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Regional spread of three distinct genotypes of *Mycoplasma pneumoniae* and different timing of macrolide-resistant strain appearance among genotypes between 2011 and 2013 in Yamagata, Japan

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ABSTRACT

Background: We previously revealed that several multiple-locus variable-number tandem-repeat analyses (MLVA) and P1 types of *Mycoplasma pneumoniae* (*M. pneumoniae*) cocirculated between 2011 and 2013 in Yamagata, Japan. However, the regional spread of *M. pneumoniae* infection by genotype is not reported yet. It remains unclear whether there is a difference in the spread of macrolide-resistant *M. pneumoniae* among genotypes.

Methods: Genotypes were labeled according to 4-locus (Mpn 13, 14, 15, and 16) MLVA and P1 types. A total of 208 strains belonging to three major genotypes, i.e., type 4-5-7-2, 1; 4-5-7-3, 1; and 3-5-6-2, 2c, were analyzed by combining with the information of macrolide resistance-associated mutation and the patients' information including residence.

Results and Discussion: The three genotypes were widely distributed over more than four cities and towns in Yamagata Prefecture, cocirculating between late 2011 and early 2013, and there was little difference in the duration of their epidemics. Timing of macrolide-resistant strain appearance during the epidemic period differed between type 4-5-7-2, 1 and type 4-5-7-3, 1, and it did not appear throughout type 3-5-6-2, 2c epidemic. These genotypic differences can account for the variation in the prevalence of macrolide resistance-associated mutations in each of the studied areas.

Key words: *Mycoplasma pneumoniae,* molecular typing, P1 type, MLVA, macrolide resistance

Introduction

Mycoplasma pneumoniae (*M. pneumoniae*) is a common cause of upper and lower respiratory tract infections, particularly in children and young adults[1),2)]. This pathogen is responsible for up to 20% of all cases of community-acquired pneumonia (CAP)[3)] and 30% of pediatric cases of CAP[4),5)]. *M. pneumoniae* infections can be treated using macrolides (MLs) as first-line antibiotics[6)-8)]. However, since the year 2000, ML-resistant *M. pneumoniae* harboring a point mutation in its genome region coding the 23S rRNA domain V (ML resistance-associated mutation) has increased worldwide, contributing to increasing global public

health concerns[6), 9)].

Molecular typing of *M. pneumoniae*, including 4-locus (Mpn13, 14, 15, and 16) multiple-locus variable-number tandem-repeat analysis (MLVA) and P1 gene typing based on polymorphisms in the P1 gene encoding *M. pneumoniae* P1 adhesion protein, is an effective tool to understand the epidemiology of *M. pneumoniae* infection. Using these typing methods, we reported polyclonal spread of multiple genotypes of *M. pneumoniae* in schools[10)]. In addition, we revealed that several P1 and MLVA types of *M. pneumoniae* had cocirculated between 2011 and 2013 in Yamagata Prefecture, Japan[11), 12)]. However, the regional spread of *M. pneumoniae* infection by genotype is not reported yet. It remains unclear whether there is difference in the spread of ML-resistant *M. pneumoniae* among genotypes. In this study, we present detailed geographical and temporal distribution of *M. pneumoniae* isolates between 2011 and 2013 in Yamagata Prefecture.

Methods

Study design:

Our previous study conducted between 2004 and 2014 revealed that three major MLVA types of *M. pneumoniae*, including type 4-5-7-2, type 4-5-7-3, and type 3-5-6-2, cocirculated between 2011 and 2013 in Yamagata, Japan[11)]. To elucidate the regional spread of *M. pneumoniae* infection by genotype, we performed a retrospective study of *M. pneumoniae* infection cases between 2011 and 2013 by combining the molecular typing results with the patients' information. The profile of molecular typing and the ML susceptibility of the *M. pneumoniae* strains were obtained from our previous study[11)]. The genotypes of *M. pneumoniae* were defined using a combination of MLVA and P1 types and labeled in the order of MLVA type and P1 type. A total of 208 strains belonging to three major genotypes, i.e., type 4-5-7-2, 1; 4-5-7-3, 1; and 3-5-6-2, 2c, were analyzed. The patients' information including residence, specimen collection date, age, sex, and clinical diagnosis was collected from the medical records obtained from the Yamanobe Pediatric Clinic and Katsushima Pediatric Clinic in Yamagata, Japan. This study was approved by the Yamagata University Faculty of Medicine (H27-17) and Yamagata Prefectural Institute of Public Health (YPIPHEC 16-04) ethics committees.

Statistical analysis:

We examined the differences among the three genotypes. Kruskal–Wallis test was used for age, and Fisher's exact test was used for sex, diagnosis, and prevalence of the ML resistance-associated mutation. When significant differences (P value < 0.05) were detected among the three genotypes, multiple comparisons were performed. Wilcoxon rank sum test with the Benjamini–Hochberg procedure was used for age, and Fisher's exact test with the Holm method was used for prevalence of the ML resistance-associated mutation. Statistical tests were performed using R version 3.4.1 (The R Foundation, Vienna, Austria).

Results and Discussion

We analyzed 208 strains belonging to three major genotypes, including 74 strains of type 4-5-7-2, 1, 74 strains of type 4-5-7-3, 1, and 60 strains of type 3-5-6-2, 2c. Table 1 shows the patients' information and ML resistance-associated mutation prevalence among the three major genotypes. Statistical analysis revealed that patients infected with type 4-5-7-2, 1 were significantly younger than the patients infected with type 3-5-6-2, 2c. In addition, the prevalence of ML resistance-associated mutations in type 4-5-7-2, 1 (83.8%; 62/74) was significantly higher than that in type 4-5-7-3, 1 (25.7%; 19/74) and type 3-5-6-2; 2c (0%; 0/60). However, there were no significant differences in clinical diagnosis, defined as the ratio of lower to upper respiratory tract infections, among the three genotypes, suggesting that there is no association between genotype and disease severity.

Figure 1 shows the geographical information of the three genotypes of *M. pneumoniae*, mapped according to districts (cities and towns). Of the 208 *M. pneumoniae* strains, 181 (87%) were isolated in four of the studied districts: 72 (34.6%) in Yamagata City, 48 (23.1%) in Yamanobe Town, 42 (20.2%) in Sagae City, and 19 (9.1%) in Nakayama Town. In these four districts, each of the three genotypes

Table 1. Comparison of patient characteristics and macrolide resistance-associated mutation prevalence among the three major genotypes of *Mycoplasma pneumoniae* is olated between 2011 and 2013 in Yamagata, Japan

		Genotype* of *M. pneumoniae* isolate			
		4-5-7-2, 1 (n = 74)	4-5-7-3, 1 (n = 74)	3-5-6-2, 2c (n = 60)	*P* value†
Age, median (range)		8 (1–45)	8 (3–63)	9 (1–56)	0.042‡
Sex	Male	42	40	34	0.953
	Female	32	34	26	
Diagnosis	Lower respiratory tract infection	40	31	24	0.201
	Upper respiratory tract infection	34	43	36	
Macrolide resistance-associated mutation					
	Positive	62	19	0	<0.001§
	Negative	12	55	60	

* Genotype was represented by the combination of VNTR numbers at 4 loci as Mpn13–Mpn14–Mpn15–Mpn16 and P1 type.

† Kruskal–Wallis test was used for age and Fisher's exact test was used for sex, diagnosis, and prevalence of the ML resistance-associated mutation. When significant differences (P value < 0.05) were detected among the three genotypes, multiple comparisons were performed.

‡ Significant differences were obtained between type 4-5-7-2, 1 and 3-5-6-2, 2c (P = 0.045) by multiple comparison (Wilcoxon rank sum test with the Benjamini–Hochberg procedure).

§ Significant differences were obtained between type 4-5-7-2, 1 and 4-5-7-3, 1; 3-5-6-2, 2c and between type 4-5-7-3, 1 and 3-5-6-2, 2c, respectively (P < 0.001) by multiple comparison (Fisher's exact test with the Holm method).

was isolated. In the remaining six districts (Oe Town, Asahi Town, Kaminoyama City, Kahoku Town, Higashine City, and Tendo City), one to two of the three genotypes were isolated. These findings suggest that the three genotypes of *M. pneumoniae* were widely distributed in the studied districts. Prevalence of ML resistance-associated mutation varied within each district, with 100% (2/2) in Kahoku Town; 75% (3/4) in Kaminoyama City; 52.1% (25/48) in Yamanobe Town; 44.4% (32/72) in Yamagata City; 40.5% (17/42) in Sagae City; 7.7% (1/13) in Oe Town; 5.3% (1/19) in Nakayama Town; and 0% in Higashine City, Tendo City, and Asahi Town. The observed differences in the prevalence of this mutation according to different districts may reflect differences in its prevalence according to genotype (Table 1).

Geographical and temporal information of the three genotypes of *M. pneumoniae* strains is shown in Figure 2 (a), where each year has been divided into three terms. A single genotype of type 4-5-7-3, 1 was isolated during the first and second terms of 2011, whereas the three genotypes were isolated between the third term of 2011 and the first term of 2013. Each of the three genotypes was isolated for seven terms (approximately a 28-month period) in succession, which gradually spread to multiple surrounding districts. Monthly occurrence of each genotype with or without the ML resistance-associated mutation is shown in Figure 2 (b). Type 4-5-7-2, 1 was first isolated in October 2011, whereas the ML-resistant strain was prevalent from the initial period of its epidemic. Type 4-5-7-3, 1 was first isolated in January 2011; however, it did not possess the ML resistance-associated mutation until its emergence 11 months after the initial epidemic. Furthermore, type 3-5-6-2, 2c; first isolated in December 2011 and intermittently occurring until

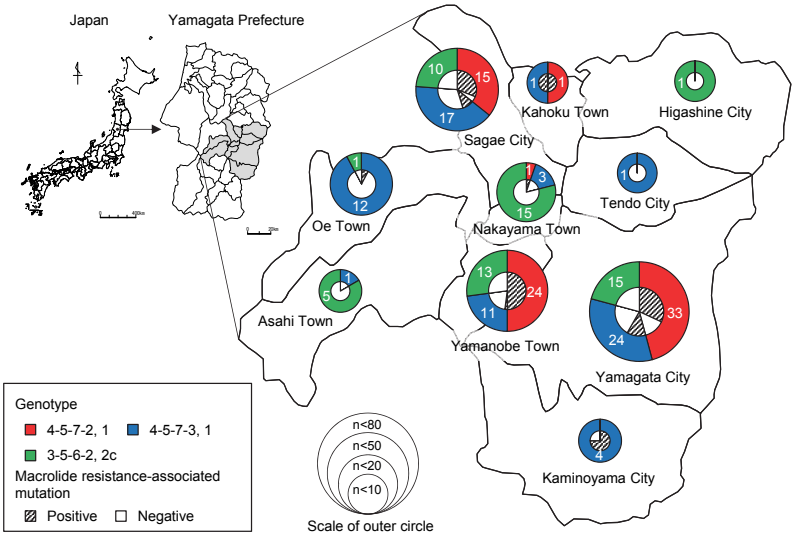

Figure 1. Geographical information of the three genotypes (type 4-5-7-2, 1; type 4-5-7-3, 1; and type 3-5-6-2, 2c) of *Mycoplasma pneumoniae* isolated between 2011 and 2013 in Yamagata Prefecture, Japan.
Gray area of the map displays the ten districts from where *M. pneumoniae* strains were derived. The proportion of genotypes and prevalence of the ML resistance-associated mutation by genotype are shown in the outer ring and inner circle, respectively. The numbers in the outer ring indicate the number of *M. pneumoniae* genotypes detected within each district.

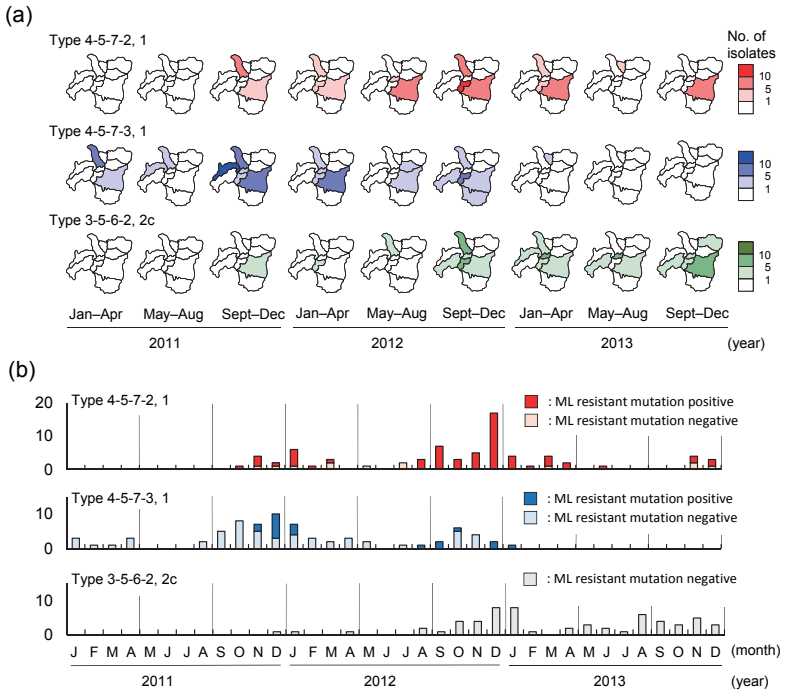

Figure 2. Geographical and temporal information of the three genotypes (type 4-5-7-2, 1; type 4-5-7-3, 1; and type 3-5-6-2, 2c) of *Mycoplasma pneumoniae* isolated between 2011 and 2013 in Yamagata Prefecture, Japan.
(a) Distribution of *M. pneumoniae* strains by district, where each year was divided into three terms. Maps correspond to the gray area shown in Figure 1.
(b) Monthly occurrence of *M. pneumoniae* strains with or without the ML resistance-associated mutation.

December 2013, did not possess the ML resistance-associated mutation throughout the entirety of its epidemic period.

Prevalence of early emergence of the ML-resistant type 4-5-7-2, 1 *M. pneumoniae* may be due to the isolation of ML-resistant type 4-5-7-2, 1 strain in Yamagata Prefecture before 2011[11),12)]. Type 4-5-7-3, 1 strain, which had not been isolated before 2011 in Yamagata Prefecture[11),12)], was ML-susceptible during the initial period of its epidemic. Therefore, emergence of ML-resistant type 4-5-7-3, 1 strain may be associated with a selective pressure resulting from therapeutic ML use. Although type 3-5-6-2, 2c strain, as well as type 4-5-7-3, 1 strain, had low prevalence in Yamagata Prefecture prior to 2011[11),12)], ML-resistant type 3-5-6-2, 2c strain did not appear throughout its epidemic period. However, we previously reported that ML resistance-associated mutation emerged after ML treatment in a child infected with type 3-5-6-2, 2c *M. pneumoniae* as well as in children infected with type 4-5-7-3, 1[13)]. Hence, our current findings suggest that type 3-5-6-2, 2c *M. pneumoniae* with a ML resistance-associated mutation has poor transmission ability compared with other genotypes. Further surveillance is needed to clarify the potential emergence of a ML-resistant type 3-5-6-2, 2c strain in this area.

There are several limitations in this study. First, this study was conducted based on *M. pneumoniae* strains isolated from patients who visited only two pediatric clinics. The two clinics are located in Yamanobe Town and Yamagata City of Yamagata Prefecture; therefore, obtained findings may not reflect the situation of the whole prefecture. Second, we could not collect clinical information on anything except clinical diagnosis; therefore, we could not sufficiently compare severity among patients who were infected with different genotypes of *M. pneumoniae*. Our study was restricted to a local area and retrospectively performed; however, no similar studies that described regional difference in the prevalence of ML resistance-associated mutation by combining the molecular typing results is reported from other areas in Japan.

In summary, this study reveals that the three distinct genotypes of *M. pneumoniae* geographically and temporally cocirculate in Yamagata Prefecture, Japan and there is little difference in the duration of their epidemics. However, the timing of emergence of the ML-resistant strain differs for each genotype. These genotypic distinctions may account for the observed differences in the prevalence of ML resistance-associated mutation in each of the studied areas. Provision of early information about the genotype of circulating in each region will help clinicians choose specific antibiotics against infection.

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