GENOMIC POPULATION OF *Streptococcus pneumoniae* 19A (SPN19A) ISOLATED IN INVASIVE DISEASE (IPD) AND NASOPHARYNGEAL CHILDREN CARRIAGE (NCC) IN PRE-PCV10 (2005-2009) AND POST-PCV10 (2011-2017) PERIODS IN BRAZIL

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BACKGROUND AND AIM

After PCV10 introduction into the national children's immunization program in 2010 in Brazil, Spn19A increased as cause of invasive pneumococcal disease (IPD) and nasopharyngeal children carriage (NCC)[1]. This study describes the genomic population structure of Spn19A strains before (2005-2007) and after (2011-2017) PCV10 introduction.

METHODS

Spn19A (n=454) collected from IPD [n=403, age groups <5-years (n=244) and ≥50-years (n=159)] through national laboratory surveillance, and from NCC [n=51, age group 1-<2-years] were MLST characterized (pre-PCV10: n=68, post-PCV10: n=386). Of these, 50 Spn19A (pre-PCV10: n=20, post-PCV10: n=30) were whole-genome sequenced (WGS). Antimicrobial resistance was defined by broth microdilution (CLSI, 2018).

RESULTS

Spn19A MLST (n=454) – IPD (n=403) and NCC (n=51) (figure 1):
- 8 clonal complexes (CCs) and 9 Singletons were identified
- Pre-PCV10 (n=68): predominance of CC1118 (51.5%, n=35)
- Post-PCV10 (n=386): predominance of CC320 (60.9%, n=235)

Spn19A WGS (n=50) – IPD (n=45) and NCC (n=5) (figure 2 and table 1):
- 11 GPSC clusters (GPSCs)[2] were identified
- GPSC1/CC320, prevalent in the post-PCV10 period and related to Spn19A expasion, was associated to high penicillin (MIC≥2.0µg/mL) and ceftriaxone (MIC≥1.0µg/mL) nonsusceptibility (87.5%, 14/16), showing mutations in the *pbp*1a+*pbp*2b+*pbp*2x genes in all strains.
- GPSC1/CC320 also presented cotrimoxazole mutations (*fol*A+*fol*P) and was the only lineage to presented the transposon Tn*2010,* which carry macrolide (*mefE+erm*B) and tetracycline (*tet*M) resistance genes, and both pilus-1 and pilus-2 genes.

Table 1. Antimicrobial resistance genes observed in Spn19A strains (n=50) whole-genome sequenced isolated from pre-PCV10 (n=20) and post-PCV10 (n=30) periods from IPD [n=45, <5y (n=28) and ≥50y (n=17)] and NCC [n=5, 1-<2y].

Antimicrobial agent	Resistance Genes	Pre-PCV10 (n=20) n (%)	Post-PCV10 (n=30) n (%)	Total (n=50) n (%)	CC / GPSC (n)
β-lactam	*pbp*1a+*pbp*2b+*pbp*2x	5 (25.0)	17 (56.7)	22 (44.0)	320/1(16), 276/10(3), 1118/204(1), 199/4(1), ST156/6(1)
	*pbp*2b+*pbp*2x	4 (20.0)	6 (20.0)	10 (20.0)	1118/204(4), 199/4(3), 9793/341(2), ST63/9(1)
	*pbp*2b	8 (40.0)	2 (6.7)	10 (20.0)	1118/204 (9), 1118/new (1)
	*pbp*1a	0 (0.0)	1 (3.3)	1 (2.0)	ST4913/16(1)
	*pbp*1a+*pbp*2b	0 (0.0)	1 (3.3)	1 (2.0)	387-1131/5(1)
	none	3 (15.0)	3 (10.0)	6 (12.0)	733/18(5), 9793/341(1)
Macrolide	*erm*B+*mef*E	3 (15.0)	13 (43.3)	16 (32.0)	320/1(16)
	*erm*B	2 (10.0)	5 (16.7)	7 (14.0)	276/10(3), 9793/341(2), 1118/204(1), ST63/9(1)
	mefE	0 (0.0)	3 (10.0)	3 (6.0)	199/4(3)
	none	15 (75.0)	9 (30.0)	24 (48.0)	1118/204(13), 733/18(5), 156/6(1), 1118/new(1), 9793/341(1), ST4913/16(1), 199/4(1), 387-1131/5(1)
Cotrimoxazole	*fol*A+*fol*P	17 (85.0)	23 (76.6)	40 (80.0)	320/1(16), 1118/204(10), 733/18(5), 9793/341(3), 199/4(2), 387-1131 (1), ST4913/16(1), ST156/6(1), 1118/new(1)
	*fol*P	2 (10.0)	5 (16.7)	7 (14.0)	276/10(3), 1118/204(2), 199/4(2)
	none	1 (5.0)	2 (6.7)	3 (6.0)	1118/204(2), ST63/9 (1)
Tetracycline	*tet*M	7 (35.0)	18 (60.0)	25 (50.0)	320/1(16), 276/10(3), 1118/204(2), 9793/341(2), ST63/9 (1), 1118/new(1)
	none	13 (65.0)	12 (40.0)	25 (50.0)	1118/204(12), 733/18(5), 199/4(4), 9793/341(1), 387-1131/5(1), ST156/6(1), ST4913/16(1)

*pbp*1a: amino acid substitution Thr_{371}→Ser/Ala and/or Pro_{342}→Thr; *pbp*2b: amino acid substitution Thr_{451}→Ala and/or Ala_{624}→Gly; *pbp*2x: amino acid substitution Thr_{338}→Ala, His_{394}→Leu and/or Leu_{546}→Val; *fol*A: amino acid substitution Ile_{100}→Leu; *fol*P: insertion or duplication of 1-2 amino acid between 58-68 positions. No strains showed the presence of *cat* gene and mutations in *rpo*B and *par*C genes.

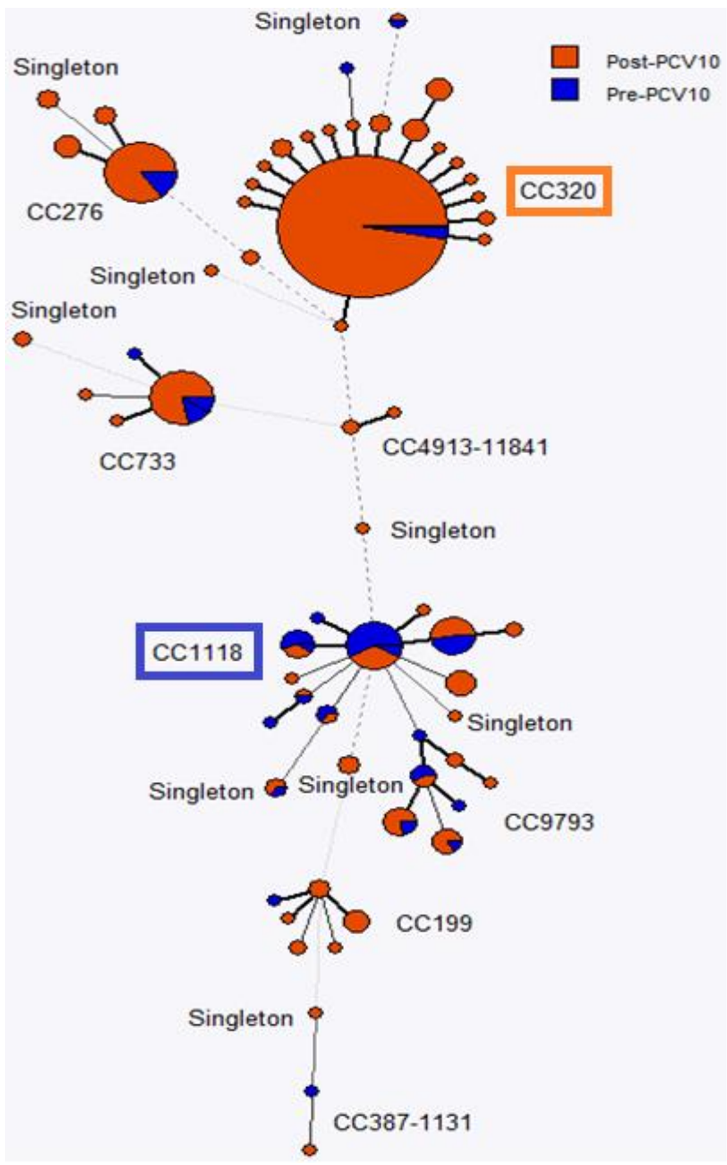

Figure 1. Minimal spanning tree (MST) of Spn19A strains (n=454) isolated from IPD [n=403, <5y (n=244) and ≥50y (n=159)] and NCC [n=51, 1-<2y] in pre-PCV10 (n=68) and post-PCV10 (n=386) periods constructed by BioNumerics software. Each circle represents a sequence type (ST); the size is proportional to the number of isolates within that particular ST.

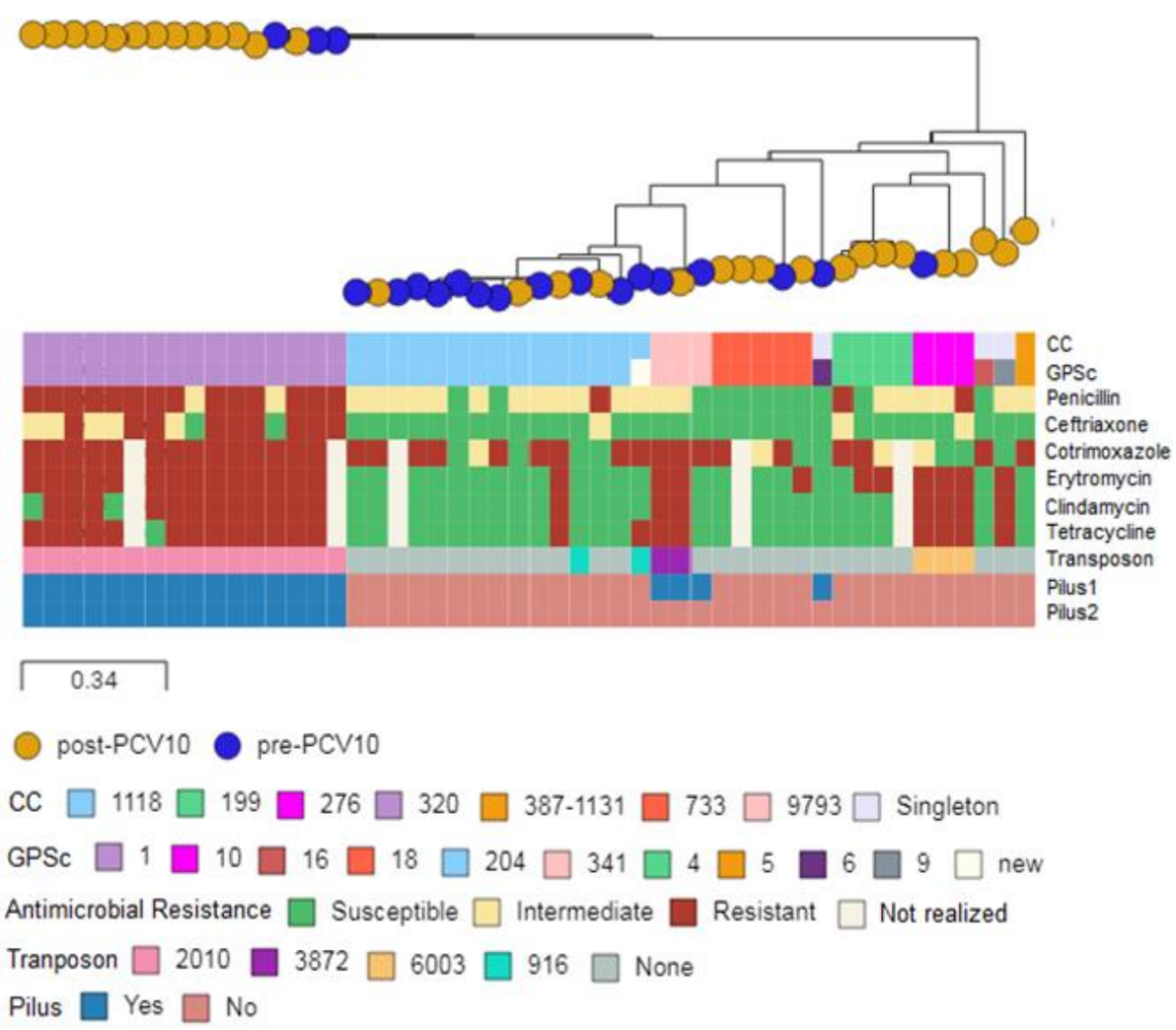

Figure 2. Comparison of Spn19A strains (n=50) whole-genome sequenced isolated from pre-PCV10 (n=20) and post-PCV10 (n=30) periods from IPD [n=45, <5y (n=28) and ≥50y (n=17)] and NCC [n=5, 1-<2y] constructed by Microreact.

CONCLUSIONS

The study showed the expansion of the lineage GPSC1/CC320-Spn19A-MDR after PCV10 introduction in Brazil. The presence of MDR strains, the transposon Tn*2010* and the pilus-1 and pilus-2 genes could have collaborate with the expansion of this lineage in the years after PCV10 introduction.

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