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Antimicrobial Resistance in *Streptococcus pneumoniae* Isolated in Canada: Comparison of Blood and Respiratory Isolates, CANWARD 2007-2014

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ABSTRACT

Background: The goal of this study was to determine the serotype distributions and antimicrobial susceptibilities of *Streptococcus pneumoniae* (SPN) collected from blood and respiratory infections across Canada in 2007-2014.

Methods: *S. pneumoniae* strains were obtained from Canadian hospitals as part of the ongoing national surveillance study, CANWARD. SPN were serotyped using the Quellung method. Antimicrobial susceptibility testing was performed using the CLSI broth microdilution method. Multi-drug resistance (MDR) was defined as resistance to ≥3 structurally unrelated classes of antimicrobials.

Results: 2,309 SPN isolates were collected during the CANWARD 2007-14 study; 1,497 (65%) were obtained from respiratory samples and 812 (35%) from blood samples. The 10 most common serotypes from respiratory isolates were 3, 11A, 19A, 22F, 19F, 23A, 6C, non-typeable, 35B and 23B. The 10 most common serotypes from blood were 19A, 3, 22F, 7F, 4, 5, 12F, 11A, 8 and 9N. Antimicrobial susceptibilities were: 78% (respiratory isolates)/82% (blood isolates) for clarithromycin (CLR), 92/95% for clindamycin (CLD), 85/91% for doxycycline (DOX), 99/99% for levofloxacin, 82/89% for penicillin (PEN, oral penicillin V breakpoints), 84/87% for trimethoprim-sulfamethoxazole (SXT) and 99/100% for ceftriaxone (non-meningitis breakpoints). Isolates from blood demonstrated 4.4% MDR, primarily to CLR, CLD, DOX, PEN and SXT in serotype 19A. A variety of MDR patterns were observed in 8.6% of respiratory isolates, predominantly in serotypes 15A, 19A, 19F and non-typeable isolates. MDR in respiratory isolates was statistically significantly higher than in blood isolates (p<0.001).

Conclusions: The top 10 SPN serotypes causing respiratory versus blood infections in Canada in 2007-2014 were different, with the exception of 3, 11A, 19A and 22F. SPN from respiratory samples demonstrated lower antimicrobial susceptibilities and higher MDR in a greater diversity of serotypes than SPN isolated from blood.

BACKGROUND

Streptococcus pneumoniae is a Gram-positive pathogen responsible for a number of both noninvasive (pneumonia, otitis media) and invasive (meningitis, bacteremia) manifestations of disease [1]. Over 90 different capsular types of *S. pneumoniae* have currently been described; these serotypes differ in their ability to invade the bloodstream based on their ability to illicit an immune response, resist phagocytosis and avoid complement [2]. For this reason, invasive and noninvasive serotypes of *S. pneumoniae* tend to differ.

Episodes of invasive pneumococcal disease are often transient; however, pneumococcal carriage is frequently long-term. Due to this lengthy occupation of the nasopharynx, these noninvasive serotypes are exposed to prolonged antimicrobial pressure, which can lead to the selection of antimicrobial resistant strains. Studies have shown that there is a correlation between the frequency of serotype isolation from the nasopharynx and the likelihood of antimicrobial resistance [2].

The objective of this study was to determine the serotype distributions and antimicrobial susceptibilities of *S. pneumoniae* collected from blood and respiratory infections across Canada in 2007-2014.

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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 2014, inclusive. In brief, tertiary-care medical centres were asked to submit clinical isolates (consecutive, one per patient per infection site) from both inpatients and outpatients attending hospital clinics, emergency rooms, surgical/medical wards and intensive care units. Centres submitted their first 100 respiratory isolates and first 10 blood isolates per month for 10 months. Isolates were shipped to the coordinating laboratory (Health Sciences Centre, Winnipeg, Canada) where they were subcultured onto appropriate media and stocked in skim milk at -80°C.

Antimicrobial Susceptibility Testing and Serotyping

Antimicrobial susceptibility testing was performed on 2,309 *S. pneumoniae* isolates using broth microdilution and methods described by the Clinical and Laboratory Standards Institute (CLSI) [3]. Minimum inhibitory concentrations were interpreted using CLSI criteria [4]. MDR was defined as resistance to ≥3 antimicrobial classes. Isolates resistant to ≥5 antimicrobial classes were considered XDR. Serotyping was performed using the Quellung reaction, with pool, group, type and factor specific antisera (Statens Serum Institut, Copenhagen, Denmark).

CONCLUSIONS

1. The ten most common serotypes varied between respiratory and blood *S. pneumoniae*. Serotypes 3, 11A, 19A and 22F were common between respiratory and blood top ten serotypes.
2. Respiratory isolates demonstrated lower antimicrobial susceptibilities than isolates from blood. Respiratory isolates also demonstrated a higher percentage of MDR (8.6%) than blood isolates (4.4%), with more MDR patterns in a greater diversity of serotypes (17 vs. 10).
3. The most common MDR serotypes were 15A, 19A, 19F and non-typeable. Two serotype 19A isolates (one respiratory and one blood) were found to be XDR, with resistance to clarithromycin, clindamycin, doxycycline, levofloxacin, penicillin and trimethoprim-sulfamethoxazole.

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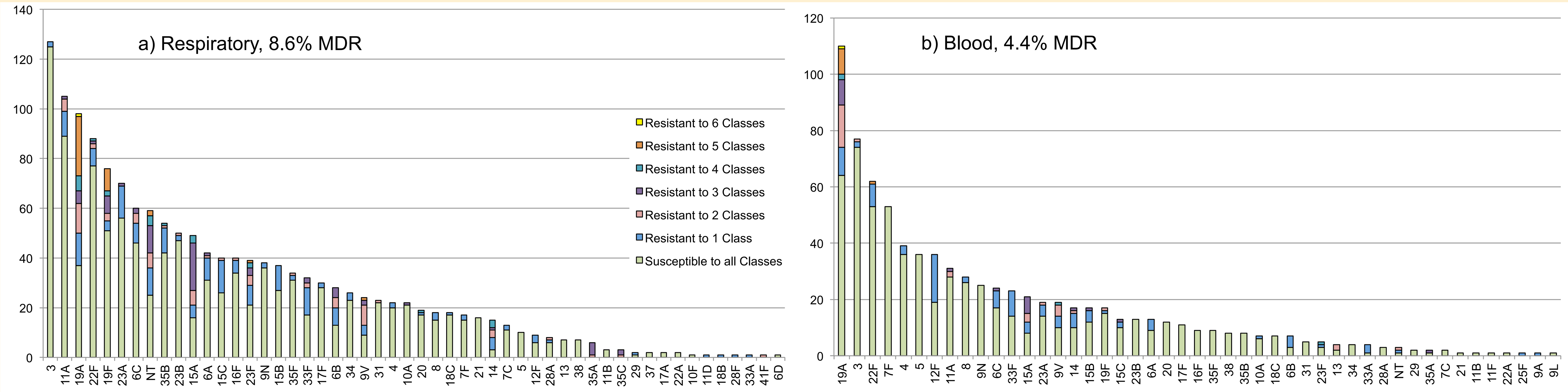
RESULTS

Figure 1. Serotypes identified from a) respiratory isolates and b) blood isolates; organized from most common to least common, broken down by number of isolates resistant to 0 – 6 classes of antimicrobials (β-lactams, macrolides, lincosamides, fluoroquinolones, tetracyclines and folate pathway inhibitors).



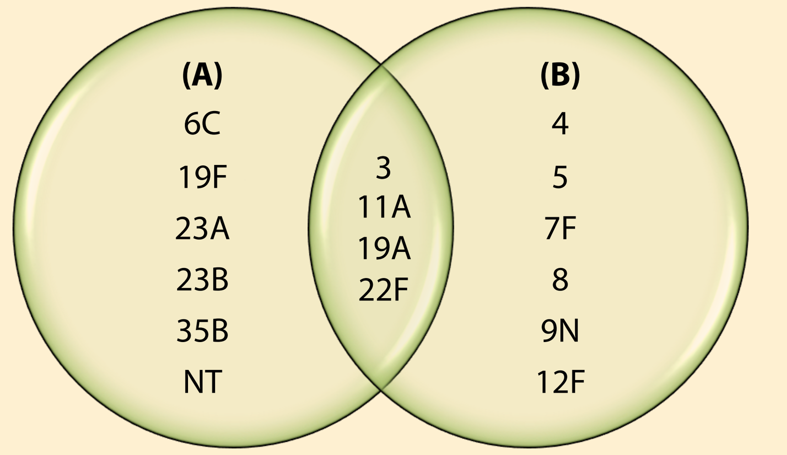


Figure 2. Differences between the ten most common serotypes demonstrated by (A) respiratory *S. pneumoniae* and (B) blood *S. pneumoniae*.

Of the 2,309 *S. pneumoniae* isolates collected in 2007-14, 1,497 (65%) were obtained from respiratory samples, while 812 (35%) were obtained from blood samples. The top 10 serotypes collected differed between respiratory and blood isolates (Figure 2). Respiratory isolates demonstrated 8.6% MDR, which was statistically significantly higher than blood isolates (4.4%, p<0.001). Ten blood serotypes were MDR, most commonly 15A and 19A. Seventeen respiratory serotypes were MDR, with 15A, 19A, 19F and non-typeable isolates being most common. Only serotype 19A demonstrated an XDR pattern.

Table 2. Antimicrobial susceptibilities of *S. pneumoniae* collected from respiratory and blood samples.

	Antimicrobial Susceptibilities (%)	
	Respiratory	Blood
Clarithromycin	77.7	81.6
Clindamycin	91.7	95.0
Ceftriaxone (meningitis)	99.1	99.7
Ceftriaxone (non-meningitis)	99.7	100
Doxycycline	85.1	90.9
Levofloxacin	98.7	99.3
Penicillin (meningitis)	80.6	87.8
Penicillin (non-meningitis)	99.8	100
Trimethoprim-sulfamethoxazole	83.5	87.4

Table 2. Respiratory and blood serotypes demonstrating MDR (resistance to 3 or more antimicrobial classes) or XDR (resistance to 5 or more antimicrobial classes, italicized) patterns.

MDR/XDR Pattern(s) in Respiratory Isolates	Number of Respiratory Isolates	Serotype	Number of Blood Isolates	MDR/XDR Pattern(s) in Blood Isolates
CLR, CLD, DOX (7)	7	**6A/B/C**	1	CLR, CLD, DOX (1)
CLR, PEN, SXT (2) *CLR, CLD, DOX, PEN, SXT (1)*	3	**9V**	-	-
CLR, CLD, DOX (1)	1	**11A**	1	CLR, CLD, DOX (1)
CLR, CLD, DOX, LEV (1) CLR, CLD, DOX, PEN (1) CLR, CLD, DOX, SXT (1)	3	**14**	1	CLR, PEN, SXT (1)
CLR, CLD, DOX (17) CLR, DOX, PEN (1) CLR, DOX, SXT (1) CLR, CLD, DOX, PEN (2)	21	**15A/B/C**	7	CLR, CLD, DOX (7)
CLR, CLD, DOX (7) CLR, DOX, PEN (2) CLR, DOX, SXT (2) CLR, CLD, DOX, SXT (4) CLR, CLD, PEN, SXT (2) CLR, DOX, PEN, SXT (1) *CLR, CLD, DOX, PEN, SXT (28)* *CLR, CLD, DOX, LEV, PEN, SXT (1)*	47	**19A/F**	21	CLR, CLD, DOX (6) CLR, DOX, PEN (2) CLR, PEN, SXT (1) CLR, CLD, DOX, SXT (1) CLR, DOX, PEN, SXT (1) *CLR, CLD, DOX, PEN, SXT (9)* *CLR, CLD, DOX, LEV, PEN, SXT (1)*
CLR, PEN, SXT (1) CLR, CLD, DOX, SXT (1)	2	**22F**	1	*CLR, CLD, DOX, PEN, SXT (1)*
CLR, CLD, DOX	7	**23A/F**	1	CLR, DOX, PEN , SXT (1)
CLR, CLD, DOX	2	**33F**	-	-
CLR, DOX, SXT (4) CLR, CLD, DOX, SXT (1)	5	**35A/B/C**	1	CLR, DOX, SXT (1)
CLR, CLD, DOX (9) CLR, DOX, SXT (1) CLR, PEN, SXT (1) CLR, CLD, DOX, SXT (2) CLR, DOX, LEV, SXT (2) *CLR, CLD, DOX, PEN, SXT (2)*	17	**NT**	-	-