan; PTG, Portugal; CN, Canada.
‡MIC, MIC; PCG, penicillin G; CTX, cefotaxime; MEPM, meropenem.

Technical Appendix Table 2. Mapping and assembly statistics

Isolate name	Mapping to the *Streptococcus pneumoniae* G54 genome		No. of contigs	N50	Length of the longest contig	No. of bases in contigs	No. of contigs >1K	No. of bases in contigs >1K
	Depth of coverage	Breadth of coverage						
USA15A-16	304	99.2	141	51192	103936	2048435	73	2042765
USA15A-10	191	99.2	160	47739	233907	2091039	71	2083988
USA15A-5	232	99.2	138	47835	143850	2048562	73	2041473
USA15A-13	282	98.5	144	53272	117879	2035279	72	2029302
USA15A-14	325	98.4	153	56296	152786	2032756	74	2027545
USA15A-17	300	99.2	155	56559	233907	2091321	69	2082546
USA15A-11	260	99.2	146	45009	233947	2093768	76	2085899
USA15A-18	348	99.2	140	51042	144023	2048081	74	2041778
USA15A-12	344	99.2	135	47739	144057	2049657	73	2044263
USA15A-21	560	99.3	162	42946	143978	2043675	79	2037099
USA15A-19	408	99.3	135	44285	117742	2046667	77	2038963
USA15A-9	347	99.3	143	44792	152913	2046100	79	2040668
USA15A-20	672	99.2	147	51192	187683	2042774	75	2036720
USA15A-6	248	98.6	162	44792	152699	2099233	83	2090928
USA15A-8	220	99.2	168	53272	233922	2087910	74	2079149
USA15A-15	179	99.2	159	45009	233754	2089415	73	2082391
USA15A-7	222	98.5	132	47085	117879	2031971	74	2026455
UK15A-1	132	99.3	214	58499	229361	2037703	68	2031997
UK15A-2	182	99.3	160	62277	197139	2048111	67	2044751
UK15A-3	119	99.1	195	48666	143748	2044407	75	2038369
UK15A-4	126	99.3	191	53272	176414	2084663	74	2073611
UK15A-5	151	99.3	248	53272	193632	2077034	72	2069614
UK15A-6	103	99.3	187	54688	192035	2073297	74	2067279
UK15A-7	155	99.3	226	48060	178057	2048686	78	2042765
UK15A-8	149	99.3	241	51192	225219	2116554	69	2109936
UK15A-9	88	99.2	178	47835	118013	2071299	79	2065697
UK15A-10	253	99.0	182	53271	118454	2044161	69	2042157
UK15A-11	118	98.6	196	56406	180443	2065809	72	2060528

Isolate name	Mapping to the *Streptococcus pneumoniae* G54 genome		No. of contigs	N50	Length of the longest contig	No. of bases in contigs	No. of contigs >1K	No. of bases in contigs >1K
	Depth of coverage	Breadth of coverage						
UK15A-12	200	99.3	173	58499	225347	2039728	66	2034520
UK15A-13	77	99.3	206	51192	156502	2079916	75	2071096
UK15A-14	146	99.3	232	50391	178296	2051027	78	2044565
UK15A-15	99	99.3	171	53856	178235	2050989	70	2046995
UK15A-16	100	99.3	186	51192	176297	2079776	71	2072361
UK15A-17	125	99.3	262	53271	193632	2079982	71	2070191
UK15A-18	123	99.3	246	61081	225434	2082353	72	2070637
UK15A-19	117	98.8	192	58499	117503	2035192	67	2030023
UK15A-20	89	99.1	200	51192	176387	2041622	71	2036381
UK15A-21	117	99.3	204	60794	225263	2077499	66	2068802
UK15A-22	150	99.4	240	58444	228921	2077747	68	2068774
UK15A-23	106	98.6	193	57846	236394	2042302	64	2038575
UK15A-24	133	99.3	211	53634	118472	2052430	75	2047401
UK15A-25	97	99.3	164	63724	176634	2047916	69	2044551
UK15A-26	133	99.3	189	56827	143871	2049031	76	2045888
UK15A-27	92	99.3	185	58499	193731	2036346	67	2031070
UK15A-28	89	99.3	233	57830	193867	2051939	71	2045227
UK15A-29	117	99.3	205	51068	176372	2054460	76	2050083
UK15A-30	136	98.8	220	51192	118294	2081000	80	2075119
UK15A-31	204	99.3	273	51116	176371	2075659	82	2070386
UK15A-32	96	99.2	151	53856	177486	2051253	75	2044746
UK15A-33	131	99.1	235	56192	193632	2048628	66	2041010
UK15A-34	118	99.3	212	49154	143224	2081720	71	2072929
UK15A-35	127	99.3	209	55844	193497	2078248	71	2071923
UK15A-36	154	99.3	261	46979	178092	2055562	83	2045448
UK15A-37	122	99.3	255	53272	177499	2045811	79	2038719
UK15A-38	217	99.3	234	51448	177962	2050295	80	2044954
UK15A-39	144	99.3	285	53272	159120	2077351	76	2069143
UK15A-40	110	99.1	169	57310	143842	2049091	72	2045941
UK15A-41	145	99.3	191	53272	109932	2051289	75	2047008
UK15A-42	140	99.1	202	53272	143917	2047090	75	2043437
UK15A-43	124	99.3	245	43693	176416	2076837	78	2070426
UK15A-44	111	99.3	235	55868	193632	2077733	69	2066489
UK15A-45	112	99.3	207	58455	225479	2077621	70	2071045
UK15A-46	114	99.3	167	48430	118059	2054430	74	2048233
UK15A-47	121	98.4	197	56827	146082	2035926	66	2032991
UK15A-48	111	99.3	197	56706	221207	2079591	64	2071838
UK15A-49	55	99.1	175	56854	153042	2047339	68	2041772
UK15A-50	51	99.1	202	56853	152737	2046932	64	2043140
UK15A-51	130	99.3	249	50897	123903	2049366	76	2042712
UK15A-52	56	99.1	184	56523	152583	2045056	68	2041360
UK15A-53	76	99.1	157	56310	117880	2049111	66	2044879
UK15A-54	77	99.2	361	51030	132164	2110478	80	2085286
UK15A-55	120	99.3	171	58499	193632	2048872	66	2044600
UK15A-56	131	99.1	199	61010	118168	2041390	69	2037074
UK15A-57	70	99.1	207	51192	143883	2047636	73	2042891
UK15A-58	102	99.3	200	57876	212581	2079069	67	2070199
USA15A-1	180	99.2	152	49187	131386	2117994	77	2113421

Isolate name	Mapping to the *Streptococcus pneumoniae* G54 genome		No. of contigs	N50	Length of the longest contig	No. of bases in contigs	No. of contigs >1K	No. of bases in contigs >1K
	Depth of coverage	Breadth of coverage						
USA15A-2	359	99.2	129	57876	144296	2051367	69	2048072
USA15A-3	211	99.2	135	53272	116057	2090483	70	2086286
USA15A-4	226	99.2	148	46449	147861	2101459	74	2095036
MR15A-2_PC118	59	99.2	123	62362	236423	2087922	58	2077854
MR15A-3_PC126	48	99.2	107	74297	196965	2088018	57	2084746
MR15A-1_PC13	55	99.2	113	74297	187507	2089105	60	2086082
MR15A-4_PC131	37	99.2	115	73668	237180	2091099	58	2086559
MS15A-6_PC283	60	98.9	73	88601	205515	2055844	42	2054130
MR15A-5_PC206	51	99.2	86	76986	281100	2091251	48	2088027
MS15A-8_PC358	47	98.9	69	76464	205483	2054767	48	2053053
MS15A-11_PC723	60	98.9	78	76981	230301	2056696	49	2053450
MS15A-1_PC16	57	99.2	118	73668	187380	2091927	59	2083695
MR15A-6_PC282	53	99.2	113	74102	273917	2092055	51	2085832
MS15A-4_PC239	52	99.3	161	57166	236577	2125628	66	2115140
MS15A-5_PC273	53	99.2	110	87855	236295	2090064	49	2086830
MR15A-20_PC313	37	99.2	112	67201	144493	2092043	59	2087708
MR15A-7_PC324	69	99.2	109	74198	281111	2088707	58	2084579
MR15A-21_PC342	46	99.2	116	57166	187366	2089651	62	2083161
MS15A-7_PC307	69	99.2	91	74131	281906	2090717	47	2086830
MR15A-8_PC376	53	99.2	107	68791	281098	2084758	57	2079883
MR15A-9_PC418	62	99.2	101	73034	280619	2088689	52	2083075
MR15A-10_PC441	64	99.0	90	73668	207867	2087893	54	2082810
MS15A-9_PC443	51	98.9	110	73704	188362	2085036	55	2078197
MR15A-11_PC495	48	99.2	102	89777	200421	2132327	51	2126700
MR15A-22_PC518	46	99.2	100	62808	236982	2130579	66	2125771
MR15A-12_PC572	39	99.2	130	76941	208418	2089200	61	2083124
MR15A-13_PC618	48	99.1	103	63383	230465	2090072	66	2083524
MR15A-14_PC620	53	99.2	106	69164	272921	2090665	53	2083387
MR15A-23_PC624	46	99.1	136	51524	166238	2091029	71	2083783
MR15A-15_PC638	66	99.1	91	68716	279775	2091648	51	2087003
MR15A-16_PC646	63	99.1	92	69042	228810	2090923	54	2085392
MR15A-17_PC654	59	99.2	94	67201	235803	2087351	52	2080862
MR15A-24_PC662	40	99.0	128	73952	236444	2075919	55	2070184
MR15A-18_PC686	54	99.2	137	57004	198691	2125849	73	2117678
MS15A-3_PC228	53	99.2	120	67201	236104	2126272	60	2121183
MR15A-19_PC718	53	99.2	105	63747	200061	2087635	58	2081976
MS15A-10_PC702	49	98.9	81	67201	241587	2056026	52	2051754
MR15BC-2_PC267	45	93.5	117	53186	236869	2141903	67	2134165
MR15BC-3_PC516	50	93.3	83	72791	157706	2137629	58	2134657
MR15BC-1_PC227	54	92.7	113	69382	182768	2175295	56	2167627
MR19A-8_PC297	33	91.5	105	66211	199669	2042136	59	2036366
MR19A-9_PC396	39	91.5	87	69312	199865	2039606	52	2035554
MR19A-10_PC576	57	91.6	96	74991	211348	2041305	53	2037811
MR19A-11_PC641	44	91.5	82	68832	211347	2061370	53	2056600
MR19A-2_PC345	47	91.6	120	72932	188518	2117172	56	2112601
MR19A-3_PC381	52	91.6	109	94314	197604	2077760	47	2076395
MR19A-4_PC475	42	91.6	104	67202	197594	2082008	52	2075788
MR19A-5_PC505	52	91.7	106	69709	294446	2080140	53	2076838

Isolate name	Mapping to the *Streptococcus pneumoniae* G54 genome		No. of contigs	N50	Length of the longest contig	No. of bases in contigs	No. of contigs >1K	No. of bases in contigs >1K
	Depth of coverage	Breadth of coverage						
MR19A-6_PC543	21	91.5	138	57261	162486	2075625	75	2069618
MR19A-7_PC583	46	91.6	101	80323	197714	2080190	53	2074623
MR19A-1_PC93	39	91.6	110	64498	196287	2102697	53	2098658
MR19F-2_PC306	50	91.7	96	61356	288923	2060117	55	2055310
MR19F-3_PC463	59	91.6	105	67940	171348	2079289	56	2074660
MR19F-1_PC49	49	91.7	88	66608	289458	2039739	52	2035015
MR23F-1_PC329	45	92.3	137	61061	162764	2102475	61	2096379
MR35B-1_PC129	48	91.5	78	89013	155318	2016531	46	2015165
MR35B-2_PC216	59	91.4	76	88920	145836	2011423	43	2010919
MR35B-3_PC291	62	91.4	58	90869	141297	2012520	39	2011858
MR35B-4_PC357	56	92.6	73	90899	209053	2099214	41	2098690
MR35B-5_PC393	57	90.7	61	72699	154064	1998062	43	1998062
MR35B-8_PC540	36	91.4	164	75362	142333	2011760	49	2006412
MR35B-6_PC574	36	91.3	75	72710	115364	2013350	50	2012442
MR35B-7_PC640	43	90.6	262	45845	93591	2001819	75	1999736
MR6A-1_PC240	24	91.5	104	58856	165469	2028967	69	2020675
MR6B-1_PC140	47	92.8	127	48692	133952	2109566	78	2103163
MR6D-1_PC80	37	93.3	98	74289	130520	2139735	60	2137312
MRUT-3_PC676	39	91.2	359	49208	130039	2043252	81	2036159
MRUT-1_PC192	40	90.8	78	74395	195591	2049166	56	2046931
MRUT-2_PC400	49	90.9	94	81424	194089	2073449	48	2068790
PMEN15A-25	53	99.3	83	87810	282871	2061197	46	2058561
CN15A-1	112	99.2	98	61054	186488	2056469	59	2051810
CN15A-2	254	99.2	103	59932	203611	2054693	57	2051869
CN15A-3	186	99.2	83	73950	292862	2060598	49	2056702
CN15A-4	110	98.6	82	74405	280305	2079105	50	2076726
CN15A-5	148	99.2	85	74079	292589	2060269	48	2056975
CN15A-6	247	99.2	113	63398	241787	2058721	62	2055025
CN15A-7	197	99.2	87	74264	159798	2061429	48	2057766

Technical Appendix Table 3. Antimicrobial resistance genes and pilus determinants

Isolate name	*tetO*	*tetM*	*tetM* stop codon insertion	*ermB*	*ermTR*	*mef*	*folA* mutation	*folP* insertion	*pili1*	*pili2*
USA15A-16	–	+	–	+	–	–	–	–	–	–
USA15A-10	–	+	–	+	–	–	+	+	–	–
USA15A-5	–	+	–	+	–	–	–	+	–	–
USA15A-13	–	+	–	+	–	–	–	+	–	–
USA15A-14	–	+	–	+	–	–	–	+	–	–
USA15A-17	–	+	–	+	–	–	+	+	–	–
USA15A-11	–	+	–	+	–	–	+	+	–	–
USA15A-18	–	+	–	+	–	–	–	–	–	–
USA15A-12	–	+	–	+	–	–	–	–	–	–
USA15A-21	–	+	–	+	–	–	–	–	–	–
USA15A-19	–	+	–	+	–	–	–	–	–	–
USA15A-9	–	+	–	+	–	–	–	–	–	–
USA15A-20	–	+	–	+	–	–	–	–	–	–
USA15A-6	–	+	–	+	–	–	–	+	–	–
USA15A-8	–	+	–	+	–	–	+	+	–	–
USA15A-15	–	+	–	+	–	–	+	+	–	–
USA15A-7	–	+	–	+	–	–	–	+	–	–
UK15A-1	–	+	–	+	–	–	–	–	–	–
UK15A-2	–	+	–	+	–	–	–	–	–	–
UK15A-3	–	+	–	+	–	–	+	+	–	–
UK15A-4	–	+	–	+	–	–	–	–	–	–
UK15A-5	–	+	+	+	–	–	–	–	–	–
UK15A-6	–	+	–	+	–	–	–	–	–	–
UK15A-7	–	+	–	+	–	–	–	–	–	–
UK15A-8	–	+	–	+	–	–	–	–	–	–
UK15A-9	–	+	–	+	–	–	–	–	–	–
UK15A-10	–	+	–	+	–	–	+	+	–	–
UK15A-11	–	+	–	+	–	–	–	–	–	–
UK15A-12	–	+	–	+	–	–	–	–	–	–
UK15A-13	–	+	–	+	–	–	–	–	–	–
UK15A-14	–	+	–	+	–	–	–	–	–	–
UK15A-15	–	+	–	+	–	–	–	–	–	–
UK15A-16	–	+	–	+	–	–	–	–	–	–
UK15A-17	–	+	–	+	–	–	–	–	–	–
UK15A-18	–	+	–	+	–	–	–	–	–	–
UK15A-19	–	+	–	+	–	–	–	–	–	–
UK15A-20	–	+	–	+	–	–	–	–	–	–
UK15A-21	–	+	+	+	–	–	–	–	–	–
UK15A-22	–	+	+	+	–	–	–	–	–	–
UK15A-23	–	+	–	+	–	–	–	–	–	–
UK15A-24	–	+	–	+	–	–	–	–	–	–
UK15A-25	–	+	–	+	–	–	–	–	–	–
UK15A-26	–	+	–	+	–	–	+	+	–	–
UK15A-27	–	+	–	+	–	–	–	–	–	–
UK15A-28	–	+	–	+	–	–	–	–	–	–
UK15A-29	–	+	+	+	–	–	–	–	–	–
UK15A-30	–	+	–	+	–	–	–	–	–	–
UK15A-31	–	+	+	+	–	–	–	–	–	–
UK15A-32	–	+	–	+	–	–	–	–	–	–
UK15A-33	–	+	+	+	–	–	–	–	–	–
UK15A-34	–	+	+	+	–	–	–	–	–	–
UK15A-35	–	+	+	+	–	–	–	–	–	–
UK15A-36	–	+	–	+	–	–	–	–	–	–
UK15A-37	–	+	–	+	–	–	–	–	–	–
UK15A-38	–	+	–	+	–	–	–	–	–	–
UK15A-39	–	+	–	+	–	–	–	–	–	–
UK15A-40	–	+	–	+	–	–	+	+	–	–
UK15A-41	–	+	–	+	–	–	–	–	–	–
UK15A-42	–	+	–	+	–	–	+	+	–	–
UK15A-43	–	+	+	+	–	–	–	–	–	–
UK15A-44	–	+	+	+	–	–	–	–	–	–
UK15A-45	–	+	+	+	–	–	–	–	–	–
UK15A-46	–	+	–	+	–	–	–	–	–	–
UK15A-47	–	+	–	+	–	–	+	+	–	–
UK15A-48	–	+	+	+	–	–	–	–	–	–
UK15A-49	–	+	–	+	–	–	+	+	–	–
UK15A-50	–	+	–	+	–	–	+	+	–	–
UK15A-51	–	+	–	+	–	–	–	–	–	–

Isolate name	*tetO*	*tetM*	*tetM* stop codon insertion	*ermB*	*ermTR*	*mef*	*folA* mutation	*folP* insertion	*pili1*	*pili2*
UK15A-52	–	+	–	+	–	–	+	+	–	–
UK15A-53	–	+	–	+	–	–	+	+	–	–
UK15A-54	–	+	–	+	–	–	–	–	–	–
UK15A-55	–	+	–	+	–	–	–	–	–	–
UK15A-56	–	+	–	+	–	–	–	–	–	–
UK15A-57	–	+	–	+	–	–	+	+	–	–
UK15A-58	–	+	–	+	–	–	–	–	–	–
USA15A-1	–	+	–	+	–	–	–	–	–	–
USA15A-2	–	+	–	+	–	–	–	–	–	–
USA15A-3	–	+	–	+	–	–	–	+	–	–
USA15A-4	–	+	–	+	–	–	–	+	–	–
MR15A-2_PC118	–	+	–	+	–	–	–	–	–	–
MR15A-3_PC126	–	+	–	+	–	–	–	–	–	–
MR15A-1_PC13	–	+	–	+	–	–	–	–	–	–
MR15A-4_PC131	–	+	–	+	–	–	–	–	–	–
MS15A-6_PC283	–	+	–	+	–	–	–	–	–	–
MR15A-5_PC206	–	+	–	+	–	–	–	–	–	–
MS15A-8_PC358	–	+	–	+	–	–	–	–	–	–
MS15A-11_PC723	–	+	–	+	–	–	–	–	–	–
MS15A-1_PC16	–	+	–	+	–	–	–	–	–	–
MR15A–6_PC282	-	+	–	+	–	–	–	–	–	–
MS15A-4_PC239	–	+	–	+	–	–	–	–	–	–
MS15A-5_PC273	–	+	–	+	–	–	–	–	–	–
MR15A-20_PC313	–	+	–	+	–	–	–	–	–	–
MR15A-7_PC324	–	+	–	+	–	–	–	–	–	–
MR15A-21_PC342	–	+	–	+	–	–	–	–	–	–
MS15A-7_PC307	–	+	–	+	–	–	–	–	–	–
MR15A-8_PC376	–	+	–	+	–	–	–	–	–	–
MR15A-9_PC418	–	+	–	+	–	–	–	–	–	–
MR15A-10_PC441	–	+	–	+	–	–	–	–	–	–
MS15A-9_PC443	–	+	–	+	–	–	–	–	–	–
MR15A-11_PC495	–	+	–	+	–	–	–	–	–	–
MR15A-22_PC518	–	+	–	+	–	–	–	–	–	–
MR15A-12_PC572	–	+	–	+	–	–	–	–	–	–
MR15A-13_PC618	–	+	–	+	–	–	–	–	–	–
MR15A-14_PC620	–	+	–	+	–	–	–	–	–	–
MR15A-23_PC624	–	+	–	+	–	–	–	–	–	–
MR15A-15_PC638	–	+	–	+	–	–	–	–	–	–
MR15A-16_PC646	–	+	–	+	–	–	–	–	–	–
MR15A-17_PC654	–	+	–	+	–	–	–	–	–	–
MR15A-24_PC662	–	+	–	+	–	–	–	–	–	–
MR15A-18_PC686	–	+	–	+	–	–	–	–	–	–
MS15A-3_PC228	–	+	–	+	–	–	–	–	–	–
MR15A-19_PC718	–	+	–	+	–	–	–	–	–	–
MS15A-10_PC702	–	+	+	+	–	–	–	–	–	–
MR15BC-2_PC267	–	+	–	+	–	–	+	+	–	–
MR15BC-3_PC516	–	+	–	+	–	–	+	+	–	–
MR15BC-1_PC227	–	+	–	+	–	–	–	–	–	–
MR19A-8_PC297	–	+	–	+	–	E	+	+	+	+
MR19A-9_PC396	–	+	–	+	–	E	+	+	+	+
MR19A-10_PC576	–	+	–	+	–	E	+	+	+	+
MR19A-11_PC641	–	+	–	+	–	E	+	+	+	+
MR19A-2_PC345	–	+	–	+	–	E	–	–	+	–
MR19A-3_PC381	–	+	–	+	–	E	–	–	+	–
MR19A-4_PC475	–	+	–	+	–	E	–	–	+	–
MR19A-5_PC505	–	+	–	+	–	E	–	–	+	–
MR19A-6_PC543	–	+	–	+	–	E	–	–	+	–
MR19A-7_PC583	–	+	–	+	–	E	–	–	+	–
MR19A-1_PC93	–	+	–	+	–	E	–	–	+	–
MR19F-2_PC306	–	+	–	+	–	–	–	+	+	+
MR19F-3_PC463	–	+	–	–	–	E	–	–	+	+
MR19F-1_PC49	–	+	–	–	–	E	–	+	+	+
MR23F-1_PC329	–	+	–	+	–	–	–	+	+	–
MR35B-1_PC129	–	+	–	+	–	E	–	–	+	–
MR35B-2_PC216	–	+	–	–	–	E	–	–	+	–
MR35B-3_PC291	–	+	–	–	–	E	–	–	+	–
MR35B-4_PC357	–	+	–	–	–	E	–	–	+	–
MR35B-5_PC393	–	–	–	–	–	–	–	–	+	–
MR35B-8_PC540	–	+	–	–	–	E	–	–	+	–

Isolate name	*tetO*	*tetM*	*tetM* stop codon insertion	*ermB*	*ermTR*	*mef*	*folA* mutation	*folP* insertion	*pili1*	*pili2*
MR35B-6_PC574	–	+	–	–	–	E	–	–	+	–
MR35B-7_PC640	–	–	–	–	–	–	–	–	+	–
MR6A-1_PC240	–	+	–	+	–	–	–	+	–	–
MR6B-1_PC140	–	+	–	+	–	E	–	+	+	–
MR6D-1_PC80	–	+	–	–	–	E	+	–	–	–
MRUT-3_PC676	–	+	–	–	–	E	–	+	–	–
MRUT-1_PC192	–	+	–	+	–	–	–	+	–	–
MRUT-2_PC400	–	+	–	–	–	E	–	+	–	–
PMEN15A-25	–	+	+	+	–	–	–	–	–	–
CN15A-1	–	+	–	+	–	–	–	–	–	–
CN15A-2	–	+	–	+	–	–	–	–	–	–
CN15A-3	–	+	–	+	–	–	–	–	–	–
CN15A-4	–	+	–	+	–	–	–	–	–	–
CN15A-5	–	+	–	+	–	–	–	–	–	–
CN15A-6	–	+	–	+	–	–	–	+	–	–
CN15A-7	–	+	–	+	–	–	–	–	–	–

Technical Appendix Table 4. Core genome changes that separate clade-I-MNS from the rest of clade I

Gene name	Sequence ID
ABC transporter [Streptococcus pneumoniae]	WP_001814375.1
ABC transporter ATP binding protein [Streptococcus pneumoniae]	SNJ41425.1
ABC transporter ATP binding protein [Streptococcus pneumoniae]	WP_083990072.1
ABC transporter permease [Streptococcus pneumoniae]	WP_054383052.1
ABC transporter substrate binding lipoprotein [Streptococcus pneumoniae]	CJG46427.1
ABC-2 type transporter family protein [Streptococcus pneumoniae GA52612]	EJG83970.1
acyltransferase family protein [Streptococcus pneumoniae]	SNL85654.1
alanine aminotransferase, partial [Streptococcus pneumoniae]	KXW50488.1
alanyl-tRNA synthetase [Streptococcus pneumoniae]	CKG69103.1
aldo/keto reductase [Streptococcus pneumoniae]	WP_001269452.1
α-acetolactate decarboxylase [Streptococcus pneumoniae]	CJV90221.1
α-amylase [Streptococcus pneumoniae GA60080]	EJH11203.1
aminotransferase [Streptococcus pneumoniae GA11184]	EHD26622.1
anaerobic ribonucleotide reductase [Streptococcus pneumoniae]	CJA41997.1
cell wall surface anchor family protein [Streptococcus pneumoniae]	COK42161.1
chlorohydrolase [Streptococcus pneumoniae]	CYH35105.1
ecoEI R domain protein [Streptococcus pneumoniae GA13723]	EHZ24329.1
endo-α-N-acetylgalactosaminidase [Streptococcus pneumoniae]	CKF12265.1
exopolyphosphatase [Streptococcus pneumoniae GA47562]	EJH24984.1
glycosyl hydrolase family 20 (GH20) protein [Streptococcus pneumoniae]	CIV37094.1
glycosyl transferase [Streptococcus pneumoniae]	CKG70649.1
hlyIII superfamily protein [Streptococcus pneumoniae]	SNI42750.1
Holliday junction-specific endonuclease [Streptococcus pneumoniae]	SNH04621.1
hypothetical protein [Streptococcus pneumoniae]	WP_000842498.1
hypothetical protein [Streptococcus pneumoniae]	WP_050239143.1
hypothetical protein [Streptococcus pneumoniae]	WP_000977365.1
hypothetical protein CGSSpBS455_01575 [Streptococcus pneumoniae BS455]	EFL65997.1
hypothetical protein D059_00935	EOB20305.1
hypothetical protein D061_04916 [Streptococcus pneumoniae 1488]	EOB23299.1
hypothetical protein PNI0008_00610, partial [Streptococcus pneumoniae PNI0008]	ELU73227.1
hypothetical protein SP_1041 [Streptococcus pneumoniae TIGR4]	AAK75156.1
hypothetical protein, partial [Streptococcus pneumoniae]	WP_079098811.1
isoleucyl-tRNA synthetase [Streptococcus pneumoniae]	COK04924.1
membrane protein [Streptococcus pneumoniae]	CRH99656.1
oxidoreductase%2C pyridine nucleotide-disulfide class I%2C Mercury (II) reductase [Streptococcus pneumoniae]	CIV90663.1
penicillin binding protein 1A [Streptococcus pneumoniae]	WP_001040024.1
penicillin binding protein 2B, partial [Streptococcus pneumoniae]	BAA11616.1
phosphorylcholine transferase LicD [Streptococcus pneumoniae]	WP_078161399.1
PLP-dependent aminotransferase family protein [Streptococcus pneumoniae]	WP_050199184.1
PTS system IIABC components [Streptococcus pneumoniae]	COO88789.1
P-type ATPase-metal cation transport [Streptococcus pneumoniae]	CMW01002.1
sensor histidine kinase [Streptococcus pneumoniae]	WP_061753039.1
sialidase A (neuraminidase A) [Streptococcus pneumoniae]	COT00654.1
sugar ABC transporter permease, partial [Streptococcus pneumoniae]	WP_050272294.1
transcriptional regulator, GntR family [Streptococcus pneumoniae G54]	ACF56815.1
tryptophan synthase subunit α, partial [Streptococcus pneumoniae]	WP_085820027.1

Gene name	Sequence ID
TVP38/TMEM64 family protein [Streptococcus pseudopneumoniae]	WP_049513140.1
type 4 prepilin peptidase [Streptococcus pneumoniae]	CJD57248.1
UDP-N-acetylmuramyl tripeptide synthetase%2C Mur ligase [Streptococcus pneumoniae]	COH16933.1
Uncharacterized protein [Streptococcus pneumoniae]	SNK82086.1
Uncharacterized protein [Streptococcus pneumoniae]	CIV83149.1
YwnB [Streptococcus pneumoniae]	COG36585.1

Technical Appendix Table 5. WGS-based antimicrobial resistance detection and pilus determinant detection platform

Query (No. of bp)	Accession, sequence coordinates	Supplementary information
pbp1a (2160)	AE007317, 332863–335022 (complement)	Transpeptidase domain, 333083–333913
pbp2b (2057)	AE007317, 1494216–1496273 (complement)	Transpeptidase domain, 1494292–1495124
pbp2x (2253)	AE007317, 302261–304513	Transpeptidase domain, 302945–304019
ermB (100)	HG799494, 44520–44619	≧95% sequence identity predicts presence of the resistance gene
ermTR (68)	CP002121,856516–856583	≧95% sequence identity predicts presence of the resistance gene
mefA (1218)	U70055, 314–1531	≧98% sequence identity predicts presence of the resistance gene
mefE (1218)	U83667, 1–1218	≧98% sequence identity predicts presence of the resistance gene
tetM (100)	HG799494, 42545–42644	≧95% sequence identity predicts presence of the resistance gene
tetM (1935)	HG799494, 41018–42952	a deletion of two nucleotides at codon 339, generating a premature stop codon
tetO (100)	FM178797, 1754–1853 (complement)	≧95% sequence identity predicts presence of the resistance gene
folA (507)	AE007317,1412861–1413367 (complement)	I100L (common) and D92R substitutions confer trimethoprim resistance
folP (945)	AE007317, 268022–268966	various insertions of 1–2 codons between bases 168 and 201 of *folP* confer sulfamethoxazole resistance and intermediate cotrimoxazole resistance
rrgA-1 (100)	CP000921, 463577–463676	≧95% sequence identity predicts presence of pili-1
pitB-1 (100)	CP000921, 1003530–1003629	≧95% sequence identity predicts presence of pili-2

Technical Appendix Table 6. PBP1a transpeptidase domain sequences that were newly identified in this study

Sequences
>new1
SMKPITDYAPALEYGVYDSTASIVHDVPYNYPGTDTPLYNWDHVYFGNITIQYALQQSRNVTAVETLNKVGLDRAKTFLNGLGIDY PSMHYANAISSNTTESNKKYGASSEKMAAAYAAFANGGIYHKPMYINKIVFSDGSEKEFSDAGTRAMKETTAYMMTEMMKTVLTY GTGRGAYLPWLPQAGKTGTSNYTDEEIEKYIKNTGYVAPDEMFVGYTRKYSMAVWTGYSNRLTPIVGDGFLVAAKVYRSMITYLS EDTHPEDWTMPDGLFRNGEFV
>new2
SMKPITDYAPALEYGVYDSTATIVHDEPYNYPGTDIPVYNWDRGYFGNITLQYALQQSRNVPAVETLNKVGLNRAKTFLNGLGIDY PSLHYSNAISSNTTESDQKYGASSEKMAAAYAAFANGGTYYKPMYIHKVVFSDGSEKEFSNVGTRAMKETTAYMMTDMMKTVLT YGTGRGAYLPWLPQAGKTGTSNYTDEEIEKYIKNTGYVAPDEMFVGYTRKYSMAVWTGYSNRLTPLVGNGLTVAAKVYRSMMTY LSEGSNPEDWNIPEGLYRNGEFV
>new3
SMKPITDYAPALEYGVYDSTASIVHDVPYNYPGTDTPLYNWDHVYFGNITIQYALQQSRNVTAVETLNKVGLDRAKTFLNGLGIDY PSMHYANAISSNTTESNKKYGASSEKMAAAYAAFANGGIYHKPMYINKIVFSDGSEKEFSDAGTRAMKETTAYMMTDMMKTVLSY GTGRNAYLAWLPQAGKTGTSNYTDEEIEKYIKNTGYVAPDEMFVGYTRKYSMAVWTGYSNRLTPIVGDGFLVAAKVYRSMMTYL SEGSNPEDWNIPEGLYRNGEFV
>new4
TMKPITDYAPAIEYGIYDSTATMVNDIPYNYPGTSTPVYNWDRAYFGNITLQYALQQSRNVPAVETLNKVGLDRAKNFLNGLGIDYP DMHYSNAISSNTTESNKQYGASSEKMAAAFAAFANGGIYHKPMYINKIVFSDGSEKEFSDAGTRAMKETTAYMMTEMMKTVLSY GTGRNAYLAWLPQAGKTGTSNYTDEEIENHIKTSQFVAPDELFAGYTRKYSMAVWTGYSNRLTPLVGNGLTVAAKVYRSMMTYL SEGSNPEDWNIPEGLYRNGEFV
>new5
TMKPITDYAPAIEYGVYDSTATMVNDIPYNYPGTSTPVYNWDRAYFGNITLQYALQQSRNVPAVETLNKVGLDRAKNFLNGLGIDY PDMHYSNAISSNTTESNKQYGASSEKMAAAFAAFANGGIYHKPMYINKIVFSDGSEKEFSDAGTRAMKETTAYMMTEMMKTVLSY GTGRNAYLAWLPQAGKTGTSNYTDEEIENHIKTSQFVAPDELFVGYTRKYSMAVWTGYSNRLTPLVGNGLTVAAKVYRSMMTYL SEGSNPEDWNIPEGLYRNGEFV

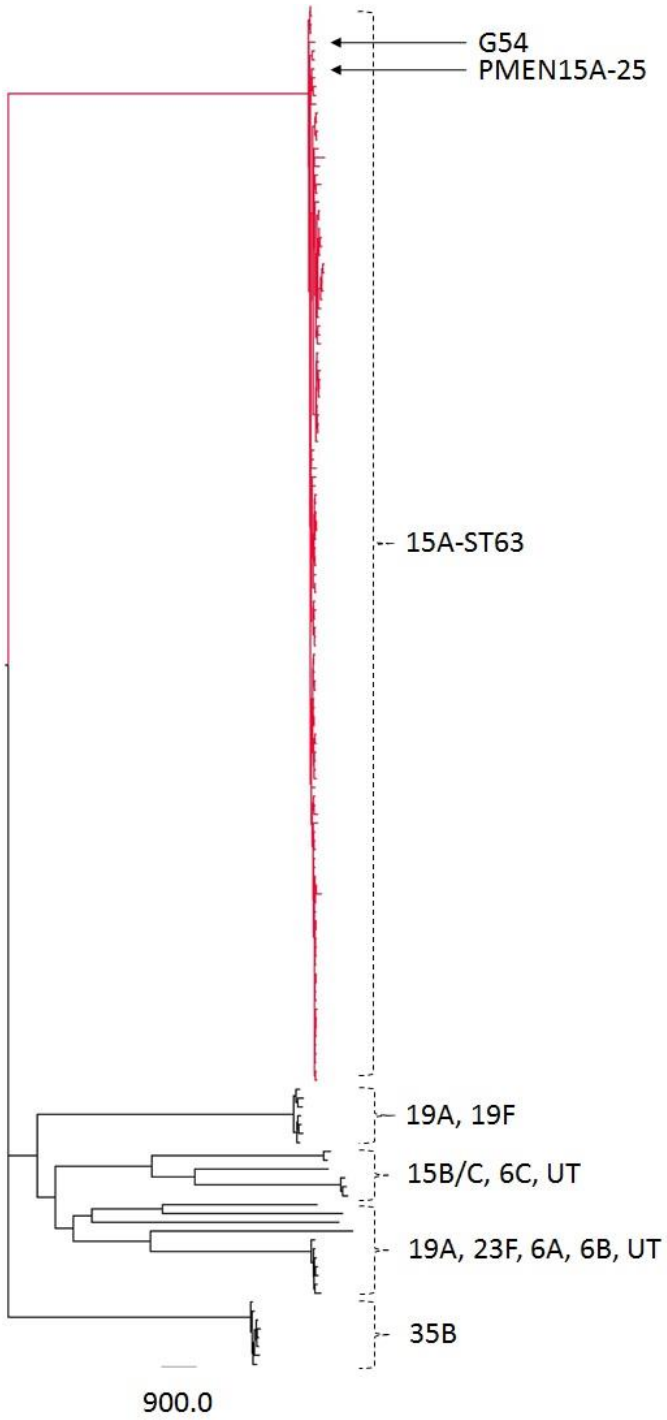
Technical Appendix Table 7. PBP2b transpeptidase domain sequences that were newly identified in this study

Sequences
>new1
TNVFVPGSVVKAATISSGWENGVLSGNQTLTDQPIVFQGSAPIYSWYKLAYGSFPITAVEALEYSSNAYMVQTALGIMGQTYQPN MFVGTSNLESAMEKLRSTFGEYGLGTATGIDLPDESTGFVPKEYSFANYITNAFGQFDNYTPMQLAQYVATIANNGVRVAPRIVEG IYGNNDKGGLGDLIQQLQPTEMNKVNISDSDMSILHQGFYQVAHGTSGLTTGRAFSNGAAVSISGKTGTAESYVEGGQEANNTNA VAYAPSDNPQIAVAVVFPHNTN
>new2
TNVFVPGSVVKAATISSGWENGVLSGNQTLTDQPIVFQGSAPIYSWYKLAYGSFPITAVEALEYSSNAYMVQTALGIMGQTYQPN MFVGTSNLETAMGKLRATFGEYGLGAATGIDLPDESTGFVPKEYSFANFITNAFGQFDNYTPMQLAQYVATIANNGVRLAPHIVEG IYDNNDKGGLGELIQAIDTKEINKVNISESDMAILHQGFYQVSHGTSPLTTGRAFSDGATVSISGKTGTGESYVAGGQEANNTNAVA YAPTENPQIAVAVVFPHNTN
>new3
TNVFAPGSVVKAATISSGWENGVLSGNQTLTDQSIVFQGSAPINSWYTQAYGSFPITAVQALEYSSNAYMVQTALGLMGQTYQPN MFVGTSNLESAMGKLRSTFGEYGLGSATGIDLPDESTGFVPKDYSFANYITNAFGQFDNYTPMQLAQYVATIANDGVRVAPRIVE GIYGNNDKGGLGDLIQQLQPTEMNKVNISDSDMSILHQGFYQVAHGTSGLTTGRAFSNGAAVSISGKTGTAESYVEGGQEANNTN AVAYAPSDNPQIAVAVVFPHNTN
>new4
TNVFVPGSVVKAATISSGWENGVLSGNQTLTDQSIVFQGSAPINSWYTQAYGSFPITAVQALEYSSNAYMVQTALGLMGQTYQPN MFVGTSNLESAMGKLRSTFGEYGLGSATGIDLPDESTGFVPKDYSFANYITNAFGQFDNYTPMQLAQYVATIANDGVRVAPRIVE GIYGNNDKGGLGDLIQQLQPTEMNKVNVSDSDMSILHQGFYQVAHGTSGLTTGRAFSNGAAVSISGKTGTAESYVEGGQEANNT NAVAYAPSDNPQIAVAVVFPHNTN

Technical Appendix Table 8. PBP2x transpeptidase domain sequences that were newly identified in this study

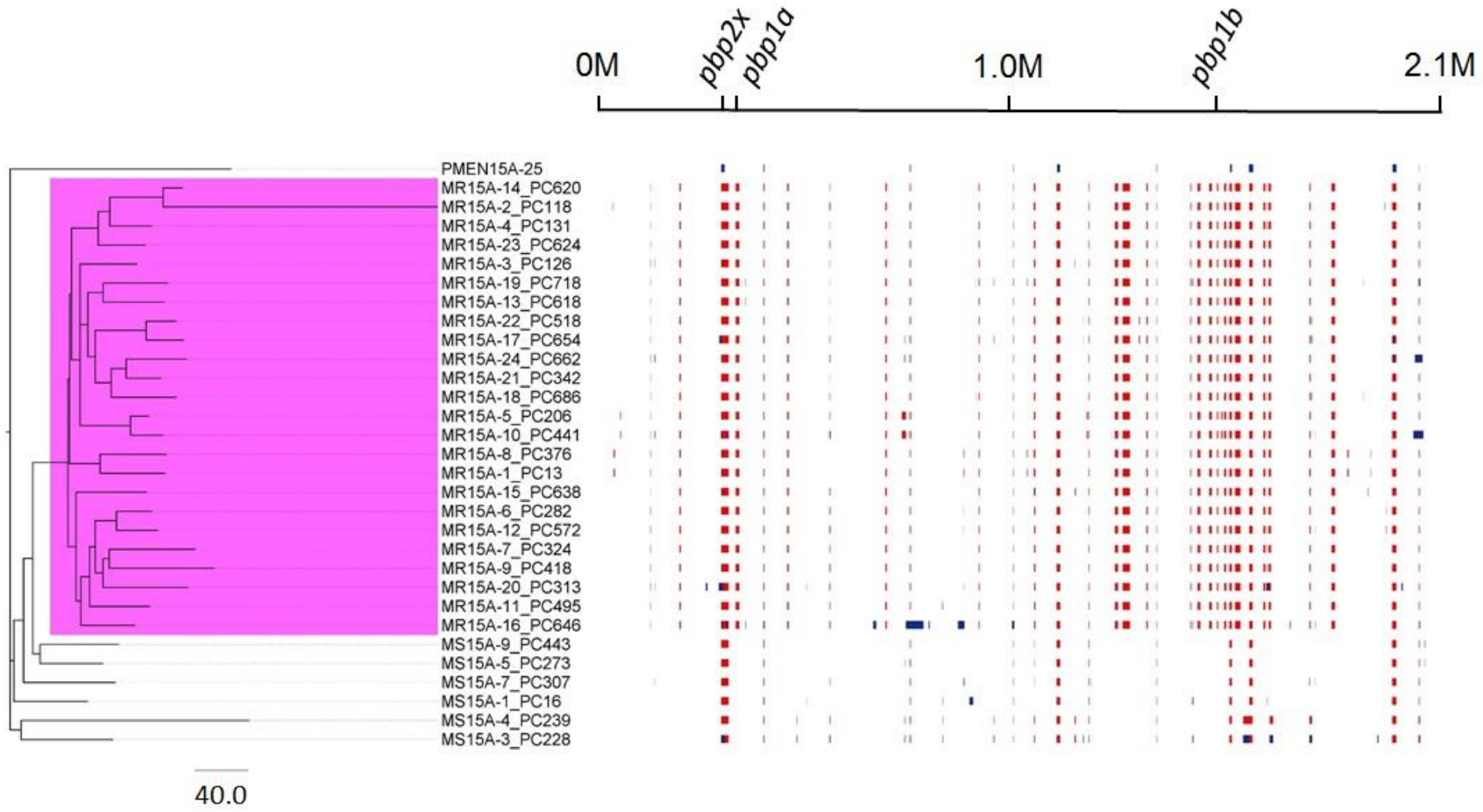
Sequences
>new1
GTDGIITYEKDRLGNIVPGTEQVSQQTVDGKDVYTTISSPLQSFMETQMDAFQEKVKGKYMTATLVSAKTGEILATTQRPTFDADT KEGITEDFVWRDILYQSNYEPGSPMKVMMLAAAIDNNTFPGGEVFNSSELKIADATIRDWDVNEGLTGGRMMTFSQGFAHSSNV GMTLLEQKMGDATWLDYLNRFKFGVPTRFGLTDEYAGQLPADNIVNIAMSAFGQGISVTQTQMLRAFTAIANDGVMLEPKFISALY DPNDQSVRKSQKEIVGNPVSKEAASVTRDHMVMVGTDPTYGTMYNHSTGKATVNVPGQNVALKSGTAEIADEKNGGYLTGSTN NIFSVVSMHPAENPDFILYV
>new2
GKDGIITYEKDRLGNIVPGTEQVSQQTVDGKDVYTTISSTLQSFMETQMDAFLEKVKGKYMTATLVSAKTGEILATTQRPTFNADTK EGITEDFVWRDILYQSNYEPGSAMKVMTLASSIDNNTFPSGEYFNSSEFKIADATTRDWDVNDGLTTGGMMTFLQGFAHSSNVG MSLLEQKMGDATWLDYLSRFKFGVPTRFGLTDEYAGQLPADNIVSIAQSSFGQGISVTQTQMLRAFTAIANDGVMLEPKFISAIYDT NNQSVRKSQKEIVGNPVSKEAASTTRNHMILVGTDPLYGTMYNHYTGKPIITVPGQNVAVKSGTAQIADEKNGGYLVGSTNYIFSV VTMNPAENPDFILYV
>new3
GKDGIITYEKDRLGNIVPGTEQVSQQTVDGKDVYTTLSSPLQSFMETQMDAFLEKVKGKYMTATLVSAKTGEILATTQRPTFNADT KEGITEDFVWRDILYQSNYEPGSAFKVMMLASSIDNNTFPSGEYFNSSEFKIADATTRDWDVNEGLTTGGMMTFSQGFAHSSNV GTSLLEQKMGDATWLDYLKRFKFGVPTRFGLTDEYAGQLPADNIVSIAQSSFGQGISVTQTQMLRAFTAIANDGVMLEPKFISAIYD TNNQSVRKSQKEIVGNPVSKEAASTTRNHMILVGTDPLYGTMYNHYTGKPIITVPGQNVAVKSGTAQIADEKNGGYLVGSTNYIFS VVTMNPAENPDFILYV
>new4
GTDGIITYEKDRVGNIVPGTELVSQQTVDGKDVYTTLSSPLQSFMETQMDAFLEKVKGKYMTATLVSAKTGEILATTQRPTFNADT KEGITEDFVWRDILYQSNYEPGSAMKVMTLAASIDNNTFPSGEYFNSSEFKIADATTRDWDVNEGLTTGGMMTFLQGFAHSSNVG MSLLEQKMGDATWLDYLKRFKFGVPTRFGLTDEYAGQLPADNIVSIAQSSFGQGISVTQTQMLRAFTAIANDGVMLEPKFISAIYDT NNQSVRKSQKEIVGNPVSKEAASTTRNHMILVGTDPLYGTMYNHYTGKPIITVPGQNVAVKSGTAQIADEKNGGYLVGSTNYIFSA VTMNPAENPDFILYV
>new5
GTDGIITYEKDRLGNIVPGTELVSQQTVDGKDVYTTLSSPLQSFMETQMDAFLEKVKGKYMTATLVSAKTGEILATTQRPTFNADT KEGITEDFVWRDILYQSNYEPGSAMKVMTLASSIDNNTFPSGEYFNSSEFKIADATTRDWDVNEGLTTGGMMTFLQGFAHSSNVG MSLLEQKMGDATWLDYLKRFKFGVPTRFGLTDEYAGQLPADNIVSIAQSSFGQGISVTQTQMLRAFTAIANDGVMLEPKFISAIYDT NNQSVRKSQKEIVGNPVSKEAASTTRNHMILVGTDPLYGTMYNHYTGKPIITVPGQNVAVKSGTAQIADEKNGGYLVGSTNYIFSA VTMNPAENPDFILYV
>new6
GKDGIITYEKDRLGNIVPGTEQVSQQTVDGKDVYTTLSSPLQSFMETQMDAFLEKVKGKYMTATLVSAKTGEILATTQRPTFNADT KEGITEDFVWRDILYQSNYEPGSAFKVMMLASSIDNNTFPSGEYFNSSEFKIADATTRDWDVNAGLTTGGMMTFLQGFVHSSNVA TSLLEQKMGDATWLDYLKRFKFGVPTRFGLTDEYAGQLPADNIVSIAQSSFGQGISVTQTQMLRAFTAIANDGVMLEPKFISAIYDT NNQSVRKSQKEIVGNPVSKEAASTTRNHMILVGTDPLYGTMYNHYTGKPIITVPGQNVAVKSGTAQIADEKNGGYLVGSTNYIFSV VTMNPAENPDFILYV
>new7
GTDGIITYEKDRLGNIVPGTEQVSQQTVDGKDVYTTLSSPLQSFMETQMDAFLEKVKGKYMTATLVSAKTGEILATTQRPTFNADT KEGITEDFVWRDILYQSNYEPGSAMKVMTLASSIDNNTFPSGEYFNSSEFKIADATTRDWDVNAGLTTGGMMTFLQGFAHSSNVG MSLLEQKMGDATWLDYLKRFKFGVPTRFGLTDEYAGQLPADNIVSIAQSSFGQGISVTQTQMLRAFTAIANDGVMLEPKFISAIYDT

Sequences
NNQSVRKSQKEIVGNPVSKEAASTTRNHMILVGTDPIYGTMYNHYTGKPIITVPGQNVAVKSGTAQIADEKNGGYLVGSTNYIFSV VTMNPAENPDFILYV

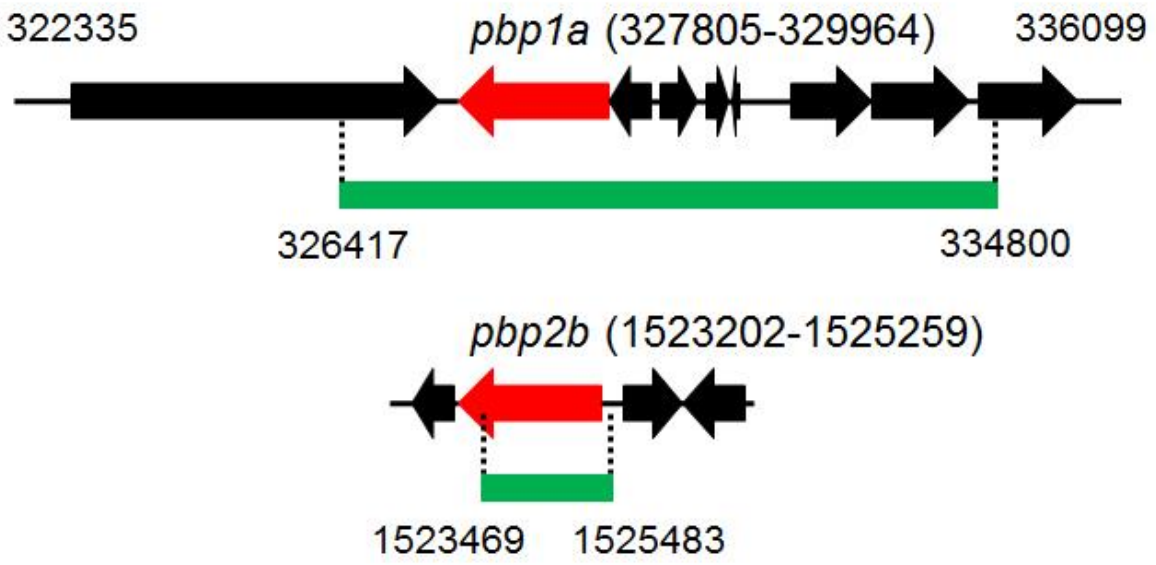


Technical Appendix Figure 1. The phylogenic tree was created using all Japanese and global isolates. All of the Japanese serotype 15A-sequence type (ST) 63 (meropenem-susceptible and meropenem-non-

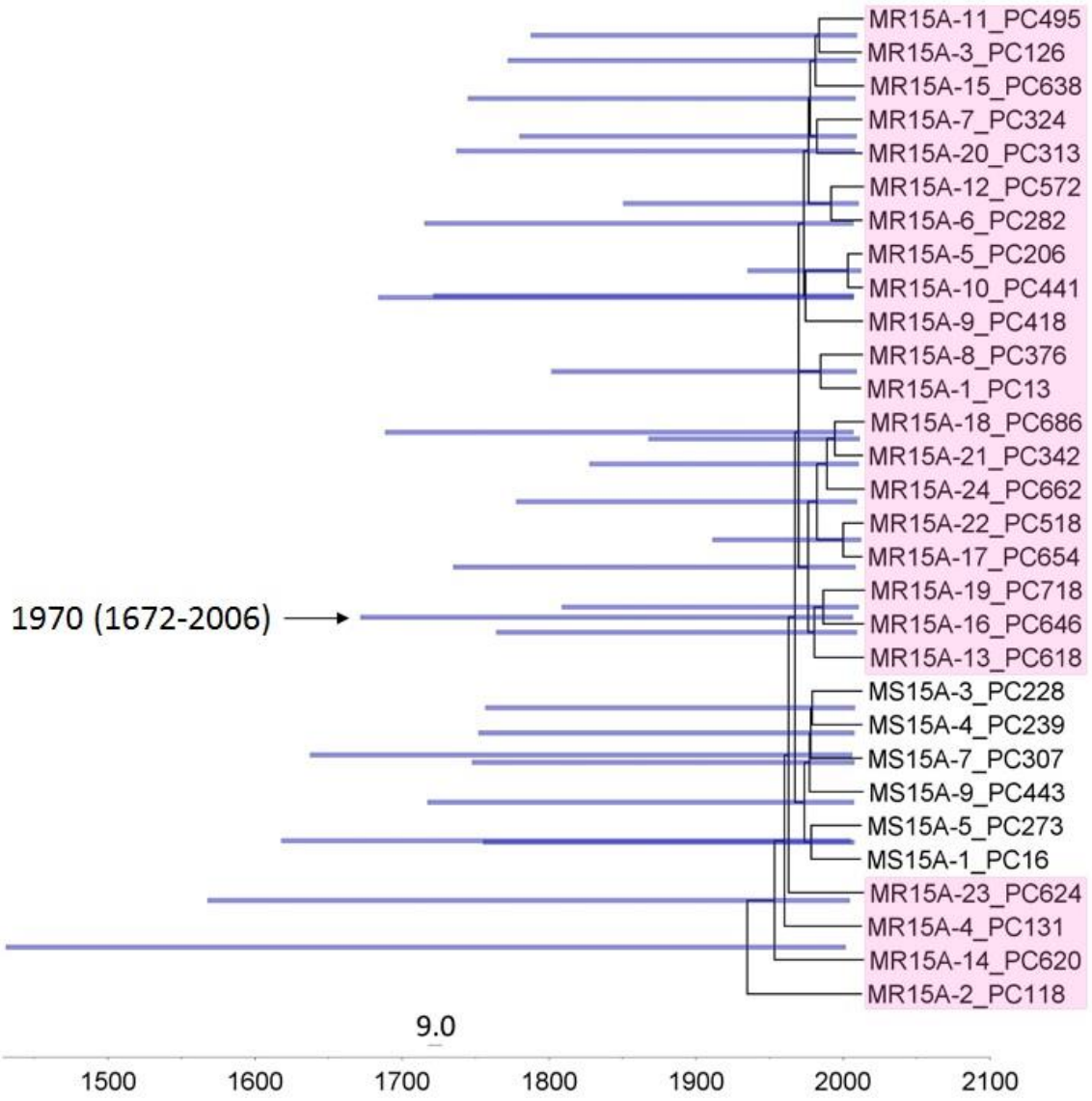
susceptible) isolates were included in the same clade (red branch). None of the meropenem-non-susceptible serotype isolates except for 15A were included in the clade. This fact indicates that there was not an ancestral isolate of the meropenem-non-susceptible serotype 15A-ST63 isolate that had a serotype other than 15A within the tested isolates.



Technical Appendix Figure 2. The phylogenic tree created by Gubbins using all of the clade-I isolates (Figure 1) generated a Japanese meropenem-non-susceptible serotype 15A-sequence type (ST) 63-specific clade (highlighted in pink). All of the meropenem-non-susceptible serotype 15A-ST63 isolates were included in the clade, and none of the meropenem-susceptible serotype 15A-ST63 isolates were. In this analysis, PMEN15A-25 was used as an outgroup isolate. The block chart on the right shows the predicted recombination sites in each isolate. Blue blocks are unique to a single isolate, while red blocks are shared by multiple isolates.



Technical Appendix Figure 3. Sketch of the predicted recombination sites including *pbp1a* and *pbp2b*, respectively. Green blocks show the recombination sites. Each number shows the sequence coordinates using *Streptococcus pneumoniae* G54 (NCBI Reference Sequence: NC_011072.11). These two recombination sites were shared by all of the meropenem-non-susceptible serotype 15A-ST63 isolates and were not found in any meropenem-susceptible serotype 15A-ST63 isolates.



Technical Appendix Figure 4. The result of an estimation of the date at which meropenem-non-susceptible serotype 15A-ST63 originated. The “MR” (all of which are colored in pink) and “MS” prefixes in front of the isolate names indicate “Japanese meropenem-non-susceptible” and “Japanese meropenem-susceptible,” respectively. The blue bars showed the 95% credibility intervals. The blue bar indicated with an arrow is the target credibility interval at the node the MR- and MS-serotype 15A-ST63 strains were separated. The years to the left of the arrow show the node age and its 95% credibility interval.