MAJOR ARTICLE

Microbiology of Bronchoalveolar Lavage Fluid in Children With Acute Nonresponding or Recurrent Community-Acquired Pneumonia: Identification of Nontypeable *Haemophilus influenzae* as a Major Pathogen

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Background. Precise etiologic diagnosis in pediatric community-acquired pneumonia (CAP) remains challenging.

Methods. We conducted a retrospective study of CAP etiology in 2 groups of pediatric patients who underwent flexible bronchoscopy (FOB) with bronchoalveolar lavage (BAL); children with acute nonresponsive CAP (NR-CAP; $n = 127$) or recurrent CAP (Rec-CAP; $n = 123$). Procedural measures were taken to limit contamination risk and quantitative bacterial culture of BAL fluid (significance cutoff point, $\geq 10^4$ colony-forming units/mL) was used. Blood culture results, serological test results, nasopharyngeal secretion findings, and pleural fluid culture results were also assessed, where available.

Results. An infectious agent was detected in 76.0% of cases. In 51.2% of infections, aerobic bacteria were isolated, of which 75.0%, 28.9%, and 13.3% were *Haemophilus influenzae*, *Moraxella catarrhalis*, and *Streptococcus pneumoniae*, respectively. Most (97.9%) of the *H. influenzae* strains were nontypeable (NTHi). *H. influenzae* was detected in 26.0% of NR-CAP cases and 51.2% of Rec-CAP cases, whereas *Mycoplasma pneumoniae* was the predominant pathogen in the NR-CAP group (accounting for 34.9% of cases) but not in the Rec-CAP group (19.3%). Viruses were found in 30.4% of cases, with respiratory syncytial virus, parainfluenzaviruses, and influenzaviruses detected most frequently. Mixed infections were found in 18.9% of NR-CAP cases and 30.1% of Rec-CAP cases.

Conclusions. A variety of microorganisms were isolated with frequent mixed infection. NTHi was one of the major pathogens found, especially in association with recurrent CAP, possibly because of improved detection with the FOB with BAL procedure. This suggests that the burden of pediatric CAP could be reduced by addressing NTHi as a major causative pathogen.

Pneumonia results from the host inflammatory response to infection of the distal airways and the lung alveoli [1]. The clinical and radiological presentations of community-acquired pneumonia (CAP) are diverse and might reflect a variety of microbiological etiologies [2, 3]. This was suggested by the epidemiology of CAP in Canada after the introduction of universal vaccination with the 7-valent pneumococcal conjugate vaccine (PCV7), which showed no decrease in non-lobar pneumonia despite an important decrease in lobar pneumonia [4].

There are few comprehensive studies of the microbiological etiology of CAP in children [5], mainly because of difficulties in obtaining reliable samples [6].

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Direct sampling from the lung and lower airways can only be achieved with invasive methods, such as transthoracic needle aspiration (TNA) and puncture of pleural effusion [7]. In adults, sputum samples are considered to be a valuable, noninvasive alternative for bacterial detection, but it is often not feasible to obtain a sputum sample from children. Therefore, most pediatric studies are based on samples obtained from the upper respiratory tract (URT) and blood samples. However, the URT is frequently colonized by bacterial pathogens [3, 7], making it difficult to discriminate commensal organisms and acute pathogens. Moreover, although blood is normally sterile and blood cultures are highly specific, the sensitivity of blood cultures in childhood CAP is low (4%–18%) [6, 7]. Serological tests for the detection of viruses and atypical microorganisms are of limited use in clinical practice because of the need for convalescent-phase serum samples.

Flexible bronchoscopy (FOB) with bronchoalveolar lavage (BAL) is an option if noninvasive samples from the lower respiratory tract (LRT) are impossible to obtain or for selected patients with nonresponse to antibiotic treatment [8, 9]. BAL fluid has the advantage of being suitable for multiple detection methods, including microscopy, aerobic culture, viral culture, and polymerase chain reaction (PCR). Protected brushes cannot pass the pediatric flexible bronchoscope, but the risk of oropharyngeal contamination can be reduced considerably with careful technique [10]. Interpretation of BAL results is more reliable with the use of quantitative bacterial culture [1, 7, 8, 11, 12]. The reliability of FOB plus BAL used with these precautions has been demonstrated, with some authors reporting specificities of 97%–100% for potential pathogens in relation to actual pneumonia [7, 13]. Moreover, FOB plus BAL has been proven to be safe, even in critically ill children [8, 14].

In this retrospective study, we assessed the etiology of CAP in 2 groups of pediatric patients who underwent FOB plus BAL: children with acute nonresponsive CAP and children with recurrent CAP. Among children, these CAP features account for a significant proportion of physician and emergency department visits, hospitalizations, and antibiotic prescribing in the community. In addition to BAL fluid culture, blood cultures, serological testing, culture and PCR results of nasopharyngeal aspirates of nasopharyngeal secretions (NPA), and pleural fluid culture were assessed, where available.

METHODS

Study Design

This was a retrospective analysis of pediatric patients who underwent FOB plus BAL from January 2005 through December 2007. The study was approved by the ethics committee of Universitair Ziekenhuis Brussel (UZ Brussels, Belgium). Patients selected for analysis had acute nonresponsive community-acquired (broncho)pneumonia (NR-CAP group) or recurrent community-acquired (broncho)pneumonia (Rec-CAP group). NR-CAP case patients were defined according to current pediatric CAP guidelines [15] as patients with persistent fever (>38.5°C) and persistent elevated infection parameters in the peripheral blood, associated in some cases with worsening consolidation visible on chest radiographs after at least 48 h of antibiotic treatment. The Rec-CAP group had an episode of CAP with a history of at least 2 other CAP episodes. Exclusion criteria included the presence of severe and chronic conditions (such as cystic fibrosis, primary ciliary disease, immune deficiency, bronchopulmonary dysplasia, cardiopathy, neuromuscular diseases, asplenia, and tuberculosis) and nosocomial respiratory tract infection.

Retrospective analyses were conducted of demographic data, antibiotic use before FOB plus BAL, and the results of a broad microbiological investigation.

Sampling Procedures

FOB and BAL were performed according to European Respiratory Society recommendations [8]. A pediatric flexible fiberoptic bronchoscope (BF3C20 Olympus) was inserted orally to avoid nasal contamination. Aliquots of saline solution (0.9%) were instilled and reaspirated in the diseased lobar or segmental bronchus (maximum volume, 3 mL/kg body weight). The first bronchial lavage fraction was discarded. All subjects were sedated with intravenous midazolam, atropinesulphate, and tramadol hydrochloride; were transcutaneously monitored for oxygen saturation and heart rate; and received supplementary oxygen as needed during the procedure.

Blood cultures and serological testing were performed according to routine procedures when CAP was diagnosed, before starting antibiotic treatment. NPA was performed routinely in children <4 years of age with CAP with use of a flexible sterile catheter (Vaginal Catheter Ch.8; Medinorm) introduced into a nostril up to the nasopharynx. At this level, saline solution (2 mL; 0.9%) was instilled and immediately reaspirated. Pleural fluid was obtained from patients with a significant amount of pleural fluid for safe puncture, using routine disinfection and puncture methods.

Microbiology

Samples were processed using standard techniques and interpreted as listed in Table 1. For *Haemophilus influenzae* biotyping and capsular typing using a PCR and agglutination technique were performed at Centre Hospitalier Universitaire St Pierre, Brussels. Nontypeable *H. influenzae* (NTHi) strains were distinguished from nonhemolytic *Haemophilus haemolyticus* [16, 17]. β-lactamase activity was assessed using a nitrocephin-based test, and antibiotic susceptibility was determined by the Etest technique (Biomérieux). Serotyping and antibiotic susceptibility testing of *Streptococcus pneumoniae* was performed as described elsewhere [18].

Table 1. Overview of Microbiological Investigations Performed on Samples and Diagnostic Criteria

Specimen	Infectious agent	Assay	Diagnostic criteria
BAL fluid	Aerobic bacteria	Culture	$\geq 10^4$ cfu/mL (cut-off value)
	Viruses	Culture	Growth
	Respiratory viruses[a]	Multiplex PCR	Positive
	Atypical[b]	Multiplex PCR	Positive
NPA	Viruses	Culture	Growth
	Respiratory viruses[a]	Multiplex PCR	Positive
	Atypical[b]	Multiplex PCR	Positive
Blood	Aerobic bacteria	Culture	Growth
	Viruses[c]	Complement fixation	Acute titer ≥ 120[d] or 4-fold increase
	Mycoplasma pneumoniae	Complement fixation	Acute titer ≥ 320[d] or 4-fold increase
Pleural fluid	Bacteria	Culture	Growth

NOTE. BAL, bronchoalveolar lavage; cfu, colony-forming units; NPA, nasopharyngeal aspirate; PCR, polymerase chain reaction.

[a] Influenzavirus A and B; parainfluenza virus types 1, 2, 3; respiratory syncytial virus; human metapneumovirus subtypes A and B; coronavirus 229E and OC43.

[b] *M. pneumoniae* and *Chlamydophila pneumoniae*.

[c] Serological testing for influenzavirus A and B, adenovirus, picornavirus, and respiratory syncytial virus.

[d] Reverse titer.

Viral culture and an in-house multiplex PCR (mPCR) were used on BAL fluid and NPA to detect respiratory viruses and atypical microorganisms (*Mycoplasma pneumoniae* and *Chlamydophila pneumoniae*). Serological tests were performed by the complement fixation technique.

Statistical Analysis

The χ^2 test with Yate's correction or, when one of the subjected figures was <5, the Fisher's exact test were used to compare positivity rates between the 2 groups. Statistically significant differences were defined as those for which the probability of occurrence was $\leq 5\%$.

RESULTS

Study Population

Of the 700 children who had FOB plus BAL, 250 children—127 with NR-CAP and 123 with Rec-CAP—met the inclusion criteria. The median age and sex ratio in both groups were similar (Table 2). Most patients with NR-CAP (77%) had received antibiotic treatment within 48 h before FOB plus BAL, whereas most patients with Rec-CAP (70%) had not been treated (Table 2).

Microbiology

A pathogen was detected in approximately three-quarters of children in both groups (Table 3). Exclusively bacterial pathogens were found in 20.5% of the NR-CAP group and 38.2% of the Rec-CAP group, and mixed infection was found in 18.9% and 30.1%, respectively. All bacterial pathogens except 3 *S. pneumoniae* were isolated from BAL specimens, whereas only 2 blood cultures and 2 pleural fluid cultures were positive. In both patients with positive blood cultures, *S. pneumoniae* was isolated from the blood cultures, whereas *H. influenzae* and *M. catharralis* were isolated from BAL specimens from the same patients. In 1 patient, *Staphylococcus aureus* was isolated from pleural fluid culture, whereas BAL yielded the same organism in combination with *Enterobacter aerogenes*. In the latter case, *S. pneumoniae* was isolated from the pleural fluid specimen, but BAL and blood cultures remained negative.

Table 2. Patient Characteristics and Antibiotic Use in the 48 h Before Flexible Bronchoscopy With Bronchoalveolar Lavage

Group	No. of patients	Age, months		Sex, %		Receipt of antibiotic $\leq$48h before flexible bronchoscopy with bronchoalveolar lavage, no. (%) of patients	
		Median	Range	Female	Male	Yes	No
NR-CAP	127	33.1	1.0–171.6	49	51	98 (77)	29 (23)
Lobar pneumonia	53	37.1	5.0–171.6	47	53	46 (87)	7 (13)
Bronchopneumonia	64	29.1	1.0–136.5	50	50	42 (66)	22 (34)
Pneumonia and pleural effusion	10	47.7	3.0–93.3	50	50	10 (100)	0 (0)
Rec-CAP	123	33.1	4.0–170.6	43	57	29 (24)	86 (70)[a]

NOTE. NR-CAP, nonresponding community-acquired pneumonia; Rec-CAP, recurrent community-acquired pneumonia.

[a] Antibiotic use unknown in 8 children.

Table 3. Distribution of Pathogens Detected in Both Groups

Variable	No. (%) of patients		
	NR-CAP (n = 127)	Rec-CAP (n = 123)	P [a]
Patients with ≥1 pathogen detected	95 (74.8)	95 (77.2)	NS
Exclusively aerobic bacteria	26 (20.5)	47 (38.2)	.003
Exclusively viruses	21 (16.5)	9 (7.3)	.04
Exclusively atypical microorganisms	24 (18.9)	4 (3.2)	<.001
Mixed infection	24 (18.9)	37 (30.1)	NS
Patients with no pathogen detected	32 (25.1)	28 (22.8)	NS

NOTE. NR-CAP, nonresponding community-acquired pneumonia; NS, not significant; Rec-CAP, recurrent community-acquired pneumonia.

[a] By χ^2 test.

Overall, aerobic bacteria were isolated from 128 (51.2%) of 250 patients with infection; 75.0% of the aerobic bacteria isolated were *H. influenzae*. Bacterial pathogens were found significantly more often in patients with Rec-CAP than in patients with NR-CAP (Table 3), but in both groups, *H. influenzae* was the predominant bacteria, being isolated in 26.0% of NR-CAP cases and 51.2% Rec-CAP cases (Table 4). *H. influenzae* was the sole pathogen isolated in 12.6% of the NR-CAP cases and 17.8% of the Rec-CAP cases.

Among bacterial isolates, *H. influenzae* was the sole bacterial pathogen isolated in 66 (68.8%) of 96 cases (27 NR-CAP cases and 39 Rec-CAP cases) (Table 4). In the remaining cases, *H. influenzae* was found with another bacterial pathogen (*Moraxella catarrhalis* in 15 cases, *S. pneumoniae* in 6 cases, and *S. aureus* in 3 cases). *H. influenzae* was found in 21 mixed bacterial-viral infections, 11 mixed bacterial-atypical infections, and 5 mixed infections with *Mycoplasma* species and a respiratory virus (Table 5).

Of the 96 *H. influenzae* isolates, 94 (97.9%) were NTHi, and 2 were lost for typing. Three isolates were initially misidentified by agglutination technique as being encapsulated strains, 2 type e and 1 type b. However, PCR results were negative in all 3 cases, resulting in a definitive identification as NTHi.

The majority of *H. influenzae* isolates (77.1%) were β-lactamase negative, including 20 of 33 isolates from NR-CAP cases and 54 of 63 isolates from Rec-CAP cases. Two β-lactamase–negative isolates were intermediate susceptible to ampicillin (minimum inhibitory concentration [MIC], 2 mg/L), but no β-lactamase–negative, fully ampicillin-resistant strain was detected.

After *H. influenzae*, *M. catarrhalis* and *S. pneumoniae* were the next most common bacterial pathogens, accounting for 28.9% and 13.4% of cases, respectively, in which aerobic bacteria were isolated. *M. catarrhalis* was isolated in 8.7% of NR-CAP cases and 21.1% of Rec-CAP cases, whereas *S. pneumoniae* was isolated in 6.3% of NR-CAP cases and 7.3% of Rec-CAP cases (Table 4). Although *S. pneumoniae* was isolated in only 17 cases, a variety of pneumococcal serogroups-serotypes (SGT) was found, with a predominance of SGT 19, 23, and 6, which were isolated in 4, 2, and 2 cases, respectively. All 17 *S. pneumoniae* isolates were fully susceptible to parenterally administrated penicillin (MIC, ≤2 μg/mL), whereas 3 isolates were intermediate susceptible to orally administrated penicillin (MIC, 0.12–1 μg/mL).

M. *catarrhalis* and *S. pneumoniae* were mostly found with another bacterial pathogen in 26 (70.3%) of 37 and 12 (70.6%) of 17 cases, respectively (Table 4). Mixed infections were found in 14 (37.8%) of 37 *M. catarrhalis* and 7 (41.2%) of 17 *S. pneumoniae* cases (Table 5).

Positive bacterial cultures were obtained significantly more often from patients who did not receive antibiotic treatment 48 h before FOB plus BAL than from patients who received antibiotic treatment; a positive bacterial culture was found in 83 of 115 cases (21 [72.4%] of 29 patients with NR-CAP and 62 [72.1%] of 86 patients with Rec-CAP) who received no antibiotics, compared with 40 of 127 patients pretreated with antibiotics (26 [26.5%] of 98 patients with NR-CAP and 14 [48.3%] of 29 patients with Rec-CAP) (P <.001).

Atypical microorganisms alone were detected significantly more often in patients with NR-CAP than in patients with Rec-CAP (Table 3). *M. pneumoniae* was detected in 34.9% of the samples from patients with NR-CAP that were tested and in 19.3% of the samples from patients with Rec-CAP that were tested, and *C. pneumoniae* was detected in 5.6% of Rec-CAP cases (Table 4). Viruses were found in 30.4% of cases overall. Exclusive viral infection was reported in 16.5% of NR-CAP cases and 7.3% of Rec-CAP cases (Table 3); overall, respiratory syncytial virus (RSV), parainfluenzaviruses, influenzaviruses, adenovirus, and human metapneumovirus were detected most frequently (Table 4). Viral coinfection was found in 11 (8.7%) of 127 NR-CAP and 5 (4.1%) of 123 Rec-CAP cases.

DISCUSSION

We assessed 250 otherwise healthy children with either NR-CAP or Rec-CAP to determine the etiology of the infection. A heterogeneous group of patients was included, which reflected pediatric CAP cases that often require hospitalization in developed countries.

An infectious agent was detected in 76% of cases, compared with detection in 17%–91% of cases in previous studies of pediatric CAP [5, 14, 19, 20]. However, most of these studies involved URT samples, and thus had limited specificity for detecting bacterial pathogens, with few studies involving samples of the LRT or lung aspirates [3, 21, 22]. Among the aerobic bacteria, which were isolated in approximately half of infections,

Table 4. Overview of Pathogens Detected

Pathogen	NR-CAP (n = 127)			Rec-CAP (n = 123[a])			P [b]
	Mono culture	Coinfection	Total, no. (%) of patients	Mono culture	Coinfection	Total, no. (%) of patients	
Aerobic bacteria	33	14	47 (37.0)	54	27	81 (65.8)	<.001
Haemophilus influenzae	27	6	33 (26.0)	39	24	63 (51.2)	<.001
Moraxella catarrhalis	2	9	11 (8.7)	9	17	26 (21.1)	.009
Streptococcus pneumoniae	2	6	8 (6.3)	3	6	9 (7.3)	NS
Staphylococcus aureus	2	3	5 (3.9)	0	5	5 (4.1)	NS
Escherichia coli	0	1	1 (0.8)	1	1	2 (1.6)	NS
Enterobacter species	0	2	2 (1.6)	1	0	1 (0.8)	NS
Pseudomonas aeruginosae	0	0	0 (0)	0	1	1 (0.8)	NS
β-hemolytic streptococci	0	0	0 (0)	0	2	2 (1.6)	NS
Haemophilus haemolyticus	0	2	2 (1.6)	1	1	2 (1.6)	NS
Viruses	30	11	41 (32.3)	30	5	35 (28.5)	NS
Respiratory syncytial virus	8	7	15 (11.8)	6	1	7 (5.7)	NS
Parainfluenzaviruses	5	1	6 (4.7)	8	1	9 (7.3)	NS
Influenzaviruses	5	3	8 (6.3)	2	2	4 (3.3)	NS
Adenovirus	5	1	6 (4.7)	1	3	4 (3.3)	NS
Human metapneumovirus	3	1	4 (3.1)	4	1	5 (4.1)	NS
Picornavirus	2	4	6 (4.7)	1	0	1 (0.8)	NS
Coronaviruses	1	3	4 (3.1)	2	0	2 (1.6)	NS
Cytomegalovirus	1	1	2 (1.6)	2	2	4 (3.3)	NS
Enterovirus (not polio)	0	0	0 (0)	4	1	5 (4.1)	NS
Rhinovirus	0	1	1 (0.8)	0	0	0 (0)	NS
Atypical microorganisms	37			19			.015
Mycoplasma pneumoniae			37/106 tested (34.9)			16/83 tested (19.3)	.03
Chlamydophila pneumoniae			0/58 tested (0)			3/54 tested (5.6)	NS

NOTE. NR-CAP, nonresponding community-acquired pneumonia; Rec-CAP, recurrent community-acquired pneumonia; coinfection: cases in which at least 2 infective agents of the same microbiological group were isolated; NS, not significant.

[a] n = 122 for viruses.

[b] By χ^2 test.

we found a predominance of NTHi in both groups of children; approximately three-quarters were *H. influenzae,* of which 97.9% were NTHi. To our knowledge, this is the first such reported finding since those of Shann et al [22] and Weinberg et al [23] in the 1980s. Since then, studies involving children with CAP or LRT infection (using serological testing or TNA) have suggested a possible role for NTHi in pediatric pneumonia but have failed to define it as a significant pathogen [24]. For example, in a recent study involving 34 children with CAP, 16 cases were attributed to *S. pneumoniae* and no case of *H. influenzae* infection was found using TNA [25]. However, the study included a small number of patients and investigated lobar CAP only. It is also possible that NTHi infection was missed in previous studies of pediatric CAP because the methodology was not appropriate for NTHi detection. NTHi does not easily invade the bloodstream or pleura [22, 26], and misclassification of NTHi strains as Hib strains using the slide agglutination technique has been reported [27]. Also, in many TNA studies, the culture media used were not appropriate for detecting *H. influenzae* and, if *H. influenzae* was found, strains were not serotyped [22].

The reliability of our finding that NTHi is frequently involved as a pathogen in childhood CAP is supported by the sterility of the LRT in healthy subjects and the finding that, in children with unilateral CAP, BAL culture of the contralateral side has negative results [10, 12], as well as by the established pathogenicity of NTHi in another normally sterile site, the middle ear. NTHi is associated with persistent, nonresponsive acute otitis media (AOM) [28, 29], possibly attributable to the choice of treatment (amoxicillin is used as first-line treatment), leading to the selection of β-lactamase–positive *H. influenzae* [29]. This was not the case in our study, because 77% of NTHi isolates were β-lactamase negative. In AOM, NTHi has a clear association with recurrence >1 month after the completion of antibiotic therapy, with an incidence of 32% in third and subsequent episodes [29]. NTHi infection contributes to complications in AOM and is clinically indistinguishable from AOM caused by *S. pneumoniae* [29]. It is therefore possible

Table 5. Distribution of Pathogens in Mixed Infections

Pathogen(s)	NR-CAP		Rec-CAP	
	Mono culture	coinfection[a]	Mono culture	coinfection[a]
Bacteria and virus	8	3	16	7
Haemophilus influenzae and virus	6	1	10	4
Adenovirus	2	…	…	…
RSV	1	…	2	…
Influenzavirus A	1	…	…	1
Parainfluenzavirus	2	…	4	1
hMPV	…	1	1	…
Cytomegalovirus	…	…	1	…
Parainfluenzavirus/ adenovirus	…	…	1	…
Enterovirus/ influenzavirus B	…	…	1	…
hMPV/adenovirus/ influenzavirus B	…	…	…	1
Adenovirus/CMV	…	…	…	1
Streptococcus pneumoniae and virus	…	1	1	1
hMPV	…	1	1	…
coronavirus OC43	…	…	…	1
Moraxella catarrhalis and virus	1	2	2	3
RSV	1	…	…	…
Coronavirus OC43	…	1	…	1
hMPV	…	1	1	…
Coronavirus 229E	…	…	1	…
Parainfluenzavirus	…	…	…	2
Staphylococcus aureus and virus	1	2	…	3
RSV/influenzavirus A	1	…	…	…
hMPV	…	1	…	…
Parainfluenzavirus	…	…	…	2
Coronavirus OC43	…	1	…	…
hMPV/adenovirus/ influenzavirus B	…	…	…	1
Other bacteria and virus	…	…	3	3
Atypical microorganism and bacteria	3	1	6	3
Mycoplasma pneumoniae and				
H. influenzae	2	1	5	2
S. pneumoniae	…	1	…	…
M. catarrhalis	…	…	1	2
S. aureus	1	…	…	…
Chlamydophila pneumoniae and				
H. influenzae	…	…	…	1
S. pneumoniae	…	…	…	1
M. pneumoniae and virus	3	0	2	0
Bacteria and atypical microorganism and virus	4	2	3	0
H. influenzae and *M. pneumoniae* and virus	3	…	2	…
Influenzavirus A	1	…	…	…
RSV	1	…	…	1
Parainfluenzavirus	1	…	…	…
Adenovirus/ parainfluenzavirus	…	…	…	1
S. pneumoniae and *M. pneumoniae* and virus	…	…	2	…
Influenzavirus B	…	…	1	…
Parainfluenzavirus and RSV	…	…	1	…
M. catarrhalis and *M. pneumoniae* and virus	…	1	1	…
Influenzavirus A/ coronavirus OC43	1	…	…	…
RSV/parainfluenzavirus	…	1	…	…
RSV	…	…	1	…

NOTE. hMPV, human metapneumovirus; NR-CAP, nonresponding community-acquired pneumonia; Rec-CAP, recurrent community-acquired pneumonia; RSV, respiratory syncytial virus.

[a] Coinfections are mentioned for each bacterial pathogen.

that, as in AOM, NTHi is not an innocent bystander in the LRT. Indeed, it is now increasingly recognized that NTHi is capable of causing invasive disease, particularly neonatal sepsis, and is also a cause of CAP, bacteremia and, to a lesser extent, meningitis [30, 31].

Moreover, we are confident that the risk of contamination in BAL samples was limited considerably, because various technical measures were taken to avoid contamination by URT secretions and a significance cutoff value was used for bacterial BAL culture [1, 8, 10–12]. Although a significance cutoff of $\geq 10^3$ colony-forming units (cfu)/mL has been shown to be reliable in adults [7, 32], we have chosen to use a higher cutoff value of $\geq 10^4$ cfu/mL, because colonization of the URT is more common in children. The finding that only 2.8% of the *Haemophilus* strains detected in our study were nonhemolytic *H. haemolyticus*, whereas previous studies showed that the prevalence of nonhemolytic *H. haemolyticus* in throat swab samples obtained from healthy children or adults was 16%–21% [16, 33], also supports the reliability of our results.

Other typical bacterial pathogens for pneumonia, such as *M. catarrhalis* and *S. pneumoniae*, were detected in 28.9% and

13.3% of cases, respectively, in which aerobic bacteria were isolated. Bacterial coinfections were reported in several earlier studies, including studies that used TNA [3, 21, 22]; most such coinfections were due to *S. pneumoniae* and *H. influenzae,* although coinfections due to *H. influenzae* and *M. catarrhalis*, as recorded in our study, were also reported [22]. Although the role of each isolated pathogen cannot be defined, previous findings of coinfection and recent insights into interspecies quorum signaling suggest an individual role for each pathogen and/or possible interaction between them [34].

In Belgium, universal childhood vaccination against *H. influenzae* type b (Hib) was introduced in the 1990s and might explain the absence of any case of infection due to Hib in our study. However, because universal childhood pneumococcal vaccination using PCV7 was only started in 2007, it is very unlikely that it may have influenced our results.

The proportion of cases with an identified etiology was comparable between both groups. Bacterial etiology was found more often in Rec-CAP cases than in NR-CAP cases, probably because only approximately a quarter of patients with Rec-CAP received antibiotics before FOB plus BAL [35]. In antibiotic-pretreated patients, the diagnostic yield might have been increased with the use of bacterial serological testing [36] or quantitative PCR [37].

The relatively low detection rates for *M. pneumoniae* and *C. pneumoniae* may be related to the young age of the patients, because atypical infections are most common in children >5 years of age [20, 38, 39]. *M. pneumoniae* was a predominant pathogen in the NR-CAP group but not in the Rec-CAP group. Most of the patients with NR-CAP had been treated with penicillin or aminopenicillins, the first-line antibiotics for treatment of CAP, and the predominance of *M. pneumoniae* gives a possible reason for nonresponsiveness. Viruses were found in 30.4% of cases, with RSV, parainfluenzaviruses, and influenzaviruses being detected most frequently. This incidence is relatively low, compared with the findings of other studies [5, 20], and may have been due to omission of rhinovirus in the PCR assay. Virus detection was slightly more frequent in the NR-CAP group. Viruses were the sole pathogen more than twice as often in the NR-CAP group than in the Rec-CAP group, possibly providing another explanation for nonresponsiveness to initial antibiotic therapy. Viral coinfections, which were found in 6.4% of cases overall, are rarely reported in most studies of CAP, although one study reported an incidence of 14% for all CAP cases and 22% for viral CAP cases [5]. Mixed infections were found in 24.4% of cases, which was comparable to previous findings [19, 20, 40].

Our findings are compatible with current guidelines that recommend antibiotic treatment for all CAP cases, because an established viral or atypical etiology does not rule out a concomitant bacterial infection [15, 39]. The predominance of NTHi in Rec-CAP cases in our study suggests the use of aminopenicillins with or without β-lactamase inhibitors for the initial treatment in recurrent cases. In case of nonresponsiveness, the addition of macrolides to the initial treatment regimen is supported by the relatively high incidence of *M. pneumoniae* infection in NR-CAP cases.

In conclusion, as in previous studies, we found that a variety of microorganisms were involved in pediatric nonresponsive and recurrent CAP with frequent mixed infection. In contrast to other reports, we found NTHi to be a major bacterial pathogen, possibly because of improved detection with FOB plus BAL. This finding might explain cases of nonresponsiveness to antibiotic treatment or recurrence in pediatric CAP, similar to observations in AOM, which is frequently associated with NTHi infection. Additional research is needed to elucidate the pathogenic mechanisms of NTHi in pediatric CAP. However, our findings suggest that the burden of pediatric CAP could be reduced by addressing NTHi as a major causative pathogen in treatment and prevention strategies.

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