Genomic surveillance of invasive *Streptococcus pneumoniae* isolates (SPN) in Brazil, periods pre- (2008-2009) and post-PCV10 (2012-2013) introduction

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Background

In 2010, Brazil introduced PCV10 into the national children's immunization program. This study describes the genomic population structure of invasive SPN before and after PCV10 introduction.

Methods

As part of the Global Pneumococcal Sequencing (GPS) project, 466 (pre-PCV10:n=232, post-PCV10:n=234; <5-years old:n=310, ≥5-years old:n=156) invasive SPN collected through national laboratory surveillance were whole-genome sequenced.

Results

The study identified 65 GPS clusters (GPSCs): 49 (410/466, 88%) were GPS previously described (last updated April 2019, n=20,187 https://www.pneumogen.net/gps/assigningGPSCs.html) and 16 (56/466, 12%) were Brazilian clusters (GPSCs 204, 231, 249, 289, 311, 341, 392-394, 571, 573-575, 577, 702 and 811). Thirty-six GPSCs (55%) were non-PCV10 lineages, 18 (28%) PCV10 and 11 (17%) PCV10/non-PCV10 (GPSCs 1, 5, 10, 11, 13, 16, 18, 23, 47, 61 and 231). Eight globally-spreading lineages recognized in the previous GPS study (Gladstone et al., 2019) were found in the collection with a prevalence of 3.9% (18/466, GPSC1), 17.8% (83/466, GPSC6), 1.5% (7/466, GPSC7), 5.6% (26/466, GPSC12), 9.0% (42/466, GPSC16), 2.6% (12/466, GPSC18), 1.5% (7/466, GPSC23) and 3.0% (14/466, GPSC32), respectively, with an overall frequency of 44.9%. In both periods, the most frequent lineage was GPSC6/CC156/PMEN3/14-9V. Post-vaccine non-PCV10 lineages GPSC16/CC66/9N-15A, GPSC12/CC180/PMEN3/3 and GPSC32/CC218/PMEN24/12F increased; in <5-years old, GPSC1/CC320/DLV-PMEN14/19A, GPSC47/CC386/DLV-PMEN20/6C and GPSC51/CC458/3; and ≥5-years old GPSC3/CC53/PMEN33/8 were predominant (Figure-1).

SPN penicillin nonsusceptibility was predicted in 40%; 127 PBP combinations were identified (51 predicted MIC≥0.125 mg/L); cotrimoxazole (*fol*A+*fol*P alterations), macrolide (*mef*/*erm*B/*erm*B+*mef*) and tetracycline (*tet*M/*tet*O/*tet*S/M) resistance were predicted in 46%, 13% and 21% SPN, respectively (Table-1). In <5-years old, a reductions in penicillin (p=0.0169) and cotrimoxazole (p<0.0001) resistance and an increase in tetracycline (p=0.019) were observed. Post-PCV10, PBP15-12-18 (MIC=2mg/L) was frequent in lineage GPSC6/CC156/PMEN3/14-9V; among <5-years old the PBP13-11-16 (MIC=4mg/L) in GPSC1/CC320/DLV-PMEN14/19A and PBP2-53-77 (MIC=0.125mg/L) in GPSC47/ST386/DLV-PMEN20/6C were predominant. Compared pre-PCV10 period, no significant changes in PCV10/non-PCV10 serotypes was observed, nor any significant changes in antibiotic resistance per lineage.

In the GPSC47/CC386/DLV-PMEN20/6C lineage in the <5-year-olds was identified a possible switch recombination that resulted in a change of MDR 6B to 6C and dating revealed the event was established before vaccine implementation (1994.07 [1990.16-1997.63]) with a selection in the post-PCV10 (Figure-2).

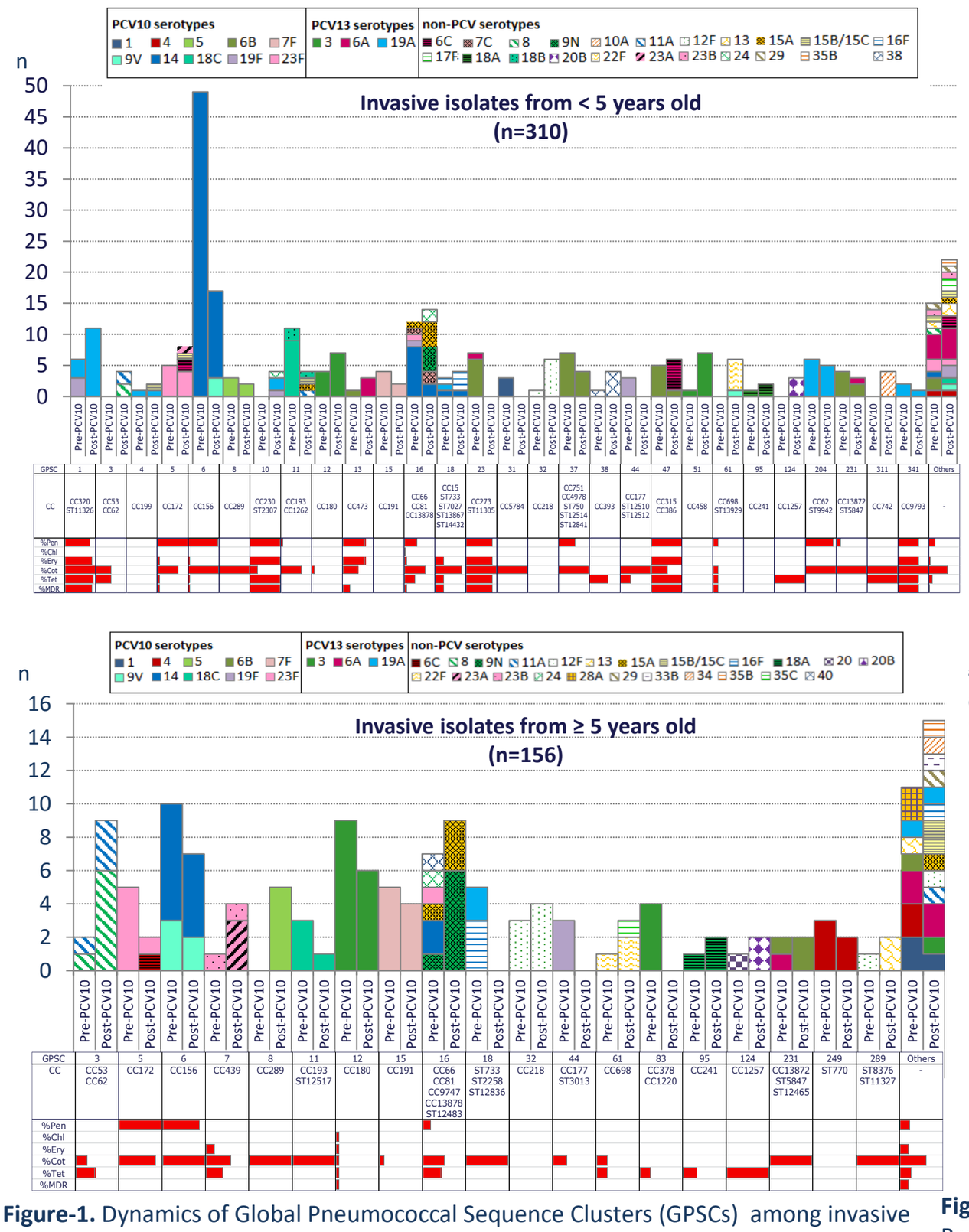

Figure-1. Dynamics of Global Pneumococcal Sequence Clusters (GPSCs) among invasive isolates from children aged <5-years old and ≥5-years old over vaccine periods in Brazil. The number of invasive pneumococcal disease is plotted by GPSC, with stratification into two vaccine periods (pre-PCV and post-PCV10), MLST clonal complex (CC) and antibiotic resistance pattern; and coloured by serotypes. The PCV10 and PCV13 serotypes are represented by solid fill while non-PCV serotypes by coloured hatched patterns.

Table-1. Antimicrobial resistance genes determinants from pneumococcal isolates in pre and post-PCV10 periods, Brazil

Antibiotic	Resistance Genes	Age <5 years (N=310)		Age ≥5 years (N=156)		Total (N=466)
		Pre-PCV10 (n=155) n (%)	Post-PCV10 (n=155) n (%)	Pre-PCV10 (n=77) n (%)	Post-PCV10 (n=79) n (%)	n (%)
β-lactam[a]	*pbp* 1A-2B-2X	89 (57)	66 (43)	18 (23)	13 (17)	186 (40)
Macrolide	*mef*	2 (1)	6 (4)	1 (1)	1 (1)	10 (2)
	*erm*B	15 (10)	15 (10)	2 (3)	3 (4)	35 (8)
	*erm*B+*mef*	2 (1)	11 (7)	0 (0)	1 (1)	14 (3)
	*rpl*D2	0 (0)	1 (1)	0 (0)	0 (0)	1 (0,2)
Cotrimoxazole	*fol*A	6 (4)	3 (2)	3 (4)	2 (3)	14 (3)
	*fol*P	24 (15)	19 (12)	12 (16)	10 (13)	65 (14)
	*fol*A+*fol*P	104 (67)	59 (38)	27 (35)	24 (30)	214 (46)
Tetracycline	*tet*O	0 (0)	0 (0)	0 (0)	2 (3)	2 (0,4)
	*tet*M	24 (15)	47 (30)	11 (14)	15 (19)	97 (21)
	*tet*S+*tet*M	0 (0)	1 (1)	0 (0)	0 (0)	1 (0,2)
Chloramphenicol	*cat*	1 (1)	0 (0)	1 (1)	1 (1)	3 (1)
Rifampin	*rpo*B1	0 (0)	1 (1)	2 (3)	0 (0)	3 (1)
Fluoroquinolone	*par*C	1 (1)	0 (0)	1 (1)	1 (1)	3 (1)

[a]Penicillin resistance prediction MIC≥0,125mg/L (51/127); Post-PVC10 period PBP15-12-18 GPSC6/CC156/PMEN3/14-9V (n=7 <5-years; n=4 ≥5-years); PBP13-11-16 GPSC1/CC320/DLV-PMEN14/19A (n=11 <5-years; n=1 ≥5-years) and PBP2-53-77 GPSC47/ST386/DLV-PMEN20/6C (n=6 <5-years).

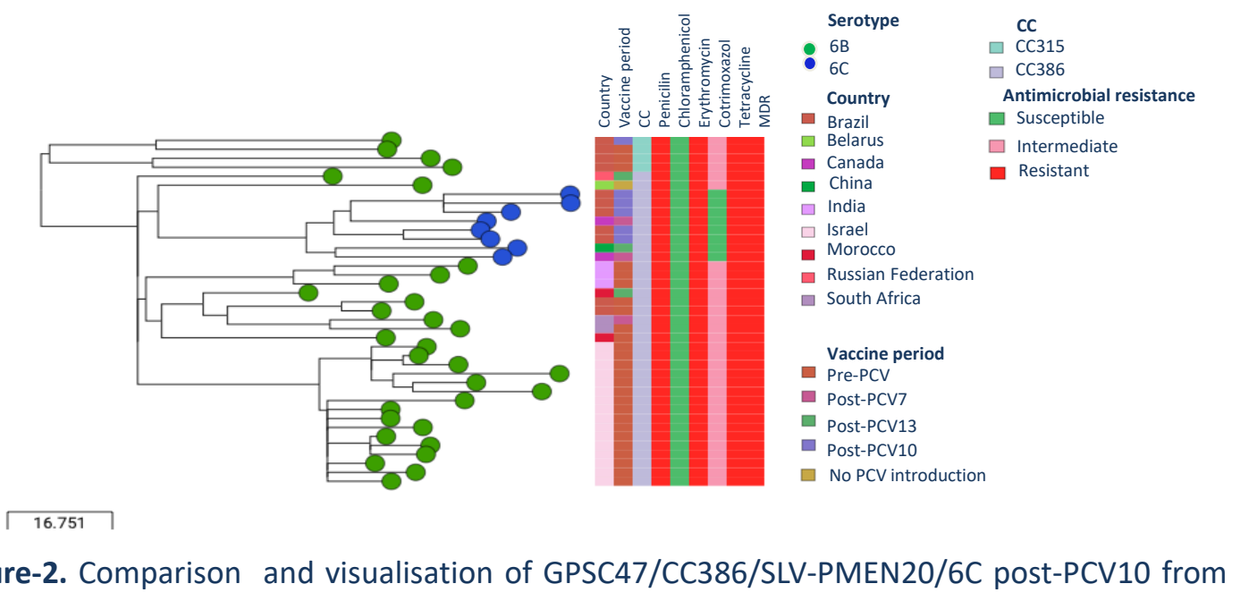

Figure-2. Comparison and visualisation of GPSC47/CC386/SLV-PMEN20/6C post-PCV10 from Brazil and other countries in the GPS project database in Microreact. To date the possible capsular switches were used the BactDating software with 100,000,000 generations on the output of Gubbins for the GPSC47 from Gladstone et al., 2019. The time to the most recent common ancestor (tMRCA) of GPSC47 is estimated at 1895.07 [1816.07-1937.83]. The tMRCA for the 6B and 6C isolates is estimated at 1994.07 [1990.16-1997.63] and the tMRCA between the 6C isolates is 1999.99 [1996.67 – 2002.91].

Conclusions

Post-PCV10, important non-PCV10 lineages, GPSC1/CC320/DLV-PMEN14/19A and GPSC47/ST386/DLV-PMEN20/6C associated with multidrug resistance and GPSC12/CC180/PMEN31/3, GPSC3/CC53/PMEN33/8 and GPSC32/CC218/PMEN24/12F were identified in Brazil.

Bibliography: Gladstone RA, Lo SW, Lees JA, Croucher NJ, van Tonder AJ, Corander J, et al. International genomic definition of pneumococcal lineages, to contextualize disease, antibiotic resistance and vaccine impact. EBioMedicine [Internet]. 2019;43:338–46. Available from: https://doi.org/10.1016/j.ebiom.2019.04.021.

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