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Characterization of Multi-Drug Resistant (MDR) and Extensively-Drug Resistant (XDR) *Streptococcus pneumoniae* (SPN) in Canadian Hospitals, 2007-2013

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REVISED ABSTRACT

Objectives: Penicillin-resistant SPN are frequently MDR and increasingly XDR. The goal of this study was to characterize penicillin-resistant and MDR/XDR SPN strains collected from blood and respiratory infections across Canada in 2007-13.

Methods: SPN strains were obtained from Canadian hospitals as part of the CANWARD study. Antimicrobial susceptibility testing using CLSI methods identified MDR strains, defined as being resistant to penicillin (MIC ≥2 µg/mL) and at least two other structurally unrelated antimicrobial classes. MDR SPN were serotyped by the Quellung method, tested for genetic relatedness by PFGE/MLST, virulence due to the presence of pili (PI-1 and PI-2), macrolide resistance genes *mef*(A/E) and *erm*(B) and mutations in penicillin-binding proteins (PBP) 1A, 2B and 2X.

Results: A total of 61 (2.8 %, N=2143) MDR SPN were identified from 2007-13, isolated from sputum (45, 73.8%) and blood (16, 26.3%). 68.9% of MDR isolates were also XDR. The most common XDR phenotype included resistance to penicillin, macrolides, lincosamides, tetracyclines and trimethoprim-sulfamethoxazole. MDR SPN serotypes included 19A (34, 55.7%), 19F (12, 19.7%), 23F (6, 9.8%), 9V (3, 4.9%), 14 (2, 3.3%), 15A (2, 3.3%), 22F (1, 1.6%) and non-typeable (1, 1.6%). Serotypes 19A/F, 9V, 14 and 22F were associated with PI-1, while only 19A/F were associated with PI-2. Macrolide resistance was associated with the presence of *mef*(A/E) (18.0%), *erm*(B) (11.5%) or both (70.5%). PFGE/MLST identified clusters of MDR SPN isolates related to PMEN clones Spain^{23F}-1 (ST81), Spain^{9V}-3 (ST156), England^{14}-9 (ST9), Sweden^{15A}-25 (ST63) and Taiwan^{19F}-14 (ST236). Sequencing of PBP genes 1A, 2B and 2X revealed amino acid substitutions in key motifs.

Conclusions: MDR/XDR SPN in Canada are most often piliated serotype 19A/F, with resistance to 5 antimicrobial classes. MDR/XDR SPN possess dual *mef*(A/E)/*erm*(B) genes, altered PBPs and are associated with the spread of variants of well-described international clones across Canada. Continual surveillance is necessary to assess the evolution of MDR/XDR SPN in Canada.

BACKGROUND

Antimicrobial and multi-drug resistance (MDR) in *Streptococcus pneumoniae* is a growing concern. Escalation of antimicrobial resistance on a global scale is due to the dissemination of several resistant and MDR international clones [1]. Extensively-drug resistant (XDR) isolates are also beginning to emerge, demonstrating resistance to high numbers of antimicrobial classes. One such clone is ST320, commonly associated with *S. pneumoniae* serotype 19A. ST320 has demonstrated high-level resistance to penicillin, in addition to higher minimum inhibitory concentrations for other antimicrobials [2].

Clonal spread is of particular importance for the propagation of both β-lactam and macrolide resistance genes. Penicillin resistance in *S. pneumoniae* is due to mutations in penicillin-binding proteins (PBPs) 1A, 2B and 2X, which reduce the affinity for β-lactam antibiotics [3]. Two mechanisms of macrolide resistance include macrolide efflux, encoded by *mef*(A/E), as well as target site inactivation through ribosomal methylation, conferred by *erm*(B) [4]. These genes are responsible for low and high-level macrolide resistance, respectively. *S. pneumoniae* strains which possess the "dual" *mef*(A/E)/*erm*(B) phenotype often demonstrate MDR [4].

Serotypes that are commonly MDR, such as 19A and 19F, also possess pneumococcal pili. Pili encoded by the genetically distinct PI-1 and PI-2 regions have been shown to assist in the early stages of colonization and adherence [5].

The purpose of this study was to phenotypically and genotypically characterize MDR and XDR *S. pneumoniae* collected from Canada in 2007-13, to determine the incidence of *mef*(A/E) and *erm*(B) macrolide resistance genes and PI-1 and PI-2 pneumococcal pilus-encoding islands, as well as characterize mutations in PBPs 1A, 2B and 2X.

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MATERIALS & METHODS

Isolate Collection:
S. pneumoniae isolates from blood and respiratory cultures were collected as a part of the CANWARD study from 2007 to 2013, inclusive. Antimicrobial susceptibility testing was performed on 2,129 *S. pneumoniae* isolates using broth microdilution and methods described by the Clinical and Laboratory Standards Institute (CLSI) [6]. Minimum inhibitory concentrations were interpreted using CLSI criteria [7]. MDR was defined as resistance to ≥3 antimicrobial classes. Isolates resistant to ≥5 antimicrobial classes were considered XDR. Isolates which demonstrated both a penicillin MIC of ≥2 µg/mL (CLSI oral Penicillin V) and MDR or XDR were selected for further characterization. A total of 61 MDR and XDR *S. pneumoniae* isolates were collected in 2007-13.

MDR/XDR SPN Characterization:
MDR/XDR SPN were serotyped using the Quellung reaction, with pool, group, type and factor specific antisera (Statens Serum Institut, Copenhagen, Denmark). To determine genetic relatedness, pulsed-field gel electrophoresis (PFGE) was performed as previously described [8]. Gels were analyzed using BioNumerics Software v3.5 (Applied Maths Inc, Austin, TX). In addition, multi-locus sequence typing (MLST) was performed using primers and methods previously described at http://spneumoniae.mlst.net. Resulting PFGE fingerprints and MLST sequence types (STs) were compared to the Pneumococcal Molecular Epidemiology Network (PMEN) clone database. To assess putative virulence, PCR to determine the presence of pneumococcal pili was performed using previously described primers [5]. The presence of *mef*(A/E) and *erm*(B) macrolide resistance genes was determined as previously described [4]. PBP 1A, 2B and 2X genes were amplified by PCR for a selection of PRSP isolates, using previously described primers [3] and sequenced using an ABI PRISM 3100-Avant Genetic Analyzer. SeqScape Software v2.5 (Applied Biosystems) was used to assemble, edit and analyze sequences.

CONCLUSIONS

1. 2.8% of *S. pneumoniae* isolates collected in Canada in 2007-13 were MDR/XDR. Included were isolates of serotype 19A, 19F, 9V, 23F, 14, 15A, 22F or nontypeable.
2. 91.2% of MDR serotype 19A isolates were also XDR, with 2 isolates resistant to six different classes of antimicrobials, including quinolones.
3. Penicillin resistance was associated with mutations in key motifs of PBP 1A, 2B and 2X. Macrolide resistance was associated with a high frequency of the dual *mef*(A/E)/*erm*(B) phenotype.
4. MDR/XDR *S. pneumoniae* from Canada are associated with well-described international PMEN clones, England^{14}-9 (ST9), Spain^{23F}-1 (ST81), Spain^{9V}-3 (ST156), Sweden^{15A}-25 (ST63) and Taiwan^{19F}-14 (ST236).
5. Pneumococcal pilus PI-1 was associated with serotypes 19A, 19F, 9V, 14 and 22F. PI-2 was associated only with serotypes 19A and 19F. Isolates with P1-2 also demonstrated PI-1 presence.

REFERENCES

1. Lynch JP and Zhanel GG. *Semin Respir Crit Care Med*. 2009; **30**(2): 210-38.
2. Pillai DR, et al. *BMC Genomics*. 2009; **10**:642-2164-10-642.
3. Nichol, KA et al. *Antimicrob Agents Chemother*. 2002; **46**(10): 3261-4.
4. Wierzbowski, A et al. *J Antimicrob Chemother*. 2014; **69**(1): 59-66.
5. Zahner, D et al. *Emerg. Infect. Dis.* 2010; **16**(6): 955-62.
6. CLSI. *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. M7-9*. Wayne, PA. CLSI 2012.
7. CLSI. *Performance standards for antimicrobial susceptibility testing; 23rd informational supplement. M100-S23*. Wayne, PA. CLSI 2013.
8. McEllistrem, MC et al. *J Clin Microbiol.* 2000; **38**(1): 351-3.

RESULTS

Table 1. MDR and XDR phenotypes demonstrated by SPN collected from CANWARD 2007-13.

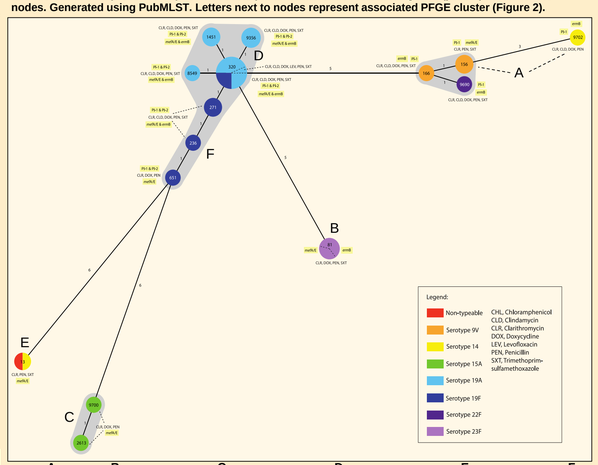
MDR/XDR Phenotype	Serotype (n)							
	9V	14	15A	19A	19F	22F	23F	NT
3 Classes:								
Clarithromycin, Doxycycline, Penicillin			1		1			
Clarithromycin, Penicillin, Trimethoprim-sulfamethoxazole	2	1		1				1
Doxycycline, Penicillin, Trimethoprim-sulfamethoxazole					1		2	
4 Classes:								
Clarithromycin, Clindamycin, Doxycycline, Penicillin		1	1					
Clarithromycin, Clindamycin, Penicillin, Trimethoprim-sulfamethoxazole				1	1			
Clarithromycin, Doxycycline, Penicillin, Trimethoprim-sulfamethoxazole				1			4	
5 Classes:								
Clarithromycin, Clindamycin, Doxycycline, Penicillin, Trimethoprim-sulfamethoxazole	1			29	9	1		
6 Classes:								
Clarithromycin, Clindamycin, Doxycycline, Levofloxacin, Penicillin, Trimethoprim-sulfamethoxazole				2				
Total Isolates	3	2	2	34	12	1	6	1

Overall, 2.8% (61) of isolates collected in 2007-13 were MDR/XDR. 42 (68.9%) MDR isolates were also considered XDR. 73.8% (45) of MDR/XDR SPN were isolated from sputum, while 26.2% (16) were isolated from blood. MDR/XDR SPN were isolated from all age groups and regions, and predominantly from men (41, 67.2%). Serotypes included 19A (34, 55.7%), 19F (12, 19.7%), 23F (6, 9.8%), 9V (3, 4.9%), 14 (2, 3.3%), 15A (2, 3.3%), 22F (1, 1.6%) and non-typeable (1, 1.6%). Macrolide resistance was associated with the presence of *mef*(A/E) (11, 18.0%), *erm*(B) (7, 11.5%) or both *mef*(A/E) and *erm*(B) (43, 70.5%). A good correlation between the presence of *erm*(B) and high-level macrolide resistance was observed (MIC >32 µg/mL). Serotypes 19A, 19F, 9V, 14, and 22F were associated with PI-1 (50, 82.0%), while only 19A and 19F were associated with PI-2 (45, 75.0%).

Table 2. Penicillin-binding protein (PBP) 1A, 2B and 2X conserved amino acid motif alterations in selected MDR/XDR SPN collected from CANWARD 2007-13.

Isolate Number	Year	PBP 1A			PBP 2B			PBP 2X			Penicillin MIC (µg/mL)
		STMK	SRNVP	KTG	SVVK	SSNT	KTGTA	STMK	AHSSNV	LKSGT	
71544	2007	-S--	----T	---	----	---A	----G	-A--	------	V----	2
72920	2007	-A--	----T	---	----	---A	-----	-A--	------	V----	2
74688	2007	-A--	----T	---	----	---A	-----	-A--	------	V----	2
80255	2008	-A--	----T	---	----	---A	-----	-A--	------	V----	2
80654	2008	-S--	----T	---	----	---A	----G	-A--	------	V----	8
81250	2008	-A--	----T	---	----	---A	-----	-A--	------	V----	2
82440	2008	-S--	----T	---	----	---A	----G	-A--	------	V----	4
86749	2009	-S--	----T	---	----	---A	----G	-AF-	------	V----	4
89945	2009	-S--	----T	---	----	---A	----G	-A--	------	V----	2
90078	2010	-A--	----T	---	----	---A	-----	-A--	------	V----	2
93289	2010	-S--	----T	---	----	---A	-----	-AF-	------	V----	4
94790	2010	-S--	----T	---	----	---A	----G	-A--	------	V----	8
98493	2011	-S--	----T	---	----	---A	----G	-A--	------	V----	4
98681	2011	-A--	----T	---	----	---A	-----	-A--	------	V----	2
101121	2012	-S--	----T	---	----	---A	----G	-A--	------	V----	4
103978	2013	-A--	----T	---	----	---A	-----	-A--	------	V----	2
104505	2013	-S--	----T	---	----	---A	-----	-A--	------	V----	4
105063	2013	-A--	----T	---	----	---A	-----	-A--	------	V----	2

Figure 1. Minimum spanning tree of MLST clonal clusters (CCs) and sequence types (STs). ST indicated in middle of node; lines infer relatedness by stating the number of differing alleles between each ST; node size proportional to number of isolates tested; grey border around nodes indicates a CC; resistance to antimicrobials and presence of pneumococcal pili and macrolide resistance genes (yellow) listed next to nodes. Generated using PubMLST. Letters next to nodes represent associated PFGE cluster (Figure 2).



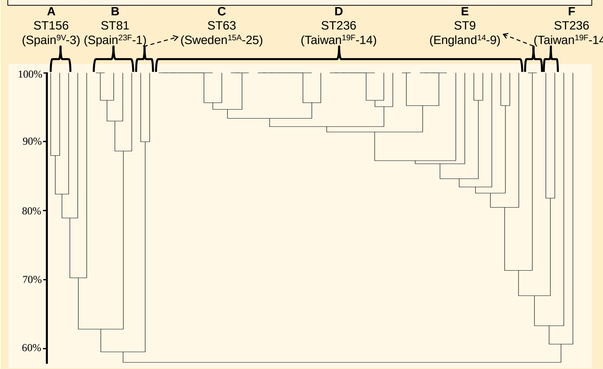


Figure 2. PFGE dendrogram of MDR/XDR SPN collected from CANWARD 2007-13, generated using BioNumerics. Bar on left indicates percent relatedness. Letters represent associated MLST clonal cluster (Figure 1); most closely related PMEN clone listed below letter.