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Etiological Agents of Lower Respiratory Tract Infections in Japanese Children

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Abstract. *To investigate the etiology of pediatric community-acquired pneumonia and bronchitis, we conducted a prospective, population-based study covering the total population <15 years of age in 16 municipalities in Hokkaido, Japan, during the period of April 2000 to March 2001. Chest radiographs were available for all cases (n=921; 398 as pneumonia and 523 as bronchitis) and paired sera for serologic assays were available for more than half of the cases. The following specimens were also collected : nasopharyngeal swabs for viral, bacteriological, mycoplasmal and chlamydial studies, blood for serology and blood culture. The children were then followed-up on days 3, 7 and 14. Specific infecting organisms were identified in a total of 853 (92.6%) out of 921 patients (398 cases of pneumonia and 523 cases of bronchitis) including 205 with mixed infection as follows: Mycoplasma pneumoniae, 252 (27.4%) patients; respiratory syncytial (RS) virus, 188 (20.4%); influenza A virus, 110 (11.9%); Streptococcus pneumoniae, 95 (10.3%); Haemophilus influenzae, 90 (9.8%); Haemophilus parainfluenzae, 35 (3.8%); Staphylococcus aureus, 29 (3.1%); adenovirus, 27 (2.9%); Moraxella catarrhalis, 12 (1.3%); Pseudomonas aeruginosa , 7 (0.8%); Chlamydia pneumoniae, 6 (0.7%); and other agents, 2 (0.2%). Mycoplasma infections were seen even in patients less than 5 years and RS and influenza A virus infections in patients more than 5 years of age. The importance of M. pneumoniae and RS virus in the etiology of lower respiratory infections in Japanese children was confirmed.*

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Microbiological investigations concerning the relative importance of the various organisms that cause lower respiratory tract infections, such as pneumonia and bronchitis, in Japanese children have not yet been completed. The most important reason for this is the difficulty of obtaining clinical specimens from children with lower respiratory tract infections and the lack of appropriate rapid diagnostic assays in routine practice (1). A variety of indirect microbiological methods have been applied to identify etiological agents from patients with pneumonia and bronchitis (2, 3).

The methods to determine etiological agents such as Gram's stain, culture of clinical specimens, serum antibody response and pneumococcal immune complexes in serum have been validated, but in the absence of a widely applicable reference standard, none is indisputably predictive of the true cause of pneumonia or bronchitis. Serological assays, direct antigen detection, PCR study and isolation of the organism are occasionally used to determine viral etiology.

Mycoplasma pneumoniae, Chlamydia pneumoniae, Streptococcus pneumoniae and other bacteria and viruses are common etiological agents that causes acute infection of the respiratory tract. Although more information is needed about outpatients who are estimated to compromise more than half of the pediatric patients with community-acquired pneumonia or bronchitis, most etiologic studies have been conducted in hospitals in industrialized countries or in clinics for outpatients in developing countries.

The aim of the present study was to investigate the etiology of all bacterial, viral, mycoplasmal, rickettsial and chlamydial cases of community-acquired lower respiratory infections such as pneumonia and bronchitis that occurred in the pediatric population of a defined geographic area, Hokkaido, in the northern part of Japan during the period of 2000 to 2001.

Patients and Methods

This was a multicenter, parallel group-trial. Sixteen geographically diverse centers in Hokkaido, in the northern part of Japan, enroled patients in the study. With regard to the proportion of urban population, age distribution, educational level, sources of living, socioeconomic status and health care services, the population is thought to be representative of that in Japan as a whole. The institutional review board of each center approved the study protocol. Informed consent was obtained for all patients from their parents before participation in the study.

To investigate the etiology of pediatric community-acquired pneumonia and bronchitis, we conducted a prospective, population-based study covering the total population <15 years of age during the April 2000 to March 2001 period. Chest radiographs were available for all cases and paired sera for serological assays were available for more than half of the cases. The following clinical specimens were also collected; nasopharyngeal swabs for viral, bacteriological, mycoplasmal and chlamydial studies, blood for serological studies and blood culture. Serological specimens and nasopharyngeal swabs were taken from more than 90% of all patients. Blood culture was taken when the patients were in febrile condition. The children were then followed up on days 3, 7, 14 and 21.

During the 12-month study period, all pediatric physicians serving the study population reported each case of suspected or confirmed pneumonia and bronchitis (community-acquired) that occurred in the study area inhabitants and filled out a simple form. All eligible patients had clinically suspected respiratory tract infections based on the presence of fever (≥38.5˚C) cough, tachypnea and nasal discharge. Pneumonia was diagnosed by the radiological finding of acute infiltrate. Bronchitis was characterized as appearance of almost similar clinical symptoms as pneumonia without lung consolidation. Roentgenographic examination of bronchiolitis showed hyperinflation of the lungs.

The microbial agents considered were *S. pneumoniae, M. pneumoniae, Moraxella catarrhalis, Haemophilus influenzae, S. pyogenes, Staphylococcus aureus, Haemophilus parainfluenzae, Candida albicans, Pseudomonas aeruginosa, Coxiella burnetii* and *C. pneumoniae*. The viruses considered were respiratory syncytial (RS) virus, rhinovirus, parainfluenza virus types 1, 2 and 3, adenovirus, cytomegalovirus, human herpesvirus 6, influenza A and B virus and coronavirus.

Diagnosis of *C. burnetii* infection was made mainly serologically by the presence of positive specific serum IgG, IgM and IgA antibodies with immunofluorescence assay (4). Detection of serum IgM antibody was performed for the diagnosis of current infection. Serum specimens for serological testing were obtained at baseline and 10 to 21 days posttherapy. Serum antibodies to *M. pneumoniae* were also determined by particle agglutination (PA), passive hemagglutination (PHA) and ELISA assays. Evidence of infection was defined as either positive serum IgM titer (≥ 1:10) at any visit or a 4-fold increase in IgG or IgA titer with ELISA. Serological study for pneumococcal antibodies was not performed because of lack of available test kits.

Nasopharyngeal swabs were collected for virological and bacteriological investigations. PCR study was also performed to detect *M. pneumoniae,* adenovirus and *C. pneumoniae* DNA (5-9). Commercially available enzyme-immunoassay (EIA) test kits were used to detect RS and influenza A virus antigens (10, 11). On the follow-up days the following data were collected; respiratory rate, chest in-drawing, temperature, general clinical state as assessed by parents and on day 14 blood was also collected for repeat serology for viral, chlamydial and mycoplasmal infections (12-14).

Differences in categorical variables between patient groups were analyzed by Fisher's exact test. The problem with multiple comparison due to several sequential statistical tests was eliminated by Bonferroni correction.

Results

Specific etiology. Of the total of 921 registered episodes, 398 were classified as pneumonia and 523 as bronchitis. Specific infecting organisms were identified in a total of 853 out of 921 (92.6%) patients (including 205 with mixed infection) with lower respiratory tract infections, as follows: *M. pneumoniae*, 252 (27.4%) patients; RS virus, 188 (20.4%); influenza A virus, 110 (11.9%); *S. pneumoniae*, 95 (10.3%); *H. influenzae*, 90 (9.8%); *S. aureus*, 29 (3.1%); *H. parainfluenzae*, 35 (3.8%); adenovirus, 27 (2.9%); *M. catarrhalis*, 12 (1.3%); *P. aeruginosa*, 7 (0.8%); *C. pneumoniae*, 6 (0.7%); and other agents, 2 (0.2%) (Table I).

Specific infecting organisms were identified in a total of 383 out of 398 (96.2%) patients with pneumonia as follows: *M. pneumoniae*, 174 (43.7%) patients; *S. pneumoniae*, 64 (16.0%); *H. influenzae,* 56 (14.0%); RS virus, 29 (7.5%); influenza A virus, 13 (3.3%); *H. parainfluenzae*, 12 (3.1%); adenovirus, 11 (2.8%); *S. aureus*; 11 (2.8%); *P. aeruginosa*, 5 (1.3%); *M. catarrhalis*, 5 (1.3%) and *C. pneumoniae*, 3 (0.8%).

Specific infecting organisms were also identified in a total of 470 out of 523 (89.9%) patients with bronchitis, as follows: RS virus, 159 (30.4%) patients; influenza A virus, 97 (18.5%); *M. pneumoniae*, 78 (14.9%); *H. influenzae*, 34 (6.5%); *S. pneumoniae*, 31 (5.9%); *H. parainfluenzae*, 23 (4.4%); *S. aureus*; 18 (3.4%); adenovirus, 16 (3.1%); *M. catarrhalis*, 7 (1.3%); *C. pneumoniae*, 3 (0.6%); *P. aeruginosa*, 2 (0.4%) and other agents, 2 (0.4%) .

Evidence of infection with one infecting organism was found in 281 (70.6%) out of 398 patients with an episode of pneumonia and in 367 (70.2%) out of 523 patients with bronchitis. Mixed infection was found in 102 (25.6%) out of 398 patients with an episode of pneumonia, as follows: infection with two organisms, 72 (18.1%) patients; with three organisms, 26 (6.5%) patients; and with four organisms, 5 (1.3%) patients. Mixed infection was also found in 103 (19.7%) out of 523 patients with an episode of bronchitis, as follows: infection with two organisms, 82 (15.7%) patients; with three organisms, 17 (3.2%) patients; and with four organisms, 4 (0.8%) patients.

The most frequent combinations were pneumococci with viruses or *M. pneumoniae*, but no statistically significant associations were found between the species or groups of species. Patients with mixed infection did not differ significantly from the other patients with respect to age, sex, presence of predefined chronic conditions, place of

Table I. *Microbiological distribution of patients with lower respiratory infections, pneumonia and bronchitis.*

	Pneumonia (n=398)	Bronchitis (n=523)	Lower Respiratory Tract Infections (n=921)
Mycoplasma pneumoniae	174 (43.7%)	78 (14.9%)	252 (27.4%)
Streptococcus pneumoniae	64 (16.0%)	31 (5.9%)	95 (10.3%)
Hemophilus influenzae	56 (14.0%)	34 (6.5%)	90 (9.8%)
RS virus	29 (7.1%)	159 (30.4%)	188 (20.4%)
Influenza A virus	13 (3.3%)	97 (18.5%)	110 (11.9%)
Hemophilus parainfluenzae	12 (3.0%)	23 (4.4%)	35 (3.8%)
Adenovirus	11 (2.8%)	16 (3.1%)	27 (2.9%)
Staphylococcus aureus	11 (2.8%)	18 (3.4%)	29 (3.1%)
Pseudomonas aeruginosa	5 (1.2%)	2 (0.4%)	7 (0.8%)
Moraxella catarrhalis	5 (1.2%)	7 (1.3%)	12 (1.3%)
Chlamydia pneumoniae	3 (0.8%)	3 (0.6%)	6 (0.7%)
Others	0 (0%)	2 (0.4%)	2 (0.2%)
Unknown	15 (3.8%)	53 (10.1%)	68 (7.4%)

Table III. *Microbiological distribution of inpatients.*

	Pneumonia (n=362)	Bronchitis (n=436)	Lower Respiratory Tract Infections (n=798)
Mycoplasma pneumoniae	157 (43.4%)	56 (12.8%)	213 (26.7%)
Streptococcus pneumoniae,	58 (16.0%)	25 (5.7%)	83 (10.4%)
Hemophilus influenzae	51 (14.0%)	28 (6.4%)	79 (9.9%)
RS virus	28 (7.7%)	146 (33.5%)	174 (21.8%)
Influenza A virus	11 (3.0%)	69 (15.8%)	80 (10.0%)
Hemophilus parainfluenzae	12 (3.3%)	23 (5.3%)	35 (4.4%)
Adenovirus	11 (3.0%)	16 (3.7%)	27 (3.4%)
Staphylococcus aureus	11 (3.0%)	18 (4.1%)	29 (3.6%)
Pseudomonas aeruginosa	5 (1.4%)	2 (0.5%)	7 (0.9%)
Moraxella catarrhalis	5 (1.4%)	7 (1.6%)	12 (1.5%)
Chlamydia pneumoniae	3 (0.8%)	3 (0.7%)	6 (0.8%)
Others	0 (0%)	2 (0.4%)	2 (0.2%)
Unknown	10 (2.8%)	41 (9.4%)	51 (6.4%)

Table II. Microbiological distribution of outpatients.

	Pneumonia (n=36)	Bronchitis (n=87)	Lower Respiratory Tract Infections (n=123)
Mycoplasma pneumoniae	17 (47.2%)	22 (25.3%)	39 (31.7%)
Streptococcus pneumoniae	6 (16.7%)	6 (6.9%)	12 (9.8%)
Hemophilus influenzae	5 (13.9%)	6 (6.9%)	11 (8.9%)
RS virus	1 (2.8%)	13 (14.9%)	14 (11.4%)
Influenza A virus	2 (5.6%)	28 (32.2%)	30 (24.4%)
Unknown	5 (13.8%)	12 (13.8%)	17 (13.8%)

treatment, presence of preceding respiratory symptoms, or presence of coexisting extrapulmonary infections (data not shown). None of the studies based on serology allowed the agents to be distinguished as primary or secondary infection.

The overall distribution by age in cases of mycoplasmal, pneumococcal, chlamydial and viral infection was consistent with the distribution of all cases of pneumonia by age, with peaks at 0-5 years. A trend toward a higher proportion at older ages was also found for mycoplasmal infections, but the difference was not statistically significant. No consistent trends by age were seen for the proportions of *S. pneumoniae*, *H. influenzae*, *H. parainfluenzae*, *S. aureus*, *M. catarrhalis*, or *Mycoplasma* infections. The proportion of patients with *Mycoplasma* infections did not increase, while that of patients with RS and influenza A viruses decreased with age, but for each age group the etiological profile was similar among inpatients and outpatients. In contrast, RS virus infection was found in 88.3% of patients aged 0-2 years and influenza A virus infection was found in 69.0% of patients aged 0-5 years.

Etiological agent by place of treatment and hospitalization rates. Mycoplasma infection was more frequent among outpatients than among inpatients (31.7% *vs.* 26.7%), a finding consistent with the accumulation of *Mycoplasma* infections in young children, who were usually treated at home (Table II). In contrast, RS virus infection was significantly more frequent among inpatients than among outpatients (21.8% *vs.* 11.4%; $p<0.05$) (Table III). Influenza A virus infection was also significantly more frequent among outpatients than among inpatients (24.4% *vs.* 10.0%; $p<0.05$). While 9.8% of outpatients and 10.4% of inpatients had pneumococcal infection, 8.9% of outpatients and 9.9% of inpatients had *H. influenzae* infection. However, no other differences between inpatients and outpatients were statistically significant.

Temporal and geographic distribution. Pneumococcal, *H. influenzae* and *M. catarrhalis* infections were observed throughout the study period. The incidence of mycoplasmal and viral infections decreased to nil in the summer season, July and August 2000 and there was a simultaneous decrease in the overall incidence of pneumonia and bronchitis.

Cases with unidentified etiology. In 68 (7.4%) of the total 921 patients, 15 (3.8%) out of 398 patients with pneumonia and 53 (10.1%) out of 523 patients with bronchitis, no evidence

was found of a specific etiological agent. Patients with infections of unidentified etiology formed no clusters by age, sex, place, or time, but they were relatively few among patients aged more than 6 years treated in hospital.

Discussion

This epidemiological study presents data on lower respiratory tract infections in Japanese children that are representative of the total population of a defined geographic area and consequently avoids selection bias. The representativity of the patients in combination with the uniform sampling and microbiological methods allowed meaningful comparisons between various groups of patients.

Although *C. pneumoniae* causes approximately 10% of general community-acquired pneumonia, its clinical symptoms in some cases are similar to those caused by other respiratory pathogens (15-18). Our results confirm many features of the etiology of lower respiratory tract infections that have been demonstrated in previous studies, in particular the high proportion of patients with mycoplasmal and viral infection (9, 16).

The significance of mixed or poly-microbial etiology has increasingly been appreciated in the literature, particularly in studies that apply new, sensitive diagnostic methods to identify infection with *Mycoplasma, Chlamydia* and viruses (19-21). *S. pneumoniae* and *M. pneumoniae* were the major etiological causes of community-acquired pneumonia in children in previous studies, together accounting for up to 60% of cases (22, 23). It is suggested that *M. pneumoniae* may predispose to secondary bacterial or viral infection.

The identification of *C. pneumoniae* infection was based on an increase in serum antibody concentration or detection of specific IgM antibodies and detection of chlamydial DNA by PCR assay (24). PA, PHA and ELISA assays were used for the diagnosis of *M. pneumoniae* infection. Although less sensitive than some newer methods, complement fixation is still widely used as the sole method for the diagnosis of viral infections apart from RS, adeno- and influenza A viruses (25). A suitable diagnostic method for studying viral respiratory tract infections in children should be the combination of direct antigen detection, detection of serum antibodies and viral isolation (26).

The etiological profile among our hospitalized children accords well with the combined information from previous studies of community-acquired pneumonia and bronchitis in essentially unselected adult patients admitted to hospital (27-29). The divergent results are partly related to endemic and epidemic causes and partly to the microbiological methods. Unlike most studies of lower respiratory tract infections, the etiology remained unidentified in a relatively small proportion of the total cases in the present study. The hospitalization rate among these patients was not higher (Table III), however, suggesting that most of the unidentified organisms caused more severe disease.

In previous studies of adult outpatients, pneumococcal infection was diagnosed in 9% to 13% of patients, which is similar to the 9.8% found by our group. *H. influenzae* infection was found in 0% to 12%, *C. pneumoniae* infection in 3% to 15%, mycoplasmal infection in 13% to 37% and viral infection in 6% to 21% of outpatients (30-31). As in the present study, trends for chlamydial and other viral infections apart from RS and influenza A viruses were not statistically significant (Table II).

RS virus sometimes cause pneumonia in young infants, but more typically cause bronchitis or bronchiolitis. *M. pneumoniae* and *C. pneumoniae* infections are common in school-aged children and are estimated to cause half of the cases or more (32). The etiological findings for younger adult patients resembled those for the older children enroled in our present study; the proportions of *Mycoplasma* and RS virus infections were, however, higher especially among children less than 5 years of age (33, 34).

In conclusion, the proportion of patients with *Mycoplasma* infections did not increase and that of patients with RS and influenza A viruses decreased with age, but for each age group, the etiological profile was completely different between inpatients and outpatients. *Mycoplasma* infections were seen even in patients less than 5 years, and RS and influenza A virus infections in patients more than 5 years of age. The results of our study confirm the importance of *M. pneumoniae* and RS virus in the etiology of community-acquired pneumonia and bronchitis in Japanese children.

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