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Comparison of Antimicrobial Resistance Patterns in *Streptococcus pneumoniae* from Blood and Respiratory Isolates in Canadian Hospitals from 2007-2016

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REVISED ABSTRACT

Objectives: The purpose of this study was to compare the epidemiology and antimicrobial susceptibility patterns of *S. pneumoniae* collected from respiratory and blood culture samples in Canada between 2007 and 2016.

Methods: *S. pneumoniae* strains were obtained from Canadian hospitals as part of the ongoing national surveillance study, CANWARD. *S. pneumoniae* 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 classes of antimicrobials (with penicillin resistance defined as MIC ≥ 2 ug/mL). Changes in antimicrobial susceptibility rates between specimen types were assessed for statistical significance (P <0.05) using the Fisher's test.

Results: 2,612 *S. pneumoniae* isolates were collected during the CANWARD 2007-16 study; 1713 (66%) were obtained from respiratory samples and 899 (34%) from blood samples. The 10 most common serotypes from respiratory isolates were 3, 11A, 19A, 22F, 23A, 19F, 6C, non-typeable, 15A and 23B. The 10 most common serotypes from blood were 19A, 3, 22F, 7F, 4, 12F, 11A, 5, 8 and 33F. Antimicrobial susceptibilities were: 77% (respiratory isolates)/81% (blood isolates) for clarithromycin (CLR) (P=0.04), 92/95% for clindamycin (CD) (P=0.0008), 85/91% for doxycycline (DOX) (P<0.0001), 99/99% for levofloxacin (P=0.1976), 80/88% for penicillin (PEN, oral penicillin V breakpoints) (P<0.0001), 84/87% for trimethoprim-sulfamethoxazole (SXT) (P=0.02) and 99/100% for ceftriaxone (non-meningitis breakpoints) (P=0.1043). Nine percent of respiratory isolates and 4.5% of blood isolates were MDR (P<0.0001). MDR was observed in 19 serotypes from the respiratory isolates and 12 serotypes from the blood culture isolates. The most common MDR serotypes (overall and in each specimen source) were 19A and 15A. The most common MDR pattern overall was resistance to CLR, CD, DOX while the most common serotype-MDR patterns were 15A resistant to CLR, CD, DOX and 19A resistant to CLR, CD, DOX, PEN, SXT.

Conclusions: Serotypes 3, 11A, 19A, and 22F were observed among the top 10 serotypes from both respiratory and blood culture isolates in Canada in 2007-2016; however, the other frequently isolated serotypes differed by specimen source. *S. pneumoniae* from respiratory samples demonstrated lower antimicrobial susceptibilities and higher MDR in a greater diversity of serotypes than *S. pneumoniae* 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 purpose of this study was to compare the epidemiology and antimicrobial susceptibility patterns of *S. pneumoniae* collected from respiratory and blood culture samples in Canada between 2007 and 2016.

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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 2016, inclusive. In brief, tertiary-care medical centres were asked to submit clinically significant 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 pathogens of the year and first 10 blood culture 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 -80C.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed on 2612 *S. pneumoniae* isolates using custom-designed, in-house produced broth microdilution panels following the methodology and quality control described by the Clinical and Laboratory Standards Institute (CLSI) [3]. Minimum inhibitory concentrations were interpreted using CLSI criteria [4]. Multi-drug resistance (MDR) was defined as resistance to ≥3 antimicrobial classes (penicillin MIC ≥ 2 µg/mL).

Serotyping

Serotyping was performed using the Quellung reaction using pool, group, type and factor commercial antisera (Statens Serum Institute, Copenhagen, Denmark) and supplementary molecular serotyping was performed with the US Centre for Disease Control's PCR multiplex method (http://www.cdc.gov/ncidod/biotech/strep/pcr.htm). Isolates for which a serotype was not determined by PCR and a Quellung reaction was not observed were confirmed as *S. pneumoniae* by *rpo*B gene sequencing.

Statistical Analysis

Changes in antimicrobial susceptibility rates and differences in demograpics by specimen type were assessed for statistical significance (P <0.05) using the Fisher's test.

CONCLUSIONS

1. Gender differences were not observed for *S. pneumoniae* bacteremias; however, *S. pneumoniae* caused significantly more respiratory infections in males than females (P=0.0012).
2. 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.
3. Respiratory isolates demonstrated lower antimicrobial susceptibilities than isolates from blood. Respiratory isolates also demonstrated a higher percentage of MDR (9.1%) than blood isolates (4.5%) with more MDR patterns in a greater diversity of serotypes (19 vs. 12).
4. The most common MDR serotypes were 15A, 19A, 19F and non-typeable. MDR 19F and non-typeable isolates were only observed in respiratory specimens.
5. The most common MDR pattern overall was resistance to CLR, CD, DOX while the most common serotype-MDR patterns were 15A resistant to CLR, CD, DOX and 19A resistant to CLR, CD, DOX, PEN, SXT.

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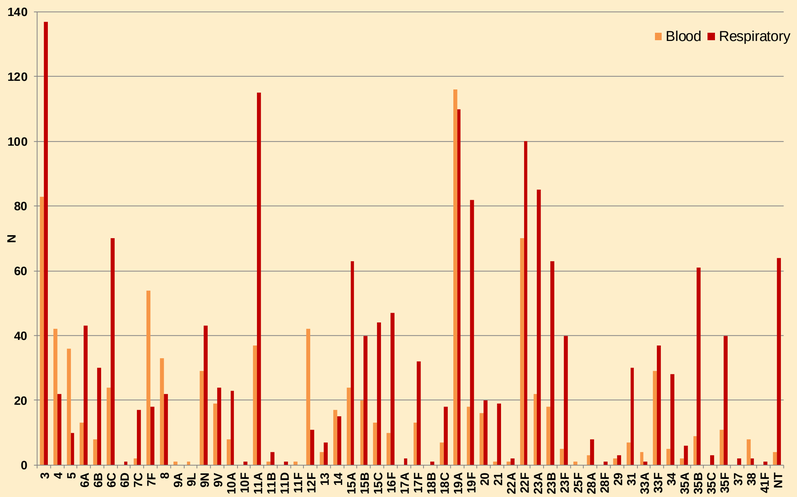
RESULTS

Figure 1. Serotype Distribution of *S. pneumoniae* Isolated from Respiratory and Blood Culture Specimens in Canadian Hospitals, 2007 - 2016



Demographics:

Of the 2,612 *S. pneumoniae* isolates collected in 2007-16, 1,713 (66%) were obtained from respiratory samples, while 899 (34%) were obtained from blood samples. The blood culture isolates were collected from 498 (55%) males and 401 (45%) females. By age, 161 (18%), 460 (62%) and 278 (31%), were isolated from individuals ≤17 years, 18-64 years and ≥65 years, respectively. The respiratory isolates were collected from 1062 (62%) males and 651 (38%) females. By age, 234 (14%), 874 (51%) and 605 (35%), were isolated from individuals ≤17 years, 18-64 years and ≥65 years, respectively.

Table 1. Antimicrobial Susceptibilities of *S. pneumoniae* Collected from Respiratory and Blood Culture Specimens

	Blood (n=860*)					Respiratory (N=1636*)					Differences in Susceptibility (P-value)
Antimicrobial Agent	%S	%I	%R	MIC_{50}	MIC_{90}	%S	%I	%R	MIC_{50}	MIC_{90}	
Ceftriaxone (non-meningitis)	99.8	0.2	0	≤0.12	≤0.12	99.2	0.6	0.3	≤0.12	0.12	NS
Ceftriaxone (meningitis)	98.6	1.2	0.3	≤0.12	≤0.12	96.6	2.6	0.8	≤0.12	≤0.12	0.004
Clarithromycin	80.8	3	16.2	≤0.03	2	77.2	3.8	19.1	≤0.03	4	0.04
Clindamycin	95.2	0.5	4.3	≤0.12	≤0.12	91.6	0.6	7.9	≤0.12	≤0.12	0.0008
Doxycycline	90.5	1.1	8.4	≤0.25	≤0.25	84.8	1.4	13.8	≤0.25	2	<0.0001
Levofloxacin	99.4	0.1	0.5	1	1	98.8	0.2	1	1	1	NS
Penicillin (non-meningitis)	99	1	0	≤0.03	0.12	98.1	1.7	0.2	≤0.03	0.5	NS
Penicillin (meningitis)	87.9	N/A	12.1	≤0.03	0.12	79.9	N/A	20.1	≤0.03	0.5	<0.0001
Penicillin (oral)	87.9	9.4	2.8	≤0.03	0.12	79.8	15.2	4.9	≤0.03	0.5	<0.0001
Trimethoprim-sulfamethoxazole	87.3	6.4	6.3	≤0.12	1	83.7	6.3	10	≤0.12	4	0.02
Vancomycin	100	N/A	N/A	≤0.25	0.25	100	N/A	N/A	≤0.25	0.25	NS

*, number of isolates which grew in broth for susceptibility testing; N/A, interpretative categories do not exist; NS, Non-significant

Multi-drug Resistance:

Respiratory isolates demonstrated significantly higher rates of multi-drug resistance than isolates from blood culture specimens (9.1% vs. 4.5%, P <0.0001). MDR in blood culture isolates was observed in twelve serotypes, most commonly 15A and 19A. Among respiratory specimens, MDR was observed in 19 serotypes with 15A, 19A, 19F and non-typeable isolates being most common. The serotype-MDR patterns are presented in detail in Table 2.

Figure 2. Differences Between the Ten Most Common Serotypes Demonstrated by *S. pneumoniae* from Respiratory and Blood Culture Specimens

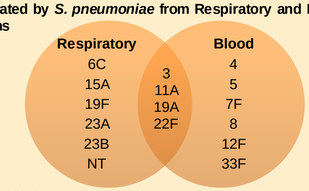


Table 2. Multi-drug Resistance in *S. pneumoniae* from Respiratory and Culture Specimens by Serotype (ST) in Canada, 2007-2016

ST	MDR Isolates (N / % by Specimen Source)		MDR Pattern(s) (N)		
	Blood	Respiratory		Blood	Respiratory
6A/B/C	1 (0.12%)	8 (0.5%)	CLR, CD, DOX	1	7
			CLR, DOX, SXT	0	1
9N	1 (0.12%)	0	CLR, DOX, SXT	1	0
9V	1 (0.12%)	3 (0.2%)	CLR, PEN, SXT	0	2
			CLR, CD, DOX, SXT	1	0
			CLR, CD, DOX, PEN, SXT	0	1
10A	0	1 (0.06%)	CLR, CD, DOX	0	1
11A	1 (0.12%)	1 (0.06%)	CLR, CD, DOX	1	1
14	1 (0.12%)	4 (0.25%)	CLR, PEN, SXT	1	1
			CLR, CD, DOX, PEN	0	1
			CLR, CD, DOX, SXT	0	1
			CLR, CD, DOX, LEV	0	1
15A	6 (0.7%)	31 (1.9%)	CLR, CD, DOX	6	27
			CLR, DOX, PEN	0	1
			CLR, CD, DOX, PEN	0	3
15B/C	2 (0.24%)	0	CLR, CD, DOX	2	0
19A	21 (2.5%)	40 (2.5%)	CLR, CD, DOX	6	5
			CLR, DOX, PEN	2	0
			CLR, PEN, SXT	1	0
			CLR, CD, DOX, SXT	1	5
			CLR, DOX, PEN, SXT	1	3
			CLR, CD, PEN, SXT	0	1
			CLR, CD, DOX, PEN, SXT	9	25
			CLR, CD, DOX, LEV, PEN, SXT	1	1
19F	0	20 (1.24%)	CLR, CD, DOX	0	3
			CLR, DOX, PEN	0	2
			CLR, DOX, SXT	0	2
			DOX, PEN, SXT	0	1
			CLR, CD, DOX, SXT	0	2
			CLR, CD, PEN, SXT	0	1
			CLR, CD, DOX, PEN, SXT	0	9
20	0	1 (0.06%)	CLR, CD, DOX	0	1
22F	1 (0.12%)	2 (0.12%)	CLR, PEN, SXT	0	1
			CLR, CD, DOX, SXT	0	1
			CLR, CD, DOX, PEN, SXT	1	0
23A	0	1 (0.06%)	CLR, CD, DOX	0	1
23F	1 (0.12%)	6 (0.4%)	DOX, PEN, SXT	0	3
			CLR, DOX, PEN, SXT	1	2
			CLR, CD, DOX, PEN, SXT	0	1
33F	0	2 (0.12%)	CLR, CD, DOX	0	2
35A/C	1 (0.12%)	7 (0.4%)	CLR, DOX, SXT	1	7
35B	0	1 (0.06%)	CLR, CD, DOX, SXT	0	1
NT	0	18 (1.1%)	CD, DOX, SXT	0	1
			CLR, CD, DOX	0	9
			CLR, DOX, SXT	0	1
			CLR, PEN, SXT	0	1
			CLR, CD, DOX, SXT	0	2
			CLR, DOX, LEV, SXT	0	2
			CLR, CD, DOX, LEV, SXT	0	2
Total	37 (4.5%)	146 (9.1%)	N/A	N/A	N/A

2439 isolates (827 blood; 1612 respiratory) were available for MDR analysis; N/A, not applicable

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