



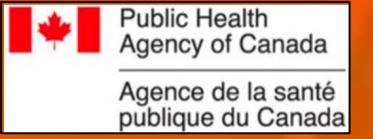
Epidemiology, Antimicrobial Resistance Patterns and Genetic Diversity of Novel PCV20 Pneumococcal Vaccine Serotypes 8, 10A, 11A, 12F, 15BC, 22F and 33F as Well as Serotypes 3 and 19A in Canada: CANWARD 2011-2020

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Introduction

PCV20 (Pevnar-20®) is a pneumococcal conjugate vaccine formulation approved for use in Canadian adults since May 2022 and in children older than 6 weeks since July 2023.¹ PCV20 covers serotypes included in PCV13 (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F) and 7 novel serotypes (8, 10A, 15BC, 11A, 12F, 22F and 33F.)

Several PCV20 serotypes have recently been shown to contribute to increased Canadian prevalence of invasive pneumococcal disease (IPD), based on 2023 data.² These include serotypes 3, 4, 9V and 22F, with increased multidrug resistance noted in serotype 19A.²

Therefore, the goal of this study was to assess demographics, antimicrobial resistance (AMR) and genetic determinants of AMR/virulence by whole genome sequencing (WGS), of isolates from the 7 novel PCV20 serotypes, plus PCV13 serotypes 3 and 19A, 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 as part of CANWARD, including demographic and clinical data (sex, age, region and infection source).

Antimicrobial susceptibility testing (AST): AST was performed using reference CLSI broth microdilution³ with custom-designed in-house panels, as previously described.⁴ MICs were interpreted as susceptible, intermediate or resistant using CLSI MIC breakpoints⁵ for 7 antimicrobial agents (chloramphenicol CHL, clarithromycin CLA, clindamycin CLI, doxycycline DOX, levofloxacin LEV, penicillin PEN and trimethoprim-sulfamethoxazole SXT). Isolates are defined as “susceptible” (S) if susceptible to all agents, “non-resistant” (NR) if intermediate for 1 or more agents, “resistant” (R) if resistant to 1/2 agents, and multidrug resistant (MDR) or extremely drug resistant (XDR), if 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).⁶

Whole Genome Sequencing (WGS): A subset of isolates from serotypes 15BC, 3 and 19 were analyzed by WGS at the DNA Core, National Microbiology Laboratory using WADE (WGS Analysis and Detection of Molecular Markers, found at github.com/phac-nml/wade) for MLST and molecular determinants of AMR (genes: chloramphenicol acetyltransferase *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 groups were assessed by Fisher’s exact test.

Acknowledgements

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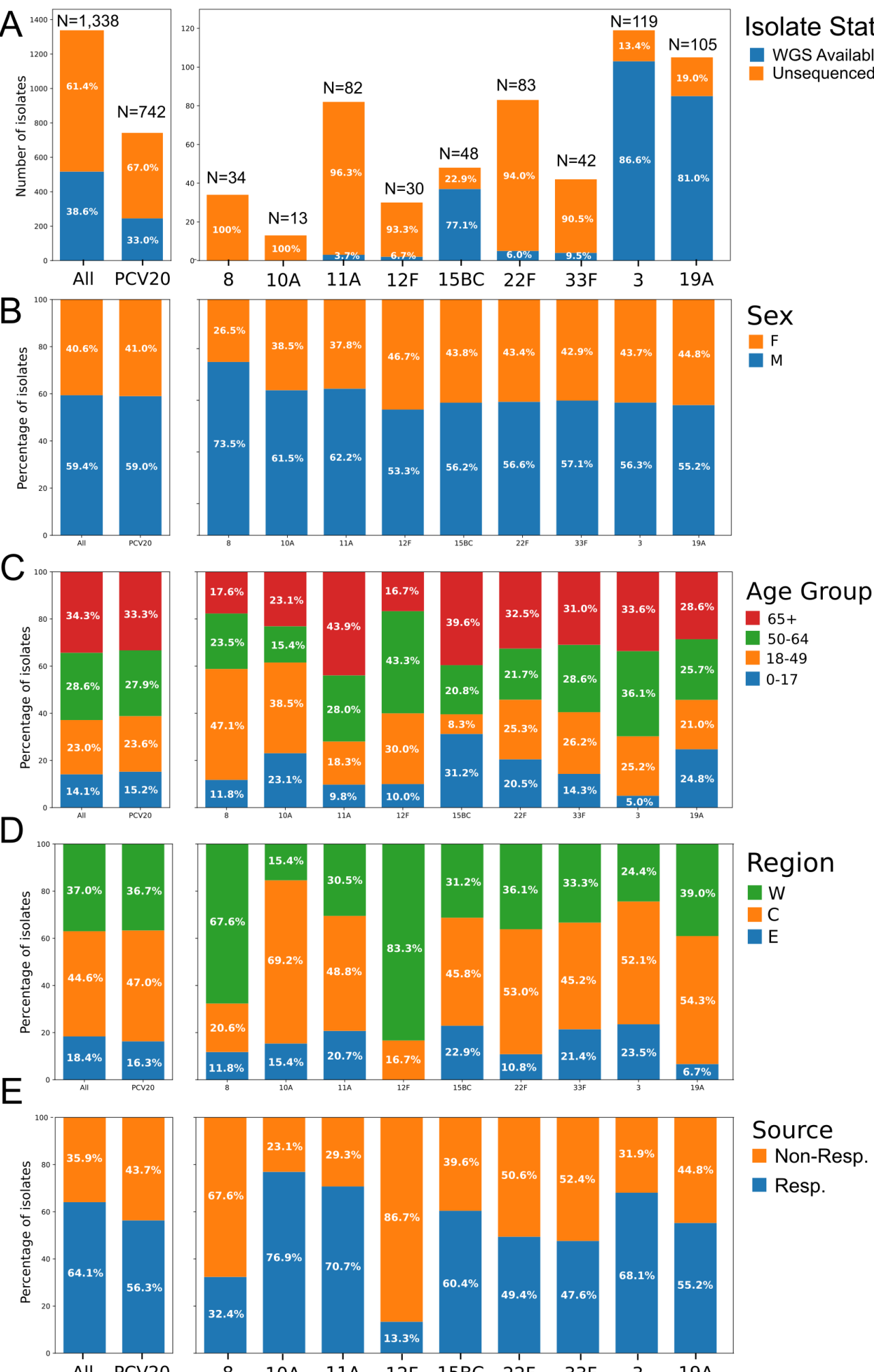


Figure 1: Epidemiology of Novel PCV20 Serotypes and Serotype 3 and 19A: Distribution of all isolates and all PCV20 serotype isolates (left panel) and nine PCV20 serotypes (right panel), by **A) Isolate Status** (WGS available or Unsequenced), **B) Sex** (Male, Female), **C) Age Group** (0-17, 18-49, 50-64, 65+), **D) Region** (West, Central, East), and **E) Source** (Respiratory, Non-Respiratory).

Results

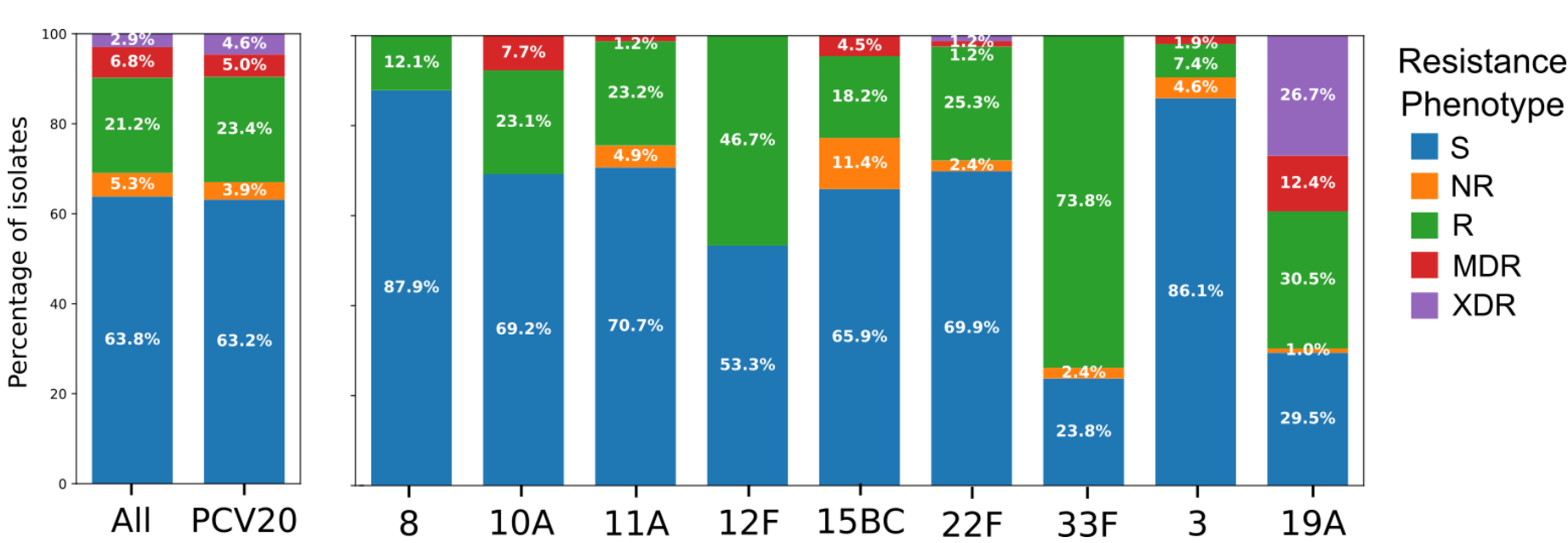


Figure 2: Antimicrobial Resistance Phenotype Profile of Novel PCV20 Serotypes and Serotype 3 and 19A: Profile of AMR for all isolates and all PCV20 isolates (left panel) and nine PCV20 serotypes.

Demographic and Clinical Characteristics of PCV20 Serotype Isolates: PCV20 serotype isolates make up 742/1,338 (55.4%) of all serotyped isolates, with WGS available for 77.1% of serotype 15BC, 86.6% of serotype 3 and 81.0% of 19A isolates (**Fig. 1A**). These three serotypes (with sufficient WGS available) are the focus of downstream WGS analyses. Serotypes 15BC and 19A were frequently observed in those under 18 (**Fig. 1C**). Serotypes 8 and 12F were frequently observed in Western Canada (**Fig. 1D**) and from non-respiratory clinical sources (**Fig. 1E**).

Resistance Phenotype Profile of PCV20 Serotype Isolates: All novel PCV20 serotypes contained isolates resistant to at least one agent, with serotypes 8 and 3 having the highest proportion of pan-susceptible (S) isolates (**Fig. 2**). The majority of serotype 19A and 33F isolates were resistant to at least one agent. 26.7% and 12.4% of serotype 19A isolates were MDR/XDR, respectively (p < 0.0001 vs. all PCV20 isolates). Serotype 22F was the only novel PCV20 serotype with XDR, with a single XDR isolate observed (1/83 or 1.2%) (**Fig. 2**).

Number of Genetic AMR and Virulence Determinants in Serotypes 15BC, 3 and 19A: The majority of serotype 3 isolates did not contain AMR determinants (91.3%), significantly lower than for all PCV20 isolates (p < 0.0001). The majority of serotype 15BC and 19A had at least one (78.4% and

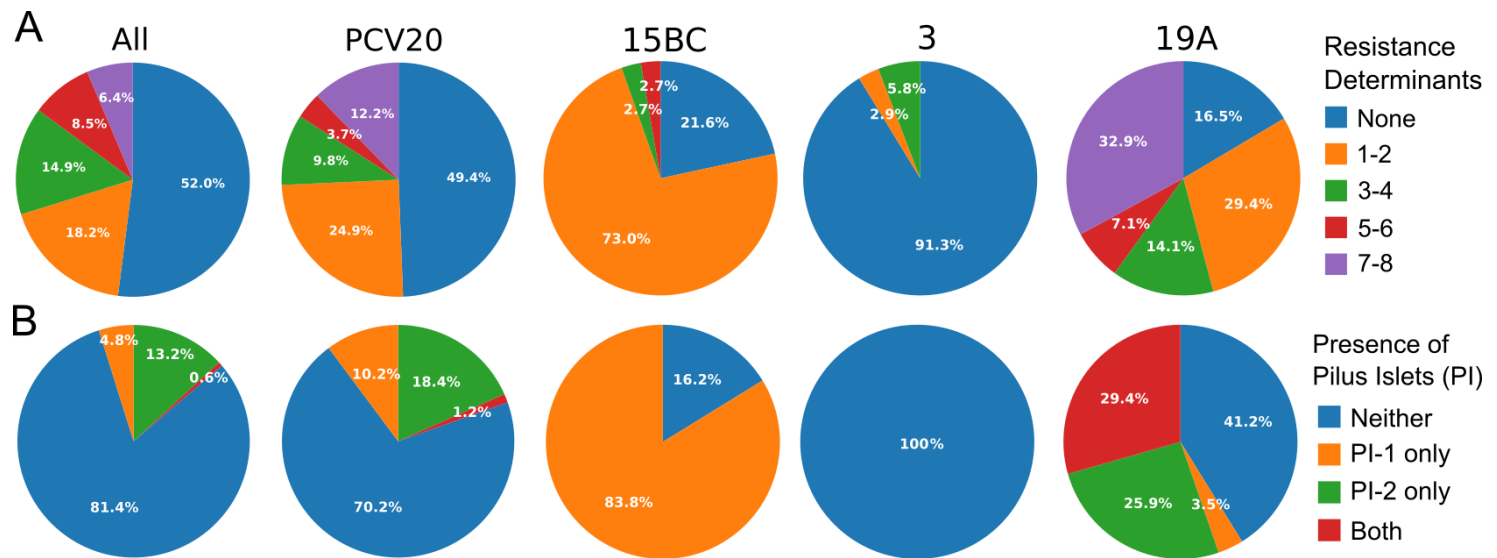


Figure 3: Profile of Genetic AMR and Virulence Determinants For Serotypes 15BC, 3 and 19A: **A)** Isolates by number of AMR/virulence determinants for all isolates, all PCV20 isolates and serotypes 15BC, 3 and 19A. **B)** Number of isolates with neither, only one of, or both virulence determinants Pilus Islet 1 and 2 (PI-1 and PI-2).

83.5% respectively), higher than for all PCV20 isolates (p < 0.0001 for both), while 32.9% of 19A isolates had 7 or more AMR determinants (**Fig. 3A**). Serotype 3 isolates contained no pilus islets, compared to 29.8% of all PCV20 isolates having one or both; 16.2% of serotype 15BC isolates contained PI-1 only. The majority of serotype 19A isolates (74.1%) contained PI-1 or PI-2 isolates, compared to 28.6% of all PCV20 isolates (p < 0.0001), and 3.5% contained both pilus islets, compared to 1.2% of all PCV isolates (**Fig. 3B**). All PCV20 novel serotypes and 3/19A isolates had virulence determinants *ply* and *lytA*.

Table 1: Presence of AMR and Virulence WGS Determinants in Sequenced Isolates from Serotypes 3, 15BC and 19A, by GPSC, MLST and MDR/XDR Phenotype

Serotype	GPSC	MLST	Total	MDR/XDR	%	Gene present								Variants present												Pilus Islet present					
						cat	%	ermB	%	mefAE	%	tetM	%	foIA	%	foIP	%	gyrA	%	parC	%	pbp1a	%	pbp2b	%	pbp2x	%	PI1	%	PI2	%
15BC	4	199	14	--	--	--	--	--	--	7	50.0	--	--	--	--	1	7.1	--	--	--	--	--	--	--	--	3	21.4	--	--	--	--
	11	1262	11	--	--	--	--	--	--	--	--	1	9.1	--	--	11	100	--	--	--	--	--	--	--	1	9.1	--	--	--	--	
	9	63	1	1	100	--	--	1	100	--	--	1	100	--	--	--	--	--	--	--	--	1	100	1	100	--	--	--	--		
	Other	Other	11	--	--	--	--	--	--	1	9.1	1	9.1	3	27.3	9	81.8	--	--	--	--	1	9.1	1	9.1	1	9.1	6	54.5	--	--
3	12	180	77	2	2.6	5	6.5	5	6.5	--	--	5	6.5	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	
	Novel	8561	14	--	--	--	--	--	--	--	--	--	--	--	--	1	7.1	1	7.1	--	--	--	--	--	--	--	--	--	--	--	
	Other	Other	12	--	--	1	8.3	1	8.3	1	8.3	1	8.3	2	16.7	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	
19A	1	320	24	24	100	--	--	24	100	24	100	24	100	24	100	24	100	--	--	2	8.3	24	100	24	100	24	100	21	87.5	24	100
		Other	4	4	100	--	--	4	100	4	100	4	100	4	100	4	100	--	--	--	--	4	100	4	100	4	100	4	100	4	100
		416	13	--	--	--	--	8	61.5	--	--	1	7.7	--	--	--	--	--	--	--	--	--	--	--	--	--	13	100	--	--	
	4	199	10	--	--	--	--	--	--	3	30.0	--	--	3	30.0	3	30.0	--	--	--	--	--	--	7	70.0	7	70.0	--	--	--	--
		Other	2	--	--	--	--	--	--	--	--	--	--	--	1	50.0	--	--	--	--	--	--	1	50.0	1	50.0	--	--	--	--	
	27	695	15	--	--	--	--	--	--	7	46.7	--	--	--	--	--	--	--	--	--	--	15	100	--	--	15	100	15	100	--	--
	Other	Other	17	6	35.3	--	--	9	52.9	1	5.9	10	58.8	3	17.6	4	23.5	--	--	--	--	5	29.4	7	41.2	4	23.5	7	41.2	--	--

Detailed Description of AMR and Virulence Determinants in Serotypes 15BC, 3 and 19A: Half of serotype 15BC (GPSC 4) isolates contained *mefAE* (**Table 1**). All 15BC (GPSC 11) contained *folP* variants, while 15BC (GPSC 9) had the only MDR/XDR isolate in this serotype and contained *ermB*, *tetM* and *pbp* variants. Several 15BC isolates with MLST from novel GPSC (8325, 8554, 8711), and GPSC 131/132, also contained *folP* variants. Most serotype 3 isolates (75/109,68.9%) were GPSC 12, including 2 (1.8%) MDR isolates. Serotype 3 had 14 isolates from MLST 8561

(novel GPSC), of which 1/14 (7.1%) had *gyrA* and *parC* variants. A small percentage of serotype 3 (GPSC 12) isolates (5/77 or 6.5%), and a single isolate (novel MLST), were the only PCV20 novel serotypes isolates with *cat* determinants. All serotype 19A (GPSC 1) isolates were MDR/XDR, containing *ermB*, *mefAE*, *tetM* genes, *folA*, *folP* and *pbp* variants and both pilus islets (**Table 1**). No serotype 19A (GPSC 4/GPSC 27) isolates were MDR/XDR. All serotype 19A MLST 2343 (novel GPSC) isolates and a single GPSC 9 isolate were MDR/XDR with *ermB/tetM* determinants.

Conclusions

A small proportion of PCV20 novel serotype isolates from serotypes 10A, 11A, 15BC and 22F are MDR/XDR. Serotype 3 isolates are rarely MDR, except for 2 isolates belonging to GPSC 12. Serotype 19A GPSC 1 isolates are all MDR/XDR, and contain a wide range of AMR determinants (genes and variants), as well as both pilus islets.