

Serotype distribution, multilocus sequencing type, and antimicrobial susceptibility of invasive *Streptococcus pneumoniae* isolates from adults in Hokkaido, Japan

Mitsuyo Kawaguchiya^a, Meiji Soe Aung^a, Noriko Urushibara^a, Nobuhide Ohashi^a, Nobuyuki Kobayashi^b, Kenji Kudo^c, Masahiko Ito^c, Nobumichi Kobayashi^a

^a Division of Hygiene, Department of Social Medicine, Sapporo Medical University School of Medicine, Sapporo, Japan.
^b Medical Laboratory and Blood Center, Asahikawa Medical University Hospital, Asahikawa, Japan.
^c Sapporo Clinical Laboratory, Inc., Sapporo, Japan.

Background

In Japan, the 23-valent pneumococcal polysaccharide vaccine (PPSV23) for adults aged >65 years was incorporated into the national immunization program in 2014. The present study aimed to investigate the molecular characteristics and antimicrobial resistance of *Streptococcus pneumoniae* isolates from adults with invasive pneumococcal disease (IPD) in Hokkaido, the northern main island of Japan.

Materials and Methods

From September 2017 to December 2023, a total of 45 pneumococcal isolates from adult patients (the mean age of the patients was 73 (range, 41–98) years, with most patients (n=31) being aged ≥65 years) with IPD (41 inpatients; 4 outpatients) were collected throughout Hokkaido, Japan. All isolates were serotyped by sequential multiplex PCR and direct sequencing. Multilocus sequence typing (MLST) scheme for pneumococcus was performed to identify the sequence types (STs). Minimum inhibitory concentrations to 11 antimicrobials were measured by broth microdilution test using a Dry Plate. Multidrug resistance (MDR) was defined as resistance to at least three classes of antimicrobials.

Results

Table 1. Molecular epidemiological characterization and non-susceptibility rates of IPD isolates.

Serotype (n,%)	ST (no. of isolates)	MLST Allelic Profile	PRSP	Number of non-susceptible isolates (% in each serotype) ^a										MDR
				PEN	ERY	TET	CLI	CXM	CEC	IPM	MEM	LVX	SXT	
PCV20 (21, 46.7%)/PPSV23 serotypes (20, 44.5%) ^b			2 (18.1)	4 (19.0)	17 (81.0)	17 (81.0)	14 (66.7)	5 (5.7)	7 (33.3)	3 (14.3)	2 (9.5)	1 (4.8)	5 (5.7)	15 (71.4)
3 (11, 24.5%)	180 (11)	7-15-2-10-6-1-22	0	0	10 (90.9)	10 (90.9)	9 (81.8)	0	0	0	0	0	0	9 (81.9)
23F (4, 8.9%)	242 (1)	15-29-4-21-30-1-14	0	1 (100)	1 (100)	1 (100)	0	1 (100)	1 (100)	1 (100)	0	0	1 (100)	1 (100)
	1437 (1)	1-32-6-6-6-1-14	0	0	0	0	0	1 (100)	1 (100)	0	0	0	1 (100)	0
	3543 (2)	1-32-4-6-6-1-14	0	1 (50.0)	2 (100)	2 (100)	2 (100)	0	2 (100)	0	0	0	2 (100)	1 (100)
6A (1, 2.2%)	14600 (1)	7-29-4-1-6-121-11	1 (100)	1 (100)	0	0	0	1 (100)	1 (100)	1 (100)	1 (100)	0	1 (100)	1 (100)
7F (1, 2.2%)	191 (1)	8-9-2-1-6-1-17	0	0	0	0	0	0	0	0	0	0	0	0
9V (1, 2.2%)	280 (1)	15-17-4-16-6-1-17	0	0	1 (100)	1 (100)	1 (100)	0	0	0	0	0	0	1 (100)
11A (1, 2.2%)	99 (1)	5-8-4-16-6-1-31	0	0	1 (100)	1 (100)	0	0	0	0	0	0	0	0
14 (1, 2.2%)	2922 (1)	1-5-4-5-5-20-8	0	0	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	0	0	0	0	1 (100)
19F (1, 2.2%)	10017 (1)	15-16-435-15-6-20-26	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	0	1 (100)
Non-vaccine serotypes (24, 53.3%)			2 (8.3)	7 (29.2)	21 (87.5)	18 (75.0)	13 (54.2)	7 (29.2)	14 (58.3)	1 (4.2)	2 (8.3)	0	12 (50.0)	20 (83.3)
23A (6, 13.3%)	338 (3)	7-13-8-6-1-6-8	0	2 (66.6)	3 (100)	3 (100)	2 (66.6)	0	3 (100)	0	0	0	0	3 (100)
	5242 (3)	7-13-8-6-1-337-8	0	0	2 (66.6)	2 (66.6)	2 (66.6)	0	3 (100)	0	0	0	0	2 (66.6)
6D (3, 12.5%)	282 (1)	30-4-2-4-4-1-1	1 (100)	1 (100)	1 (100)	1 (100)	0	1 (100)	1 (100)			0	1 (100)	0
	2923 (1)	2-13-2-5-6-121-29	0	0	1 (100)	1 (100)	0	0	0	0	0	0	1 (100)	1 (100)
	6183 (1)	1-5-2-173-6-1-14	0	0	1 (100)	1 (100)	1 (100)	0	0	0	0	0	0	1 (100)
15A (3, 12.5%)	63 (3)	2-5-36-12-17-21-14	1 (33.3)	2 (66.6)	3 (100)	3 (100)	3 (100)	2 (66.6)	2 (66.6)	1 (33.3)	1 (33.3)	0	1 (33.3)	3 (100)
34 (3, 12.5%)	1439 (1)	5-5-6-1-9-14-14	0	0	0	0	0	0	0	0	0	0	1 (100)	2 (66.6)
	7388 (2)	5-5-7-16-9-1-14	0	0	2 (100)	2 (100)	1 (50.0)	0	0	0	0	0	2 (100)	2 (100)
35B (3, 12.5%)	558 (3)	18-12-4-44-14-77-97	0	1 (33.3)	3 (100)	1 (33.3)	1 (33.3)	3 (100)	3 (100)	0	1 (33.3)	0	2 (66.6)	3 (100)
6E (2, 4.4%)	90 (2)	5-6-1-2-6-3-4	0	1 (50.0)	2 (100)	2 (100)	2 (100)	1 (50.0)	2 (100)	0	0	0	2 (100)	2 (66.6)
23B (1, 2.2%)	36 (1)	1-8-4-1-1-4-6	0	0	1 (100)	0	0	0	0	0	0	0	0	1 (100)
31 (1, 2.2%)	11184 (1)	1-2-461-16-15-155-18	0	0	0	0	0	0	0	0	0	0	1 (100)	0
37 (1, 2.2%)	447 (1)	29-33-19-1-36-22-31	0	0	1 (100)	1 (100)	0	0	0	0	0	0	0	0
Non-typeable (1, 2.2%)	2754 (1)	2-8-70-16-6-19-18	0	0	1 (100)	1 (100)	1 (100)	0	0	0	0	0	1 (100)	0
Total (rate; %)			4 (8.9)	11 (24.4)	38 (84.4)	35 (77.8)	27 (60.0)	12 (26.7)	21 (46.7)	4 (8.9)	4 (8.9)	1 (2.2)	17 (37.8)	34 (75.6)

PRSP, PEN-resistant *S. pneumoniae*; PEN, penicillin; ERY, erythromycin; TET, tetracycline; CLI, clindamycin; CXM, cefuroxime; CEC, cefaclor; IPM, imipenem; MEM, meropenem; LVX, levofloxacin; SXT, trimethoprim-sulfamethoxazole. ^aAll isolates were susceptible to vancomycin (data not shown). ^bOne serotype 6A covered by the PCV20 is not included in the PPSV23.

- Among a total of 45 isolates, serotype 3-ST180 (24.4%) was the most prevalent, followed by 23A-ST338/ST5242 (13.3%), 23F-ST242/ST1437/ST3543 (8.9%), and 6D-ST282, 15A-ST63, 35B-ST558, and 34-ST1439/ST7388 (6.7% each), collectively accounted for 73.4% of all isolates.
- Overall, the prevalence rates of PCV20- and PPSV23-serotype of all isolates were 46.7% and 46.5%, respectively.
- The rate of non-susceptibility to penicillin was 24.4%, and most of them (n=10/11, 90.9%) were also non-susceptible to erythromycin and tetracycline.
- Among the non-PPSV23 serotypes, 23A, 6D, 15A, 35B, and 6E showed MDR, along with non-susceptibility to penicillin.

Conclusions: The present study revealed the high prevalence of non-PPSV23 serotypes among isolates from IPD in adults, associated with high rates of non-susceptibility to antimicrobial agents. These findings indicated the need for continuous surveillance of *S. pneumoniae* in Japan.