Comparison of Oropharyngeal and Nasopharyngeal Swab Specimens for the Detection of *Mycoplasma pneumoniae* in Children with Lower Respiratory Tract Infection

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The oropharyngeal swab specimen was superior to the nasopharyngeal swab specimen for the detection of *Mycoplasma pneumoniae* in children with lower respiratory tract infection. The oropharyngeal loop-mediated isothermal amplification had 100% sensitivity and specificity compared with polymerase chain reaction testing, whereas the oropharyngeal rapid antigen detection test using immunochromatographic assay had relatively low sensitivity (66%) and reasonable specificity (90.7%). *(J Pediatr 2017;189:218-21).*

Early and accurate diagnosis of *Mycoplasma pneumoniae* (MP) lower respiratory tract infection (LRTI) in children is important for appropriate antibiotic treatment. Loop-mediated isothermal amplification (LAMP), a rapid nucleic acid amplification technique, has been implemented widely in Japan.[1-4] In 2013, the Japanese guidelines in children recommended LAMP as the first choice to confirm the early diagnosis of MP infection.[5] In 2013, a new rapid antigen detection test (RADT) became available in Japan.[6,7]

Oropharyngeal (OP) and nasopharyngeal (NP) swab specimens are the most frequently used samples for detecting MP in children; however, the better sampling mode of the 2 remains controversial.[8-10] We carried out a prospective study to compare OP and NP swab specimens in children with MP LRTI. Both swab samples were analyzed by the LAMP and RADT techniques in comparison with real-time polymerase chain reaction (PCR). The aims of this study were to determine the optimal sampling site for the detection of MP infection, and to evaluate the diagnostic values of the LAMP and RADT techniques.

Methods

We conducted a prospective study of children and adolescents with community-acquired LTRI. Between December 2015 and August 2016, patients (age between 1 and 18 years) who were evaluated and had signs of LRTI were enrolled. Written informed consent was provided by the parents. The study protocol was approved by the Ethics Committee of Furano Kyokai Hospital.

Paired flocked swabs from the OP (FLOQSwabs 519C; Copan Italia, Brescia, Italy) and NP (FLOQSwabs 534CS01; Copan Italia) sites were collected on the patients' first visit. Two OP swabs with a 4.5-mm tip diameter were used simultaneously to rub the posterior wall of the oropharynx under direct visualization. Two NP swabs with a 2.5-mm tip diameter were inserted into one of the nostrils; on feeling resistance at the nasopharynx, the swabs were rotated and withdrawn. These were blind-sampled in terms of the location of the swab in the nasopharynx.

DNA was extracted from the swabs using the Loopamp DNA SR Extraction Kit (Eiken Chemical, Tochigi, Japan). Real-time PCR (LightCycler Nano Real-Time PCR System; Roche Diagnostics, Tokyo, Japan) to measure MP DNA load was carried out according to Nakamura et al[11] with some modifications. The target was the 16S rRNA gene. Confirmation of MP infection was defined as a positive PCR result in either the OP or NP swab sample. The LAMP assay was conducted using the Loopamp MP DNA Amplification Kit D (Eiken Chemical). RADT was conducted using the Ribotest Mycoplasma Kit (Asahi Kasei Pharma, Tokyo, Japan), which detects MP L7/L12 ribosomal protein using an immunochromatographic assay.

Results

Fifty-eight children (36 boys), with a median age of 8.8 years (range, 1-17 years), consented to enrollment. They comprised 18 hospitalized patients and 40 outpatients. The PCR result was positive in 15 OP swab specimens and in 7 NP swab specimens; overall, 15 children (25.9%; 95% CI, 15.3%-39.0%) had confirmed MP infection. The diagnostic values of LAMP and RADT for the detection of MP compared with the PCR results are shown in the **Table**. The sensitivity of the OP LAMP was significantly higher than that of the NP LAMP ($P < .05$). The sensitivity of the OP RADT was higher than that of the NP RADT, but the difference was not statistically significant ($P = .14$).

LAMP	Loop-mediated isothermal amplification
LRTI	Lower respiratory tract infection
MP	*Mycoplasma pneumoniae*
NP	Nasopharyngeal
OP	Oropharyngeal
PCR	Polymerase chain reaction
RADT	Rapid antigen detection test

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Table. LAMP and RADT results compared with real-time PCR results for the detection of MP in children with LRTI

	OP			NP			OP			NP		
	LAMP			LAMP			RADT			RADT		
Confirmed MP infections*	Positive	Negative	Total	Positive	Negative	Total	Positive	Negative	Total	Positive	Negative	Total
Positive	15	0	15	8	7	15	10	5	15	5	15	10
Negative	0	43	43	0	43	43	4	39	43	8	35	43
Total	15	43	58	8	50	58	14	44	58	13	45	58
Sensitivity, % (95% CI)	100 (69.8-100)			53.3 (26.6-78.7)			66.7 (38.4-88.2)			33.3 (11.8-61.6)		
Specificity, % (95% CI)	100 (88.0-100)			100 (88.0-100)			90.7 (77.9-97.4)			81.4 (66.6-91.6)		
Positive predictive value, % (95% CI)	100 (69.8-100)			100 (51.8-100)			71.4 (41.9-91.6)			38.5 (13.9-68.4)		
Negative predictive value, % (95% CI)	100 (88.0-100)			86.0 (73.3-94.2)			88.6 (75.4-96.2)			77.8 (62.9-88.8)		
Accuracy, % (95% CI) %	100 (90.9-100)			86.2 (74.6-93.9)			84.5 (72.6-92.7)			69.0 (55.5-80.5)		

*Positive PCR result in either the OP or NP swab.

Figure, A compares the MP DNA load in the 15 confirmed cases in OP swab specimens versus NP swab specimens. The median MP DNA load of the 15 PCR-positive OP swab specimens was 711.3 copies/μL (range, 12.4-3749.3 copies/μL). All PCR-positive OP specimens were LAMP-positive as well. The median MP DNA load of the 7 PCR-positive NP swab specimens was 378.8 copies/μL (range, 4.5-22 050.5 copies/μL). All PCR-positive NP specimens were LAMP-positive as well. One of the 8 PCR negative-NP swab specimens was LAMP-positive. The MP DNA loads had a wider distribution range in the NP swab specimens than in the OP swab specimens. LAMP was able to detect an MP DNA load of ≥4.5 copies/μL.

Figure, B compares the MP DNA loads in the OP swab specimens from the confirmed RADT-positive and RADT-negative cases. The median MP DNA load was 1506.1 copies/μL (range, 77.-3749.3 copies/μL) in the 10 RADT-positive OP swabs and 17 copies/μL (range, 12.4-181.6 copies/μL) in the 5 RADT-negative OP swab specimens. **Figure**, C compares the MP DNA loads in the NP swab specimens of the confirmed RADT-positive and RADT-negative cases. The median MP DNA load was 2760.7 copies/μL (range, 378.8-3036.5 copies/μL) in the

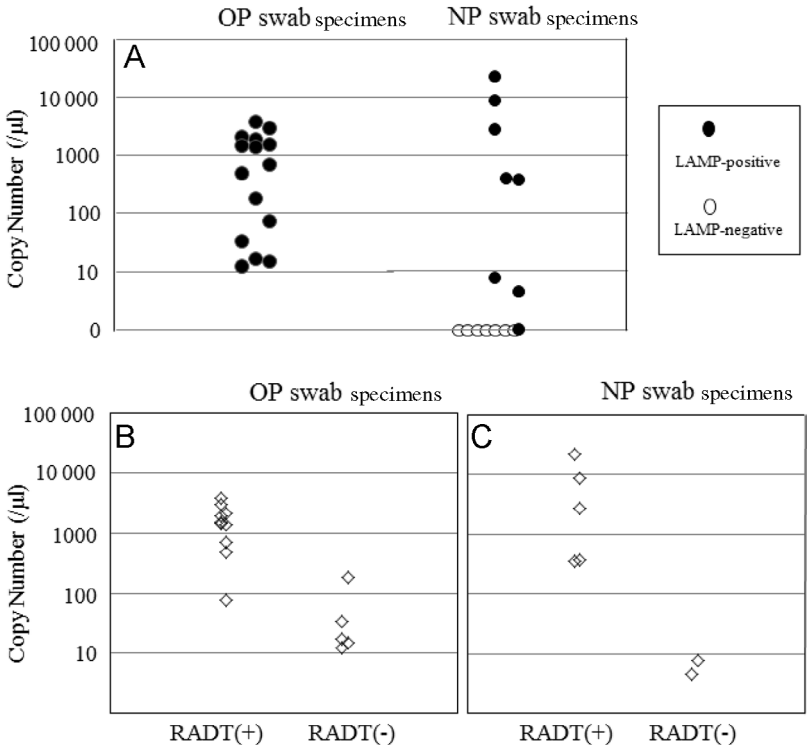

Figure. A, Comparison of MP DNA loads between OP and NP swab samples in in the 15 confirmed cases. **B,** Comparison of MP DNA loads in the OP swab specimens from the 15 confirmed cases between the RADT-positive and -negative cases. **C,** Comparison of MP DNA loads in NP swab samples from the 7 confirmed cases between the RADT-positive and -negative cases.