

## Induced sputum in the diagnosis of childhood community-acquired pneumonia

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## ABSTRACT

**Background.** The usefulness of induced sputum in searching for causative agents of pneumonia in children has not been studied.

**Methods.** The study involved 101 children, aged 6 months to 15 years, treated for community-acquired pneumonia at Turku University Hospital (Turku, Finland) from January 2006 to April 2007. Nasopharyngeal aspirate samples were first collected through both nostrils. Sputum production was then induced by inhalation of 5.0 % hypertonic saline for 5–10 minutes and sputum sample was either aspirated or expectorated. The presence and amount of bacteria and viruses in paired nasopharyngeal aspirate and sputum specimens was analyzed and compared using semiquantitative bacterial culture and quantitative PCR techniques.

**Results.** A good-quality sputum specimen was obtained from 76 children. The possible causative agent was found in 90 % of the cases. *Streptococcus pneumoniae* (46 %) and rhinovirus (29 %) were the most common microbes detected. Newly discovered viruses, human bocavirus and human metapneumovirus, were detected in 18 % and in 13 % of the children, respectively. One-fourth of all bacterial findings were only detected in sputum, and the amount of bacteria in the remainder of the sputum specimens compared to nasopharyngeal aspirate was higher in 14 % and equal in 70 % of all the comparisons. The amount of rhinovirus in sputum was higher in 82 % of the comparisons.

**Conclusions.** Sputum induction provides good-quality sputum specimens with high microbiological yield in children with community-acquired pneumonia. Induced sputum analysis can be useful in the microbiological diagnosis of childhood community-acquired pneumonia.

## INTRODUCTION

The establishment of definite microbiological diagnosis of childhood community-acquired pneumonia (CAP) is necessary but challenging. Although most cases of childhood pneumonia can be successfully treated with empirical antibiotic therapy,[1-2] identification of aetiological agent/s is essential if the child is critically ill, or if the first line antibiotic therapy fails to be effective. In addition, the increasing incidence of bacterial antibiotic resistance accentuates the importance of accurate microbiological diagnosis.[3, 4]

In adults, sputum gram stain and culture are often performed to define the microbiological diagnosis of pneumonia.[5-6] In children, sputum analysis has not been considered feasible because young children cannot produce adequate sputum samples, and in contrast to adults, the nasopharyngeal colonization of *Streptococcus pneumoniae* (19–38 %), *Haemophilus influenzae* (13–25 %), *Moraxella catarrhalis* (22–39 %), and *Staphylococcus aureus* (16–36 %) is common in children.[7-12] Previous studies in children have reported the usefulness of sputum induction in the detection of microbes normally not found in the nasopharynx such as *Mycobacterium tuberculosis* and *Pneumocystis jirovecii*. [13-14]

We performed this study to investigate the virological and bacteriological yield from induced sputum specimens from children with CAP, and to study whether analysis of paired nasopharyngeal aspirate and induced sputum specimens could be useful in the microbiological diagnosis of CAP in children.

## PATIENTS AND METHODS

### Study design and patients

This study was conducted from January 2006 to April 2007 in the Paediatric Infectious Disease Ward of Turku University Hospital (Turku, Finland). Children aged 6 months to 15 years with radiologically verified CAP were eligible for the study. Pneumonia was defined as presence of pneumonic infiltrates (alveolar or interstitial) on the chest radiograph with a simultaneous finding of signs and/or symptoms of acute infection. The clinical and laboratory data were collected through a systematic review of medical records and chest radiographs were reviewed by a paediatric radiologist according to a systematic formula.[15] The findings were classified as alveolar and/or interstitial infiltrates, hyperaeration, hilar enlargement, atelectasis, and pleural fluid. Perihilar, peribronchial infiltrates were not interpreted as pneumonic infiltrates. Study children were evaluated and treated according to local guidelines by the duty paediatrician. Intravenous benzylpenicillin (penicillin G) was recommended as the first-line antibiotic therapy for hospitalized children with pneumonia in the study hospital during the study period. Immunization against *S. pneumoniae* and influenza A and B in our society was not generally recommended during the study period.

The study was approved by the Ethics Committee of the Hospital District of South-West Finland. Signed informed consent was obtained from the parent or guardian before enrolment.

## Nasopharyngeal aspirate

A nasopharyngeal sample was aspirated with a disposable catheter connected to a mucus extractor prior to sputum induction. Nasopharyngeal aspirate was taken from both nostrils by inserting the catheter into the back wall of the nasopharynx and drawing back while applying suction with an electronic suction device to obtain a nasopharyngeal sample and to clean the nasopharynx of nasopharyngeal mucus.

## Sputum induction

Children were pretreated with inhaled salbutamol (0.15 mg/kg, maximum dosage 5 mg) delivered by a nebulizer device to prevent bronchoconstriction. Sputum production was induced by inhalation of 5.0 % hypertonic saline solution for 5 to 10 minutes.[16] Hypertonic saline was delivered by a nebulizer device (Spira Moduli, Spira Oy, Hämeenlinna, Finland) attached to oxygen. A sputum sample was obtained by aspirating the nasopharynx through the nostrils with a disposable mucus extractor, or by expectoration if the child was old enough to produce an adequate sputum sample. Specimens for microbiological studies were immediately prepared by dipping one sterile Coban swab and three sterile cotton swabs into each sputum and nasopharyngeal aspirate sample. The Coban swab was placed in a Coban Amies gel agar transport system with charcoal (Coban diagnostics, Murrieta, CA, USA) for bacterial culture; one cotton swab was applied over a microscope glass slide for bacterial gram staining; two cotton swabs were placed in sterile tubes for viral antigen detection and for viral PCR analyses; and the rest of the samples were used for *M. pneumoniae* and *S. pneumoniae* PCR. Specimens were stored at +4°C until analyzed the following working day. All microbiological studies were done on both sputum and nasopharyngeal aspirate specimens.

## Bacteriology

Gram staining and bacterial culture were performed on purulent portions of nasopharyngeal aspirate and sputum specimens using standard methods. The quality of the sputum specimen was assessed by evaluating the gram stain by light microscopy under low power (10 x lens objective). Sputum samples were considered of good quality if they had <25 squamous epithelial cells and >25 leukocytes per low-powered field.[17] Good-quality specimens were further analyzed, and a finding of more than ten bacteria per high-powered field (100 x lens objective) was reported. Sputum and nasopharyngeal specimens were cultured on chocolate and blood agar plates, and the amount of bacteria in culture was semiquantitatively defined as low (+), moderate (++) or high (+++). The following organisms were considered potential pathogens: *S. pneumoniae*, *H. influenzae*, *M. catarrhalis*, *S. aureus*, beta-hemolytic streptococci, and enterococci and Enterobacteriaceae (reported only in the absence of normal respiratory flora and in case of at least moderate amount of bacterial growth). Bacterial blood cultures were done using Bactec 2400 system (Becton Dickinson, Franklin Lakes, NJ, USA) with pediatric bottles.

For bacterial PCR studies, DNA was extracted using the High Pure PCR Template Preparation Kit (Roche Diagnostics, Mannheim, Germany) in accordance with the manufacturer's protocol for bacteria. Before DNA extraction purulent samples were homogenized by incubation with 0.1% dithiothreitol in equal amounts of the sample volume. Detection of *M. pneumoniae* DNA by PCR was done as previously described with slight modifications.[18] Quantitative *S. pneumoniae* PCR on *S. pneumoniae* culture positive samples was carried out as previously described.[19] The standard curve for *S. pneumoniae* quantification was done using genomic DNA from *S. pneumoniae*

ATCC 6314D (American Type Culture Collection, Manassas, VA, USA) in tenfold dilutions ranging from 0.4 pg of pneumococcal DNA to 40 000 pg of pneumococcal DNA per reaction.

The IgM enzyme immunoassay test for *M. pneumoniae* in serum samples was done according at the discretion of the duty paediatrician using standard methods.[18]

## Virology

Nasopharyngeal and sputum specimens were eluted from the swabs by vortexing with 1 ml of phosphate buffered saline. Nucleic acids were extracted from 550- $\mu$ l aliquots of the specimens using the NucliSens easyMag automated nucleic acid extractor (BioMèrieux, Boxtel, Netherlands) with the elution volume set to 55  $\mu$ l. Quantitative RT-PCR was used to detect enteroviruses, rhinovirus, and respiratory syncytial virus (RSV) as described previously.[20] Human metapneumovirus (hMPV) was detected by quantitative PCR using the Amplitect Quantification Kit for Metapneumovirus Genomes targeting the nucleoprotein gene of hMPV and Ambigen qPCR Mastermix (AME Biosciences, Toroed, Norway) as recommended by the manufacturer. Qualitative PCR targeting the polymerase gene of hMPV was performed as previously described. [21] Quantitative human bocavirus (HBov) PCR was done as described previously.[22] Viral antigens for influenza A and B viruses, RSV, parainfluenza virus types 1, 2 and 3, and adenovirus, were detected by time-resolved fluoroimmunoassay.[23]

## Statistics

For continuous data, comparison was performed by use of the Kruskal-Wallis test, and, for categorical data, comparison was performed by use of the  $\chi^2$  test, or Fisher's exact test. The significance level was  $P < 0.05$ . Simple kappa coefficient was calculated to assess the agreement between the induced sputum and nasopharyngeal samples. Wilcoxon signed-rank test was used to compare the *S. pneumoniae* and rhinovirus densities in paired nasopharyngeal aspirate and sputum specimens.

## RESULTS

### Patient population

A total of 133 children with radiologically verified community-acquired pneumonia were eligible for the study (Figure 1). Sputum induction was performed on 101 (76%) of these children. The final study population consisted of 76 children with a good-quality sputum sample obtained. Of the good-quality sputum specimens, 79 % had  $< 10$  squamous epithelial cells and  $> 25$  leukocytes per low-powered field, and 21 % had 10–25 squamous epithelial cells and  $> 25$  leukocytes per low-powered field. The majority of the study children had a bacterial type of pneumonia as 88 % of the children had alveolar infiltration on the chest radiograph, 71 % had serum C-reactive protein (CRP) level  $\geq 60$  mg/L, and 58 % had a white blood cell (WBC) count  $\geq 15.0 \times 10^9/L$  on admission (Table 1). Sputum induction was performed previous to administration of intravenous or oral antibiotic treatment on 49 % of the children. The sputum sample was obtained by expectoration in seven (9 %) children and by aspirating through the nostrils in 69 (91 %) children. No serious adverse events were recorded during the sputum induction but children found hypertonic saline inhalation and repeated nasopharyngeal aspirations unpleasant.

Table 1. Patient characteristics and clinical findings at presentation in children with community-acquired pneumonia.

Characteristic	Children with a good-quality sputum sample (n = 76)	Children with a poor-quality sputum sample or the quality of the sputum sample not analyzed (n = 25)	Children without sputum induction (n = 32)
Age, median years (interquartile range)	3.4 (1.7–6.8)	7.5 (3.0–10.7)	4.1 (2.0–7.5)
Male, No. (%)	36 (47 %)	15 (60 %)	15 (47 %)
Underlying condition (%)	24 (32 %)	8 (32 %)	16 (50 %)
Asthma	12 (16 %)	6 (24 %)	3 (9 %)
Prematurity (gestational age $\leq$ 36 weeks)	6 (8 %)	0 (0 %)	1 (3 %)
Atopy	2 (3 %)	0 (0 %)	1 (3 %)
Congenital heart disease	2 (3 %)	0 (0 %)	2 (6 %)
Neurological disease	2 (3 %)	2 (8 %)	13 (41 %)
Ongoing antibiotic treatment on admission (%)	11 (15 %)	5 (20 %)	8 (25 %)
Macrolide	6 (8 %)	1 (4 %)	3 (9 %)
Betalactam	3 (4 %)	4 (16 %)	3 (9 %)
Other	2 (3 %)	0 (0 %)	2 (6 %)
Symptoms and signs			
Fever $\geq$ 38.0°C (%)	74 (97 %)	25 (100 %)	30 (94 %)
Cough (%)	72 (95 %)	23 (92 %)	25 (78 %)
Dyspnoea (%)	27 (36 %)	8 (32 %)	8 (25 %)
Inflammatory parameters			
CRP level (mg/L), median (interquartile range)	128 (50–212)	142 (39–259)	107 (33–238)
WBC count ( $\times 10^9/L$ ), median (interquartile range)	18.6 (9.8–25.6)	14.5 (7.2–22.6)	12.0 (9.2–19.6)
Chest radiograph finding			
Alveolar infiltrates (%)	67 (88 %)	21 (84 %)	29 (91 %)
Interstitial infiltrates (%)	11 (15 %)	4 (16 %)	3 (9 %)
Pleural fluid (%)	8 (11 %)	1 (4 %)	0 (0 %)

### Bacteria and viruses in induced sputum samples

A possible microbial aetiology determined by bacterial culture, *M. pneumoniae* PCR, and viral antigen detection and PCR assays, was identified in 68 (90 %) of the 76 children with a good-quality sputum sample (Table 2). Evidence of bacterial infection was detected in 79 % and evidence of viral infection in 55 % of the children. A sole bacterial infection was found in 34 % and sole viral infection in 11 % of the children. Mixed infections were frequent, documented altogether in 54 % of the study children. Two or more bacteria were found in 37 %; two or more viruses in 15 %; and mixed viral-bacterial infection in 45 % of the children.

*S. pneumoniae* was the most frequently isolated bacterium, detected in almost one-half of the study children, followed by *M. catarrhalis*, *H. influenzae*, and *S. aureus* (Table 2). Compared to the culture, the sensitivities and specificities of gram stain for the detection of *S. pneumoniae*, *M.*

*catarrhalis*, and *H. influenzae* in induced sputum samples were 74 % and 73 %, 62 % and 82 %, and 46 % and 83 %, respectively. Evidence of *M. pneumoniae* infection was detected in induced sputum samples by PCR in 3 % of the study children, and by IgM serology in 7 (19 %) of the 36 children tested. Altogether, evidence of *M. pneumoniae* infection was identified in 8 (11 %) children. *S. pneumoniae* was the sole bacterium detected in 22 %, *H. influenzae* in 12 %, and *M. catarrhalis* in 5 % of the children. Previously given antibiotic treatment had a significant influence on the detection rate of *S. pneumoniae* and *H. influenzae* in sputum samples (Table 2).

Rhinovirus was the most common virus detected, identified in 29 % of the children by PCR (Table 2). HBoV was found in sputum by PCR in 14 (18 %) children and hMPV in 10 (13 %) children with pneumonia. Of the 14 children who had HBoV detected in sputum, 8 (57 %) had a high load of HBoV DNA (>10 000 copies/ml) in sputum suggesting a primary symptomatic infection.[24] RSV was identified only in four percent of the children.

The most frequently detected viral-bacterial combinations, found in 15 % and in 13 % of the children, were those of rhinovirus with *S. pneumoniae* and rhinovirus with *M. catarrhalis*, respectively. The most common bacterial combination was that of *S. pneumoniae* and *M. catarrhalis*, found in 16 % of the study children.

Table 2. Bacteria and viruses in induced sputum in children with community-acquired pneumonia.

Microbe	All children (n = 76) No. (%)	Children without preceding antibiotic treatment (n = 37) No. (%)	Children with preceding antibiotic treatment (n = 39) <sup>a</sup> No. (%)
<b>Bacterium</b>			
<i>Streptococcus pneumoniae</i> <sup>b</sup>	35 (46 %)	23 (62 %)	12 (31 %) <sup>c</sup>
<i>Haemophilus influenzae</i>	22 (29 %)	5 (14 %)	17 (44 %) <sup>d</sup>
<i>Moraxella catarrhalis</i>	21 (28 %)	11 (30 %)	10 (26 %)
<i>Staphylococcus aureus</i>	9 (12 %)	5 (14 %)	4 (10 %)
<i>Mycoplasma pneumoniae</i> <sup>e</sup>	2 (3 %)	1 (3 %)	1 (3 %)
Other bacterium <sup>f</sup>	3 (4 %)	2 (5 %)	1 (3 %)
Normal/mixed flora	11 (14 %)	5 (14 %)	6 (15 %)
Neg	2 (3 %)	0 (0 %)	2 (5 %)
Total	60 (79 %)	30 (81 %)	30 (77 %)
<b>Virus</b>			
Rhinovirus	22 (29 %)	13 (35 %)	9 (23 %)
Human bocavirus	14 (18 %)	4 (11 %)	10 (26 %)
Human metapneumovirus	10 (13 %)	3 (8 %)	7 (18 %)
Respiratory syncytial virus	3 (4 %)	0 (0 %)	3 (8 %)
Enteroviruses	2 (3 %)	1 (3 %)	1 (3 %)
Parainfluenzae type 3 virus	1 (1 %)	1 (3 %)	0 (0 %)
Influenza A virus	1 (1 %)	0 (0 %)	1 (3 %)
Influenza B virus	1 (1 %)	1 (3 %)	0 (0 %)
Adenovirus	0 (0 %)	0 (0 %)	0 (0 %)
Parainfluenzae type 1 virus	0 (0 %)	0 (0 %)	0 (0 %)
Parainfluenzae type 2 virus	0 (0 %)	0 (0 %)	0 (0 %)
Total	42 (55 %)	17 (46 %)	25 (64 %)

<sup>a</sup>Antibiotic treatment was as follows: penicillin G 68 %; penicillin G and macrolide or clindamycin 13 %; intravenous cephalosporin 9 %; intravenous cephalosporin and macrolide or clindamycin 3 %; and oral amoxicillin or macrolide 7

% . Sputum induction was performed previous to administration of one intravenous antibiotic dosage in 9 children, after two intravenous antibiotic dosages in 21 children, after three intravenous antibiotic dosages in 3 children, and after oral antibiotic treatment in 6 children.

<sup>b</sup>In one child, *Streptococcus pneumoniae* was also isolated from blood culture.

<sup>c</sup> $p = 0.006$  by  $\chi^2$  test, comparison between children with preceding antibiotic treatment and those without.

<sup>d</sup> $p = 0.004$  by  $\chi^2$  test, comparison between children with preceding antibiotic treatment and those without.

<sup>e</sup>*Mycoplasma pneumoniae* was detected by PCR.

<sup>f</sup>*Streptococcus agalactiae*, *Streptococcus pyogenes*, *Pseudomonas* like gram-negative rod.

### Findings in paired sputum and nasopharyngeal aspirate samples

There was a substantial (kappa value 0.61–0.80) or almost perfect (kappa value 0.81–1.00) agreement between the sputum and nasopharyngeal aspirate samples in most bacterial and viral findings (Table 3). The only exceptions were hMPV which had moderate agreement, and *S. aureus* which had poor agreement between the samples, as eight of the nine *S. aureus* findings were only detected in sputum. In total, 24 % of the bacterial findings were only detected in sputum specimens.

Table 3. Bacteria and viruses in paired sputum and nasopharyngeal aspirate samples.

Microbe	Bacterium or virus found in:			Simple kappa coefficient
	Nasopharyngeal mucus only	Sputum only	Nasopharyngeal mucus and sputum	
<b>Bacterium</b>				
<i>Streptococcus pneumoniae</i>	7	5	30	0.68
<i>Haemophilus influenzae</i>	1	0	22	0.97
<i>Moraxella catarrhalis</i>	4	4	17	0.74
<i>Staphylococcus aureus</i>	2	8	1	0.11
<i>Mycoplasma pneumoniae</i>	0	0	2	1.00
<b>Virus</b>				
Rhinovirus	2	0	22	0.94
Human bocavirus	2	3	11	0.78
Human metapneumovirus	3	4	6	0.58
Respiratory syncytial virus	0	0	3	1.00
Enteroviruses	0	0	2	1.00
Influenza A virus	0	0	1	1.00
Influenza B virus	0	0	1	1.00
Parainfluenzae type 3 virus	0	0	1	1.00

Semiquantitative culture results were analyzed to compare the amount of bacteria in paired sputum and nasopharyngeal aspirate samples. The amount of bacteria in sputum specimens was higher in 14 % and equal in 70 % of all the comparisons. The amount of bacteria in *S. pneumoniae* culture positive samples was also quantified by quantitative pneumolysin PCR. The amount of pneumococci in sputum was higher in 45 % of the cases ( $p = 0.891$ ). Rhinovirus was detected in higher amounts in sputum by quantitative RT-PCR in 82 % of all rhinovirus positive cases ( $p = 0.020$ ).

### Clinical findings according to aetiology

Children with *S. pneumoniae* in sputum had higher WBC counts on admission than those without *S. pneumoniae* in sputum (means  $\pm$  SD,  $21.7 \pm 8.2$  vs.  $15.3 \pm 8.1$ ;  $p < 0.001$ ). They also had higher CRP levels on admission (means  $\pm$  SD,  $161 \pm 105$  vs.  $115 \pm 96$ ) but this difference was not statistically significant. Typical findings for pneumococcal pneumonia i.e. high body temperature

( $\geq 39.0$  °C), leukocytosis (WBC count  $\geq 15.0 \times 10^9/L$ ), and high CRP level (CRP  $\geq 60$  mg/L) were found in 66 % of the study children with *S. pneumoniae*. Children with concomitant rhinovirus/pneumococcal infection (n = 11) had the highest mean WBC counts ( $24.2 \times 10^9/L$ ) and mean CRP levels (181 mg/L) on admission.

Treatment failure (fever  $\geq 38.0^\circ\text{C}$  lasting for  $\geq 48$  hours) was recorded in 6 (8 %) of the 76 children. A potential causative agent/s of pneumonia was identified in five children: *S. aureus* and *M. pneumoniae* in one; *S. aureus* with bocavirus and RSV in one; *H. influenzae* and bocavirus in one; *H. influenzae* and *M. catarrhalis* in one; and bocavirus in one child.

## DISCUSSION

The present study has three major findings. First, sputum induction in children with CAP provides good-quality sputum specimens. Second, the bacteriological and virological yield of sputum specimens is good. Third, the quantification results of the paired sputum and nasopharyngeal aspirate specimens provide support that the majority of the bacteria and viruses found in sputum most probably originated from the lower airways.

The Infectious Diseases Society of America (IDSA) and American Thoracic Society (ATS) guidelines recommend routine use of sputum gram stain and culture for adult patients needing intensive care unit admission, for patients with failure of outpatient antibiotic therapy, and for patients with pleural effusion.[6] In children with pneumonia, routine sputum analysis has not been recommended because young children cannot produce adequate sputum samples, and nasopharyngeal colonization with bacteria causing pneumonia is common in healthy children.[7-12] We aimed to overcome these obstacles by inducing the sputum production with hypertonic saline inhalation and by trying to minimize nasopharyngeal contamination by cleaning the nasopharynx of the mucus prior sputum collection.

Our finding that *S. pneumoniae* was detected in one-half of all study children, and in two-thirds of children without previous antibiotic treatment, once again confirms the major role of *S. pneumoniae* in the aetiology of childhood pneumonia.[25-27] *M. catarrhalis* was identified in almost one-third of the children, but 81 % of these cases were mixed bacterial infections, suggesting that in most cases *M. catarrhalis* may probably have been just an innocent bystander. In two previous studies in our hospital, *M. catarrhalis* infection was serologically detected in 4 %–10 % of children with community-acquired pneumonia.[26, 28] An unexpected finding was that *S. aureus* was detected in good-quality sputum specimens in 12 % of the children. All but one of these *S. aureus* cases were found in induced sputum only. In addition, two children with *S. aureus* in sputum failed to respond to first line antibiotic therapy (penicillin G), suggesting that these findings may have had true clinical implications.

There is increasing evidence of the importance of rhinovirus in lower respiratory tract infections in children and adults.[29, 30] Previous studies employing sensitive polymerase chain reaction (PCR) methods have detected rhinovirus in nasal samples in 24 % – 45 % of children with pneumonia.[26, 31] The corresponding detection rate by culture has been approximately 10 %.[28, 32] In the present study, rhinovirus was clearly the most common viral finding, detected by PCR in the sputum of 29 % of the children. The mere presence of rhinovirus in sputum, however, offers no direct evidence of the aetiology of pneumonia as 82 % of the children with rhinovirus had evidence of concomitant bacterial infection. An interesting finding was that mixed rhinovirus/pneumococcal infection seemed to induce a more severe inflammation response than sole rhinovirus or sole pneumococcal infection which is in agreement with the recent results by Jennings et al. that mixed rhinovirus/pneumococcal infection is associated with severe disease in adults.[30]

The role of newly discovered viruses, HBoV and hMPV, in the etiology of pneumonia is not fully established. In our study, these new viruses accounted for up to 42 % of the all viral cases detected. HBoV was detected in sputum samples in 18 % of the children, and hMPV in 13 %. This is in agreement with recent findings of Cilla and co-workers who detected HBoV and hMPV in nasopharyngeal aspirate samples in 14 % and 12 % of children with pneumonia, respectively.[33] In accordance with previous studies, we found a high viral co-infection rate (64 %) in children with HBoV.[33, 34] The viral co-infection rate in children with hMPV was 40 %. Whether the detection of these new respiratory viruses in children with pneumonia reflects a primary infection or

persistence of the virus after a previous infection can not be determined. However, 4 of 10 hMPV cases, and 3 of 14 HBoV cases were only detected in sputum specimens suggesting that in these cases HBoV and hMPV most probably originated from the lower respiratory tract.

RSV was found rarely, which is explained by the fact that this study was conducted during a time period when there was no RSV epidemic in the society; the winter epidemic in 2006 had just ended and the spring epidemic in 2007 had not yet started.[35]

We are aware of the limitations of our study. The major question is whether the sputum specimens are representative samples from the lower respiratory tract. Although only good-quality sputum specimens were included in the final analysis, we can not rule out the possibility that the sputum specimens may have been contaminated with nasopharyngeal bacteria. The diagnostic accuracy of induced sputum could not be determined using a gold standard, as lung aspirates were not collected and blood culture was positive only in one child. In addition, control specimens from children with upper respiratory tract infection were not collected because we do not consider sputum induction in these children ethically acceptable. Furthermore, half of the children had received antibiotic treatment before sputum collection.

The strength of the present study is that the amount of bacteria and viruses in paired nasopharyngeal aspirate and sputum samples were quantified and compared. We found that one-fourth of all bacterial findings were only detected in sputum suggesting that in these cases induced sputum analysis most probably identified the causative agent of pneumonia. In most cases the same bacterium or virus was detected in both nasopharyngeal aspirate and sputum. In these cases, the amount of bacteria in sputum determined by semiquantitative culture was equal or higher in 84 % of all the paired comparisons, and the amount of viruses in sputum by quantitative rhinovirus-, enterovirus-, RSV-, HBoV-, and hMPV -PCR was higher in 73 %. It is of note that these results appeared even though the nasopharynx was carefully cleaned of the mucus prior sputum induction and collection. However, it must be stressed that sampling was not quantitatively precise.

In conclusion, a good-quality sputum specimen can be obtained by sputum induction in the majority of children with CAP, and the microbiological yield of these induced sputum specimens is good. However, our observations do not support routine use of induced sputum analysis for all children with CAP since children found the hypertonic saline inhalation and repeated nasopharyngeal aspirations unpleasant. In addition, most children were successfully treated with empirical antibiotic therapy. In accordance with the IDSA and ATS guidelines for adults, we think that induced sputum analysis could be beneficial in children needing intensive care unit admission, in children with failure of first-line antibiotic therapy, and in children with pleural effusion.[6]

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## FIGURE LEGENDS

Figure 1. Flow diagram of study patients and sputum specimens.

