

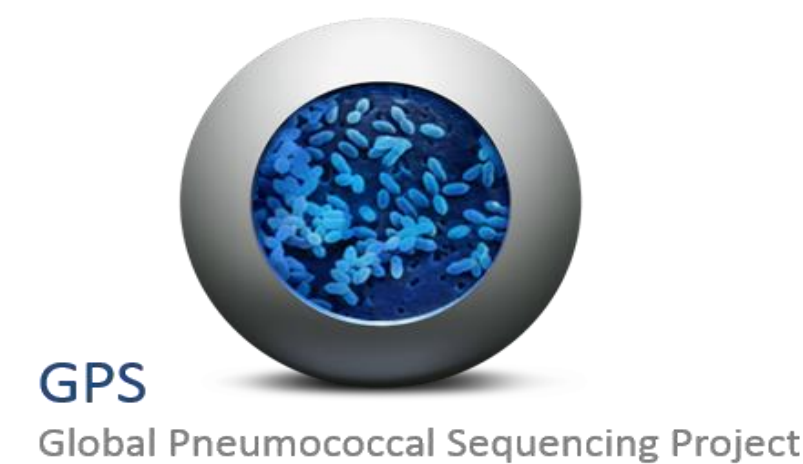


Molecular characterization of *Streptococcus pneumoniae* strains isolated from invasive disease in the pre- and post-PCV10 periods in Brazil, 2005 to 2015

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Background and aims

In Brazil, PCV10 was introduced in the National Immunization Program for children up to 2 years of age in 2010. Institute Adolfo Lutz (IAL) is the national reference laboratory for meningitis and pneumococcal diseases and performs laboratory-based epidemiological surveillance in the country. The aim of this study was to evaluate the genetic diversity among pneumococcal strains isolated in Brazil during 2005-2015.

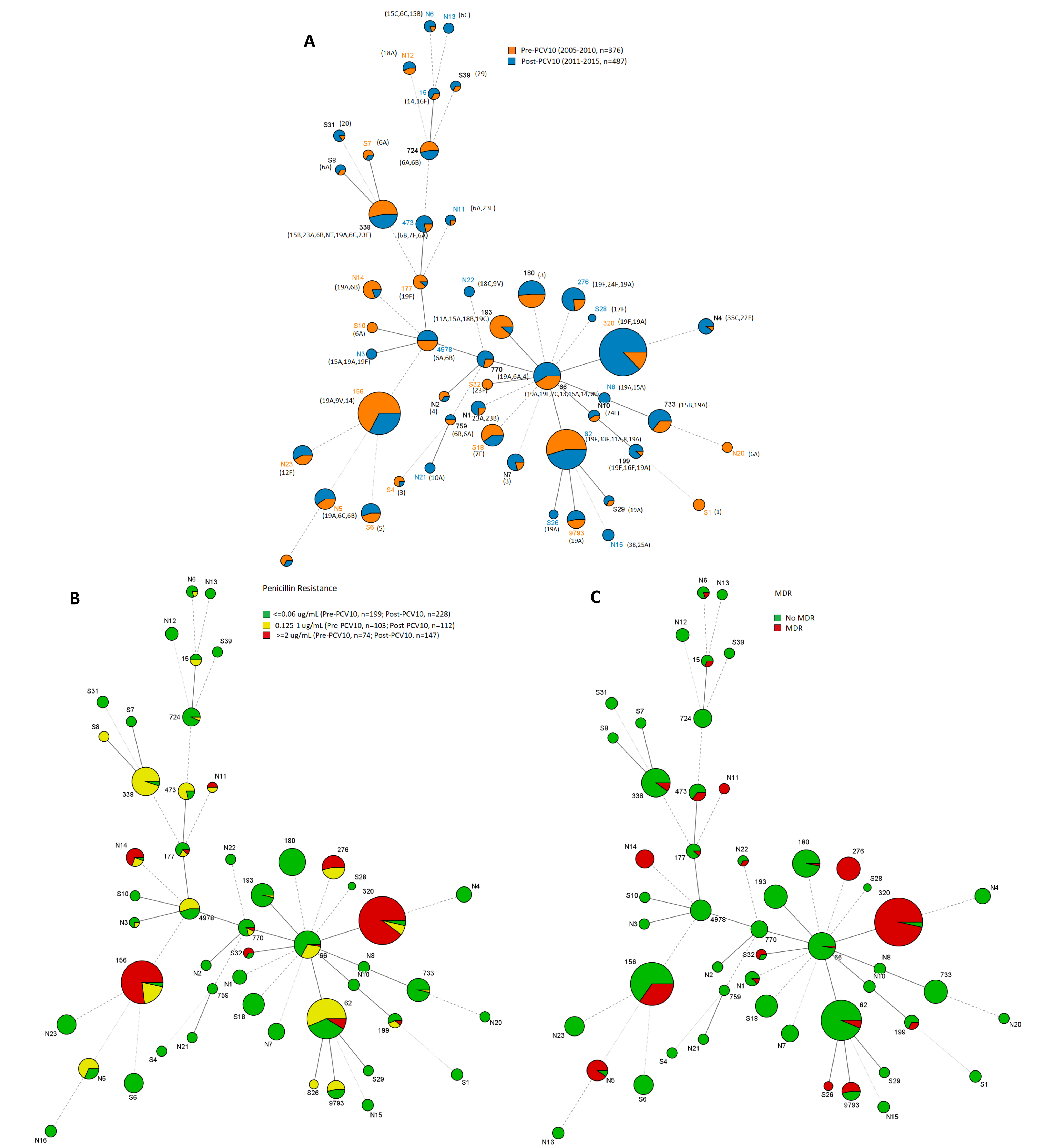
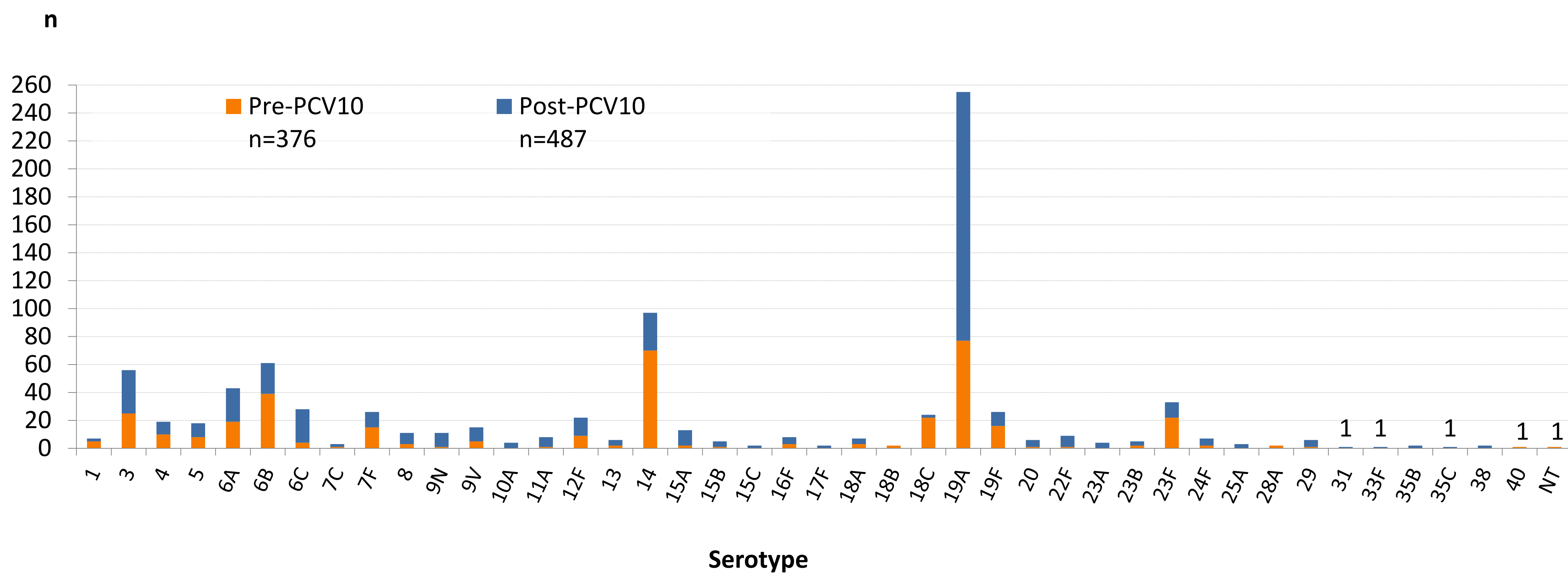
Methods

A random sample of 863 isolates was selected from a collection of 9,996 invasive pneumococcal strains, 376 **pre-PCV10 (2005-2010)** and 487 **post-PCV10 (2011-2015)** introduction. All isolates were serotyped by Quellung and antimicrobial resistance was determined by microdilution following the CLSI recommendations. Of these 863 isolates, 451 were whole-genome sequenced by the Sanger Institute as part of the Global Pneumococcal Sequencing project (www.pneumogen.net); serotype and sequence type were determined using the CDC *Streptococcus* laboratory pneumococcal typing pipeline. The remaining 412 isolates were characterized by MLST, using primers published on PubMLST (<https://pubmlst.org/spneumoniae/>). Clonal complex were identified using the BURST algorithm (http://eburst.mlst.net/v3/mlst_datasets/).

Results

A total of 245 sequence types (ST) were identified, organized in 41 clonal complexes (CC). **Pre-PCV10** introduction, the most common CC were CC156 (n=64), CC62 (n=45) and CC193 (n=24), mainly associated with serotypes 14 (90.7%), 19A (91.0%) and 18C (91.6%), respectively. **Post-PCV10**, CC320/19A isolates (n=98, 20.1%) became the most common lineage. The proportion of CC156/14 and CC62/19A isolates decreased from 15.4% to 4.9% (p<0.001) and from 10.9% to 4.7% (p<0.001), respectively. High penicillin resistance was mainly related with CC320, CC156, CC276 and CCN14. The Multi-Drug Resistance (resistance to at least 3 different classes of antibiotics, MDR) was mainly related with CC320, CC156, CC276, CCN5 and CCN14.

Figure 1: *S. pneumoniae* serotypes distribution in Pre-PCV10 (2005-2010) and Post-PCV10 (2011-2015) periods.



Minimum spanning tree drawn by Bionumerics software showing the genetic structure of *S. pneumoniae* from invasive pneumococcal disease isolated in Pre-PCV10 (2005-2010) and Post-PCV10 (2011-2015) periods in Brazil for clonal complexes (CC) with STs with more than 2 isolates. Figure A, CCs and serotypes in both periods; Figure B, Penicillin resistance and Figure C, MDR. The size of the circle is proportional to the number of isolates in each CC.

Conclusions

These isolates show considerable genetic diversity among pneumococcal strains circulating in Brazil. An expansion of the CC320/19A lineage was observed after vaccine introduction, while CC62/19A lineage decreased significantly. The CC156/14 lineage, which was the most predominant in pre-PCV10, decreased in post-PCV10 as well.

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Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Table1: Major *S. pneumoniae* clonal complex, sequence types and serotypes in Pre-PCV10 (2005-2010) and Post-PCV10 periods (2011-2015)

CC	ST	n	Pre-PCV10	Post-PCV10	Serotypes (n)
62	53	9	2	7	8 (9)
	62	6	1	5	11A (6)
	1118	23	18	5	19A (23)
	2260	1		1	19A (1)
	2878	14	10	4	19A (14)
	2880	9	6	3	19A (9)
	3308	1		1	33F (1)
	5480	1	1		19A (1)
	7034	2	1	1	19A (2)
	9799	1		1	19A (1)
	9837	6	1	5	19A (6)
	9838	1		1	19A (1)
	9940	1		1	19A (1)
	9941	1	1		19A (1)
	9942	1	1		19A (1)
66	9943	3	2	1	19A (3)
	11325	1	1		19F (1)
	12574	1		1	8 (1)
	66	21	8	13	14 (8), 9N (11), 19A (1), 19F (1)
	67	1	1		15A (1)
	73	4	1	3	15A (4)
	737	3	1	2	7C (6)
	2216	1		1	15A (1)
	11327	6	2	4	13 (4), 19A (1), 19F (1)
	12487	2	2		14 (2)
156	12837	1	1		14 (1)
	156	82	55	27	14 (73), 9V (8), 19A (1)
	162	3	2	1	9V (3)
	1556	1	1		14 (1)
	2335	1	1		14 (1)
	9668	1		1	9V (1)
	12505	1	1		14 (1)
	12506	1	1		14 (1)
	12507	1	1		14 (1)
	12508	1	1		14 (1)
	12839	1	1		14 (1)
	156~	1		1	14 (1)
177	156~~	1		1	14 (1)
	51	1	1		19F (1)
	177	3	3		19F (3)
	646	1		1	19F (1)
	3013	1	1		19F (1)
	12510	1	1		19F (1)
	12512	1	1		19F (1)
	12513	1	1		19F (1)
193	193	21	20	1	18C (19); 18B (2)
	1228	1		1	15A (1)
	1358	1	1		18C (1)
	11324	1	1		18C (1)
	12517	1	1		18C (1)
	12838	1	1		18C (1)
	193~	1		1	11A (1)
320	202	1	1		19A (1)
	236	2	2		19F (2)
	237	1		1	19A (1)
	271	1		1	19F (1)
	320	97	9	88	19A (96), 19F (1)
	1451	3		3	19A (3)
	2323	2		2	19F (2)
	4768	1		1	19A (1)
	8202	1	1		19A (1)
	8549	1		1	19A (1)
	8884	1		1	19A (1)
	9796	1		1	19A (1)
338	11326	2	2		19F (2)
	11485	1		1	19A (1)
	11486	1		1	19A (1)
	11936	1		1	19A (1)
	338	18	11	7	23F (16), 15B (1), NT (1)
	387	1		1	19A (1)
	791	3	2	1	23F (3)
	1131	1	1		19A (1)
	2777	8	2	6	6C (8)
	3163	1	1		23F (1)
	4559	1	1		23F (1)
	11318	1		1	6B (1)
N5*	11323	2	2		6C (2)
	11329	1	1		23F (1)
	11334	1		1	6C (1)
N14*	11335	1		1	6C (1)
	12835	1	1		23F (1)
	338~	1		1	23A (1)
N5*	315	7	5	2	6B (6), 19A (1)
	386	12	2	10	6C (10), 6B (2)
	11315	3	2	1	6B (3)
N14*	90	15	12	3	6B (14), 19A (1)
	12495	1	1		6B (1)

~, new ST submitted at PubMLST; * CC with predicted founder=none determined by BURST algorithm, they received "N" denomination and a sequential number 1 to 23.