



Assessment of Virulence Factors and Antimicrobial Resistance Patterns in the 7 Unique V116 Pneumococcal Vaccine Serotypes 15A, 16F, 23A, 23B, 24F, 31, 35B in Canada: CANWARD 2011-2020

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Introduction

V116 is a novel vaccine formulation approved for use in adults, a pneumococcal conjugate vaccine (PCV) covering some serotypes that were first included in **PCV13** (3, 6A, 7F, 19A), **PCV15** (22F, 33F), **PCV20** (8, 10A, 15BC, 11A, 12F), and **PPSV23** (9N, 17F, 20), as well as 7 unique V116 serotypes (15A, 16F, 23A, 23B, 24F, 31, 35B). V116 has been shown to be safe, conferring non-inferior protection for shared serotypes, plus additional coverage of unique serotypes most prevalent in older age groups.^{1,2}

Building on our previous efforts to assess potential impact of the V116 formulation³, we assessed phenotypic and genomic characteristics of Canadian isolates, for the 10-year period 2011-2020, inclusive, as part of the CANWARD Study.

Materials and Methods

Bacterial isolates: Isolates from clinical samples were forwarded to Canadian Antimicrobial Resistance Alliance (CARA) in Winnipeg by participating Canadian provincial public health and hospital laboratories (see below).

Antimicrobial susceptibility testing (AST): AST was performed using reference CLSI broth microdilution⁴ with custom-designed in-house panels, as previously described.⁵ MICs were interpreted as susceptible, intermediate or resistant using CLSI MIC breakpoints⁶ for 7 antimicrobial agents (CHL, CLA, CLI, DOX, LEV, PEN and SXT). Isolates were defined as “susceptible” (S) when susceptible to all agents, “non-resistant” (NR) when intermediate for 1 or more agents, “resistant” (R) when resistant to 1/2 agents, and “multi-drug resistance” (MDR) or “extremely drug resistant” (XDR), when resistant to 3+/5+ agents, respectively.

Serotyping: Serotyping was performed by Quellung reaction (pool, group, type and factor) using commercial antisera (SSI Diagnostica; Statens Serum Institute, Copenhagen).⁷

Whole Genome Sequencing (WGS): A subset of isolates underwent WGS at DNA Core, National Microbiology Laboratory, analyzed using WADE (WGS Analysis and Detection of Molecular Markers, found at github.com/phac-nml/wade) for MLST and detection of molecular AMR determinants (genes: chloramphenicol acetyl-transferase *cat*, rRNA methyltransferase *ermB*, macrolide efflux protein *mefAE*, ribosomal protection protein *tetM*; variants: dihydrofolate reductase *folA*, dihydropteroate synthase *folP*, DNA gyrase *gyrA*, DNA topoisomerase IV *parC*, and penicillin binding proteins *pbp1a*, *pbp2b*, *pbp2x*), and virulence (pneumolysin *ply*, autolysin *lytA*, and pilus islets **PI-1/PI-2**).

Global Pneumococcal Sequence Type (GPSC): GPSC was determined by MLST searches at www.pneumogen.net.

Statistical Analysis: Statistical differences between isolate subgroups were assessed by Fisher’s exact test.

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Results

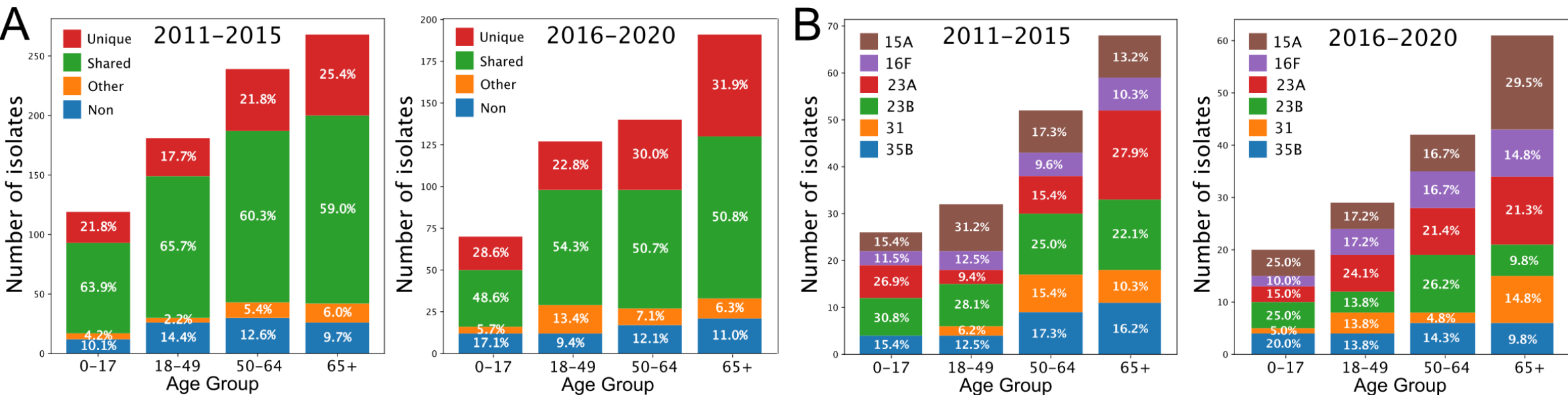


Figure 1: Distribution of V116-Unique Serotype Isolates by Age Group and 5-Year Time Period (2011-2015 and 2016-2020): **A)** All 1,338 serotyped isolates by V116 inclusion (**Unique** to V116, **Shared** with other formulations, in **Other** formulations only, and **Non** vaccine), **B)** Distribution of isolates from each of the V116-unique serotypes (N=330).

High Proportions of Serotype 15A and 35B Isolates Have AMR and Virulence Determinants: Of V116-unique serotypes, serotype 15A had the highest proportion of MDR/XDR isolates (36/67 or 53.8%, compared to 126/1,338 or 9.4% of all serotyped isolates; **p < 0.0001**). Compared to all sequenced isolates (N=517), serotype 15A had the highest proportion of isolates with presence of *ermB* (78.6% vs. 22.8%; **p < 0.0001**), *tetM* (80.4% vs. 23.2%; **p < 0.0001**), *pbp2b* (76.8% vs. 29.4%; **p < 0.0001**) and *pbp2x* (76.8% vs. 28.2%; **p < 0.0001**). High proportions of serotype 35B isolates with *pbp1a* (52.8% vs. 20.7%; **p < 0.0001**), *pbp2b* (52.8% vs. 29.4%; **p = 0.005**), *pbp2x* (47.2% vs. 28.2%; **p = 0.02**), and PI-1 (47.2% vs. 18%; **p < 0.0001**), compared to all sequenced isolates (N=517) (**Table 1**).

High Proportions of Serotype 15A and 35B Isolates Have Multiple AMR Determinants: Over 80% of serotype 15A isolates and nearly 50% of serotype 35B isolates had 3 or more AMR determinants, compared to 28% of all sequenced isolates; **p < 0.0001** and **p = 0.02**, respectively (**Figure 2**).

Table 1: Distribution of AMR and Virulence WGS Determinants in All Sequenced Isolates, All V116-Unique Isolates and Each V116-Unique Serotype

Determinant/Variant Present		All N=517		Unique N=264		15A N=56		16F N=36		23A N=56		23B N=57		31 N=23		35B N=36	
			%		%		%		%		%		%		%		%
AMR	<i>cat</i>	13	2.5	5	1.9	2	3.6	2	5.6	0	0	0	0	0	0	1	2.8
	<i>ermB</i>	118	22.8	63	23.9	44	78.6	3	8.3	13	23.2	1	1.8	1	3.2	1	2.8
	<i>mefAE</i>	79	15.3	17	6.4	3	5.4	1	2.8	1	1.8	3	5.3	1	3.2	8	22.2
	<i>tetM</i>	120	23.2	63	23.9	45	80.4	3	8.3	13	23.2	1	1.8	0	0	1	2.8
	<i>folA</i>	63	12.2	15	5.7	3	5.4	2	5.6	4	7.1	5	8.8	0	0	1	2.8
	<i>folP</i>	92	17.8	19	7.2	5	8.9	3	8.3	0	0	9	15.8	0	0	2	5.6
	<i>gyrA</i>	6	1.2	5	1.9	0	0	2	5.6	0	0	1	1.8	1	3.2	1	2.8
	<i>parC</i>	10	1.9	6	2.3	1	1.8	2	5.6	1	1.8	0	0	1	3.2	1	2.8
	<i>pbp1a</i>	107	20.7	53	20.1	17	30.4	2	5.6	13	23.2	2	3.5	0	0	19	52.8
	<i>pbp2b</i>	152	29.4	100	37.9	43	76.8	3	8.3	20	35.7	15	26.3	0	0	19	52.8
	<i>pbp2x</i>	146	28.2	80	30.3	43	76.8	6	16.7	13	23.2	1	1.8	0	0	17	47.2
Virulence	<i>lytA</i>	516	99.8	264	100	56	100	36	100	56	100	57	100	23	100	36	100
	<i>ply</i>	516	99.8	264	100	56	100	36	100	56	100	57	100	23	100	36	100
	PI1	93	18	22	8.3	5	8.9	0	0	0	0	0	0	0	0	17	47.2
	PI2	28	5.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Highlighted cells indicate statistical significance by Fisher’s exact test (p < 0.05).

Table 2: Detailed List of V116-Unique Serotype Isolates with AMR or Virulence Determinants from Older Adults (50+) (N=77)

Serotype	GPSC	Res. Pheno.	Gene present:			AMR-associated variant(s) present:					Present:	# isolates in group
			<i>ermB</i>	<i>mefAE</i>	<i>tetM</i>	<i>folA</i>	<i>folP</i>	<i>gyrA</i>	<i>parC</i>	<i>pbp</i> *	PI-1	
15A	--	R	Y		Y					Y		2
	--	MDR	Y		Y					Y		5
	6	S									Y	2
	6	R	Y								Y	1
	9	R	Y		Y					Y		5
	9	R	Y		Y		Y			Y		1
	9	MDR	Y		Y					Y		14
	140	S									Y	1
16F	135	R						Y	Y			1
	156	S								Y		2
	156	R		Y			Y			Y		1
	156	R						Y	Y	Y		1
23A	5	S								Y		1
	5	R		Y						Y		1
	5	R	Y		Y					Y		8
	5	R	Y		Y				Y	Y		1
	5	NR								Y		1
23B	--	NR								Y		1
	5	R				Y	Y			Y		1
	5	NR				Y				Y		1
	5	NR				Y		Y		Y		1
	5	NR								Y		6
	7	R		Y								1
	7	NR		Y								1
	13	NR		Y			Y			Y		1
31	57	R	Y									1
	57	R		Y								1
35B	--	NR								Y		1
	--	NR								Y	Y	1
	--	NR					Y			Y		1
	6	MDR		Y		Y	Y			Y	Y	1
	59	R		Y						Y	Y	3
	59	NR								Y	Y	5
	59	NR		Y						Y	Y	1
75	R						Y	Y			1	

*isolates positive for presence of AMR-associated variants in any of the three *pbp* genes (*pbp1a*, *pbp2b* and *pbp2x*) have been collapsed into a single variable in this table for cleaner display.

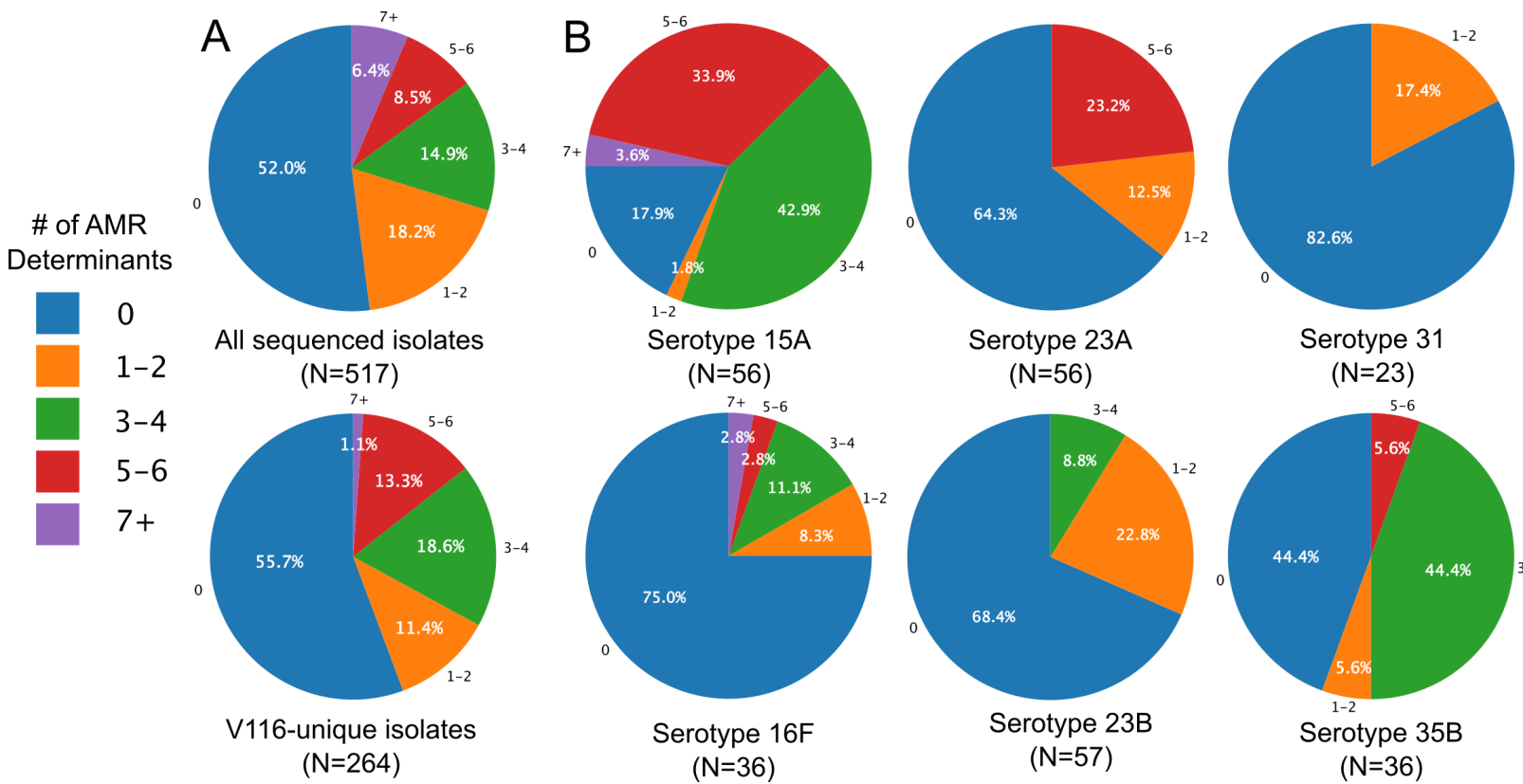


Figure 2: Distribution of Total Number of AMR Determinants Per Isolate: **A)** Total number of AMR determinants in all sequenced isolates (top) and for all V116-unique isolates (bottom). **B)** For each V116-unique serotype.

V116-Unique Serotypes 15A GPSC9 and 35B GPSC6 Are MDR: In older adults (50+), most serotype 15A isolates (28/34 or 85.2%) were R or MDR, from GPSC 6 and GPSC 9, as well as from MLST not currently assigned to GPSC. All had multiple AMR resistance determinants, including *ermB*, *tetM*, and *pbp* variants (**Table 2**). A smaller number of serotype 35B isolates from GPSC 6, GPSC 59 and GPSC 75 were R or MDR with presence of *mefAE* and *pbp* variants. Several serotype 23A GPSC 5 isolates were R, with *ermB*, *tetM* and *pbp* variants. The virulence determinant PI-1 was present in nearly half of serotype 35B isolates in adults aged 50 or older (11/24 or 45.9%), including R and MDR isolates.

Conclusions

V116 is an adult-specific pneumococcal vaccine containing 21 serotypes, of which V116-unique serotype isolates make up 330/1,338 or 24.7% of total pneumococcal isolates. These serotypes are most prevalent in older age groups (50+), particularly serotype 15A, making up nearly 30% of isolates in those aged 65 or older in the 2016-2020 period. Although no V116 isolates contained the virulence determinant PI-2 (present in 5.4% of all sequenced isolates), nearly half of serotype 35B isolates contained PI-1 (present in 18% of all sequenced isolates).