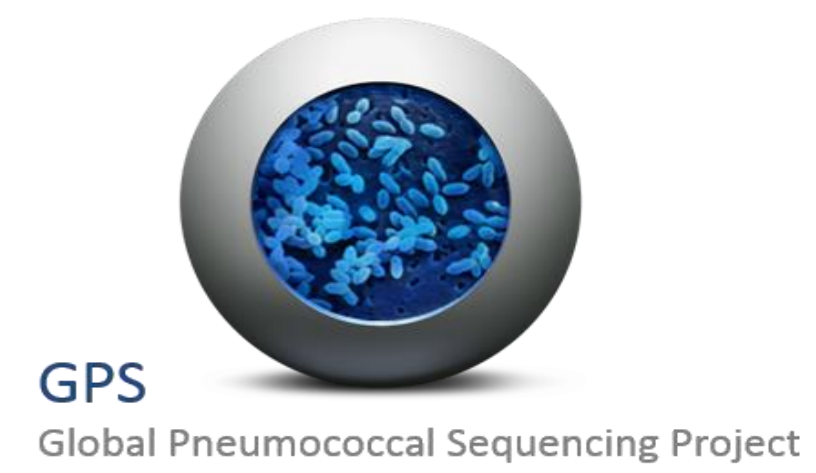


Molecular characterization of *Streptococcus pneumoniae* strains isolated from invasive disease in the pre- and post-PCV10 periods in Brazil, 2005 to 2015

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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.

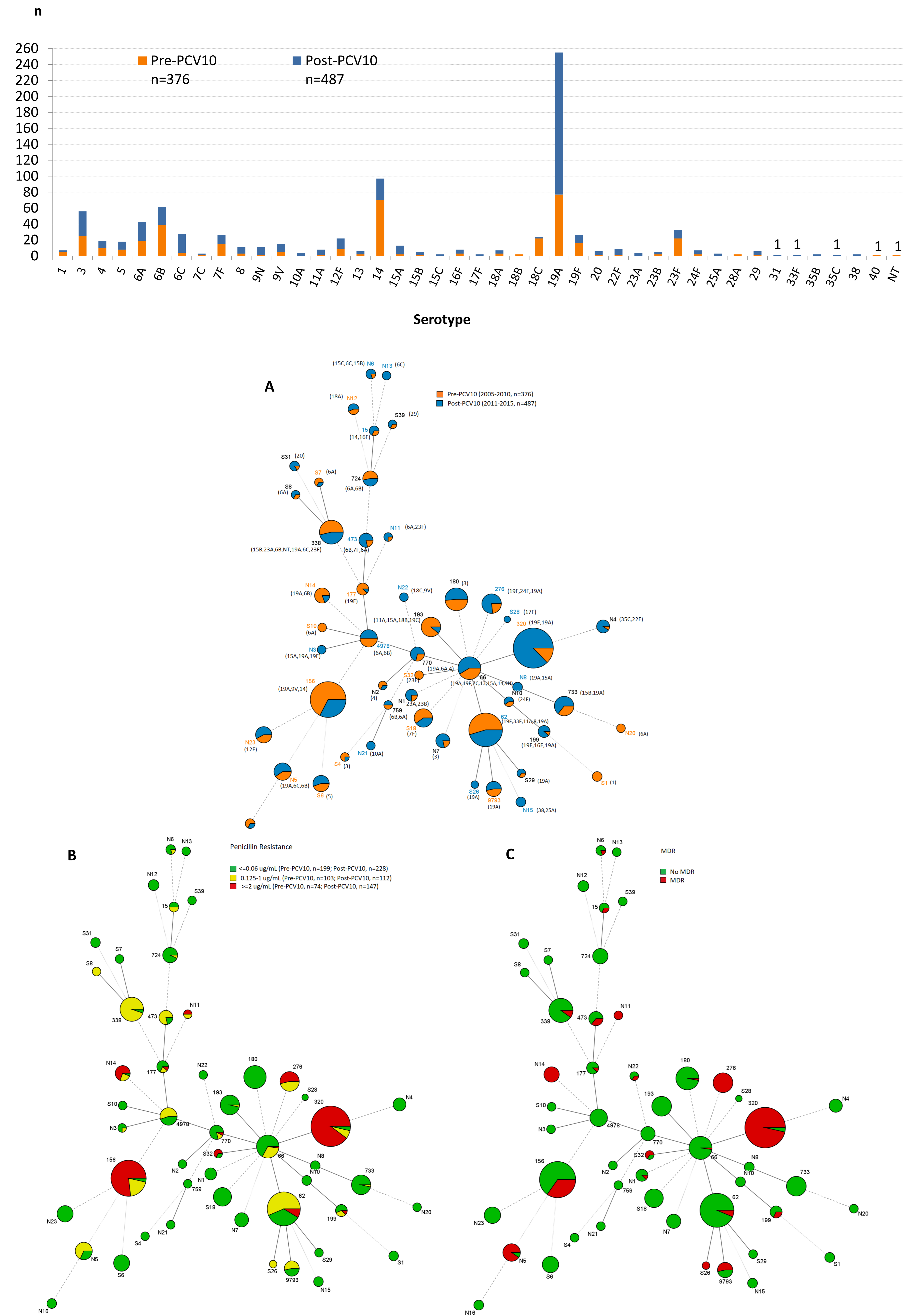
Methods

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 typing pipeline. The remaining 412 isolates were characterized by MLST, using primers published on PubMLST (<http://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 from 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.

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

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.