Jpn. J. Infect. Dis., 71, 244–246, 2018

Short Communication

Isolation Rate of *Neisseria meningitidis* in Japanese Children with Respiratory Tract Infections

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SUMMARY: Although invasive meningococcal disease is rare in Japan (0.028 cases per 100,000 population), its incidence is 10 times greater in many other countries. Colonization is a prerequisite for invasive meningococcal disease. However, no study in Japan has involved specifically analyzing the carriage rate of *Neisseria meningitidis* in children. During 5 months in 2015, the respiratory tract specimens of patients who presented to 3 hospitals with respiratory symptoms were cultured. The bacteria were identified in selective media using a meningococcal detection kit and the serogroup was identified using polymerase chain reaction analysis. In 389 patients aged ≤ 15 years with respiratory symptoms, the *N. meningitidis* isolation rate was 0.26% (1/389). The serogroup of the only child who tested positive was Y. In this study, we detected a low meningococcal isolation rate in pediatric patients. Due to increasing globalization, the risk of invasive meningococcal disease is likely increasing in Japan. Accordingly, invasive meningococcal diseases should be continuously monitored in Japan. Future large-scale studies should assess meningococcal isolation rates and corresponding serogroups.

Neisseria meningitidis causes invasive diseases and is the only pathogenic bacterium that causes outbreaks of meningitis. The prognosis of invasive meningococcal disease (IMD) is unfavorable. The mortality and long-term sequelae associated with IMD are 8–15% and 10–20%, respectively (1,2).

The incidence of IMD is as high as 1,000 cases per 100,000 population in sub-Saharan Africa, the so-called "African meningitis belt" (2,3). In the United States, the incidence of IMD was 0.18 per 100,000 in 2013 (2). In 2009, the incidences of IMD in European countries and in Australia were 0.92 and 1.2 per 100,000, respectively (4). In Japan, the law was revised after April 2013 to include not only meningococcal meningitis, but also bacteremia as IMD. Compared to recent incidence rates reported in other countries, IMD is rare in Japan; in 2014, its incidence was 0.028 per 100,000 (5). However, the risk of IMD will likely increase in Japan because of increasing globalization (1).

Meningococcal carriage is a prerequisite for IMD (2). The carriage rate of *N. meningitidis* is 5–30% in Western countries (6,7) and 10–20% worldwide (8). In Japanese adults, carriage rates of 0.4% in 2004 (7) and 0.8% in 2016 (9) were reported.

However, there have been few reports on the rate of meningococcal identification among patients with respiratory tract infections (10); moreover, no study in Japan has involved specifically analyzing children for *N. meningitidis* carriage. Therefore, we aimed to determine the rate of meningococcal isolation in children with respiratory tract infections in Japan.

From July 1 to December 1, 2015, respiratory tract specimens were collected from patients who presented with respiratory symptoms to hospitals in Chiba City, Japan. Specimens were cultured in both chocolate and blood agar media, as well as in a meningococcal-selective culture medium (Thayer-Martin agar medium). Bacteria that were cultured in a selective culture medium were identified with the meningococcal detection kit NH-20 Rapid® (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan). The polymerase chain reaction (PCR) assay was performed using primers targeting i) the conserved regulatory gene *crgA* to identify *N. meningitidis*; ii) *ciaD* to identify serogroups B, C, Y, and W; and iii) *ofg-2* to identify serogroup A (11). Patient characteristics, including age, clinical diagnoses, sources of specimen, and underlying diseases, were collected from medical records.

This study was approved by the ethics committee of Chiba University (No. 2032). All experiments were carried out in compliance with the relevant laws and guidelines and in accordance with the ethical standards of the Declaration of Helsinki.

We examined 435 specimens from 405 patients. Of these, 389 patients were ≤ 15-years-old (398 specimens)

Received May 29, 2017. Accepted December 14, 2017.
J-STAGE Advance Publication February 28, 2018.
DOI: 10.7883/yoken.JJID.2017.244

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(Table 1). Forty-four out of 398 specimens yielded positive results in the selective culture medium, and 1 *N. meningitidis*-positive specimen was detected in the specimens of the pediatric patients (≤ 15-years-old). Other bacteria that were identified in selective culture-positive specimens included *N. lactamica, Moraxella* sp., *M. catarrhalis*, *N. mucosa*, and *Neisseria* sp. in 38, 2, 1, 1, and 1 patient(s), respectively (Table 2). Thus, the respiratory tract isolation rate of *N. meningitidis* was 0.3% (1/389) among patients aged ≤ 15 years.

Table 1. Patient characteristics

No. of patients	389
Age-median year (interquartile range)	3 (1–6)
Times of examination	
Once	381
Twice	7
3 times	1
Diagnosis	
Pneumonia	93
Acute bronchitis	90
Asthma attack	70
Acute bronchitis	38
Upper respiratory infection	23
Sinusitis	11
Chronic bronchitis	9
Bacterial monitoring	7
Others	48
Underlying disease	
Bronchitis asthma	69
Severely retarded and/or neuromuscular disease	15
Atopic dermatitis and/or food allergy	5
Chromosome abnormality	5
Respiratory tract malfomation	5
Autoimmune disease	5
Congenital heart disease	4
Vesicoureteral reflux and urinary tract infection	4
Immunodeficiency	3
Preterm	2
Malignancy (neuroblastoma)	1
Others	14
No underlying disease	257

For underlying diseases, we detected 3 cases of immunodeficiency and 1 case of malignancy; no immunocompromised patient was a carrier of *N. meningitidis* (Table 1).

N. meningitidis was isolated from a 7-year-old girl with no underlying disease. She had a history of cough, sore throat, and fever for 2 days before visiting the outpatient clinic. The girl was diagnosed with acute bronchitis and prescribed clarithromycin. A sputum culture tested positive for *Neisseria* spp. 3+, alpha-hemolytic *streptococcus* spp. 3+ in the standard culture medium, and *N. meningitidis* 1+ and *N. lactamica* in the meningococcal-selective culture medium. *N. meningitidis* from the selective culture medium was identified as serogroup Y (Fig. 1). She had no symptoms of IMD and was treated with clarithromycin for her respiratory symptoms; this improved her condition. Accordingly, the presence of *N. meningitidis* was likely due to colonization.

The meningococcal isolation rate of 0.3% (1/389) in children in the current study is consistent with previous reports that included adults (7,9). It is known that the meningococcal carriage rate is age-dependent (12); based on a meta-analysis, the carriage rates in Western countries are 4.5% in infants and 23.7% in those aged 19 years (12). Due to the low carriage rate found in this study, we could not determine the age-specific isolation rates in Japanese

Table 2. The detected bacteria in selective medium

No. of patients	44
Neisseria lactamica	38
Moraxella sp.	2
N. meningitids and *N. lactamica*	1
M. catarrhalis	1
N. mucosa	1
Neisseria sp.	1

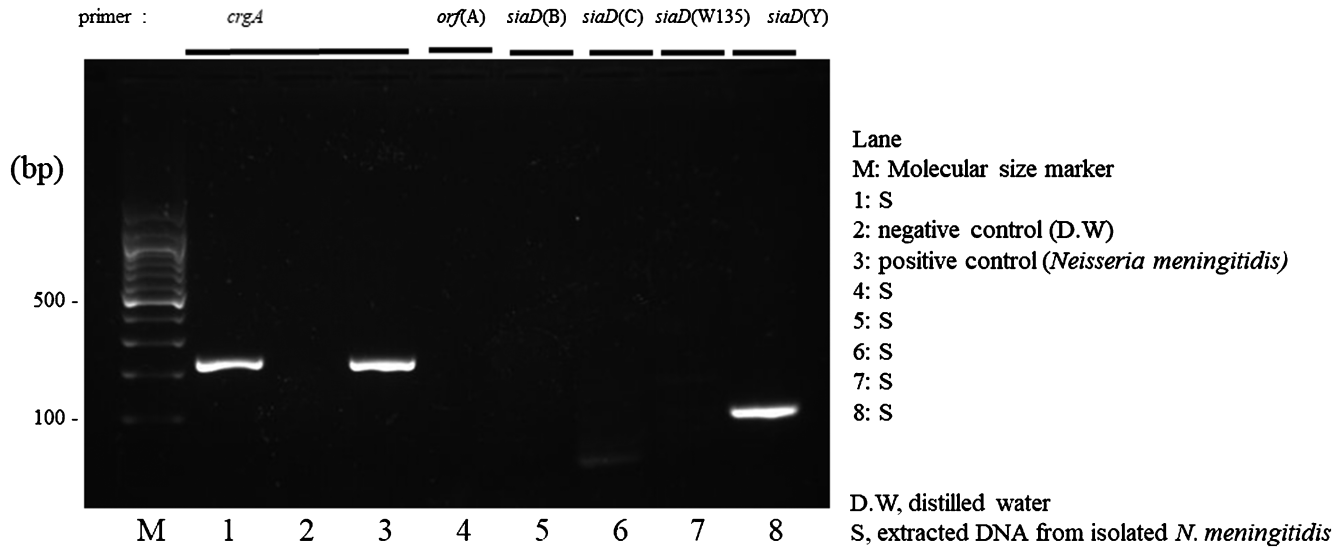

Fig. 1. Serogroup analysis. Gel electrophoresis of polymerase chain reaction samples was performed to identify the *Neisseria meningitidis* serogroup. Based on the band observed for number 8 (primers for *siaD*; 180 bp), the *N. meningitidis* serogroup was identified as Y.

children. No patient with immunodeficiency or malignancy was a meningococcal carrier; this is likely also due to the overall low isolation rate.

From April 2013 to December 2014, 59 IMD cases were reported in Japan. The serogroups of these cases were Y (42%), C (12%), W (3%), Y or W (5%), B (7%), and unknown (31%) (5). The exact serogroup distribution remains inconclusive due to the existence of serogroup-untested strains. Serogroup Y, which was detected in the current study, is the most prevalent serogroup; the quadrivalent meningococcal conjugate vaccine (including serogroups A, C, Y, and W) is expected to provide protection against this serogroup.

A relationship between *N. lactamica* and *N. meningitidis* in colonization and IMD has been reported in previous studies (4,13). Although the mechanism has not been determined, *N. lactamica* carriage can prevent IMD and meningococcal carriage (13,14). In this study, the *N. lactamica* carriage rate was 10.0% (38/389) in patients aged ≤ 15 years. This rate is not remarkably higher than that reported in other countries where IMD and meningococcal carriage rates are higher than in Japan (15). Moreover, the patient in whom *N. menigitidis* was isolated also tested positive for *N. lactamica* in this study. Thus, the low meningococcal carriage rate and low incidence of IMD in Japan is likely not only due to the *N. lactamica* carriage rate. The carriage rates of *N. lactamica* and *N. meningitidis* and the serum antibody levels against these bacteria should be investigated in a larger population.

The main limitation of this study is the relatively small number of patients and short analysis period owing to budgetary limitations. Moreover, 30% of the patients were administered antibiotics within 2 weeks before respiratory specimen culture examinations; this might have affected the isolation rate of *N. meningitidis*. Antibiotic prescriptions vary by country. A future large-scale and detailed study including information on antibiotics should compare the carriage rates of *N. meningitidis* in Japanese children with that in children in other countries.

In conclusion, of 389 pediatric patients (≤ 15-years-old) with respiratory symptoms, we detected 1 case of *N. meningitidis*, serogroup Y. The respiratory meningococcal isolation rate in this age group was 0.3% (1/389). With increasing globalization, the risk of IMD in Japan will likely also increase. This underscores the need to continuously examine IMD, the isolation rates of *N. meningitidis* in respiratory specimens, and its serogroups in Japan.

Acknowledgments This work was supported by a university grant from the Chiba University (020A0699).

Conflict of interest None to declare.

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