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Epidemiology and microbiological investigations of community-acquired pneumonia in children admitted at the emergency department of a university hospital

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ARTICLE INFO

ABSTRACT

Background: The management of children with community-acquired pneumonia (CAP) is largely influenced by the development of new molecular diagnostic tests that allow the simultaneous detection of a wide range of pathogens.
Objectives: Evaluation of a diagnostic approach including multiplex PCR assays for revisiting the epidemiology and etiology of CAP in children at hospital.
Study design: Children of all ages consulting at the Emergency Department of the University hospital of Saint-Etienne, France, during the 2012–2013 winter period were included. In addition to bacterial cultures, the following pathogens were detected using biplex commercially-available rt-PCR tests: adenovirus, respiratory syncytial virus, human metapneumovirus, bocavirus, rhinovirus/enterovirus, coronavirus, influenza viruses A and B, parainfluenza viruses, *Mycoplasma pneumoniae* and *Chlamydophila pneumonia.*
Results: From 85 patients with CAP, at least one pathogen was identified in 81 cases (95.3%), including 4 bacterial exclusive infections (4.7%), 53 viral exclusive infections (62.4%) and 24 mixed infections (28.2%). Coinfection by at least two viruses was observed in 37 cases (43.5%). Mean age was higher in the case of documented bacterial infection ($P < 0.05$). In the subgroup of viral exclusive infection, the mean age of severe cases was 2.0 years vs 3.8 years in mild and moderate cases ($P < 0.05$).
Conclusions: These findings highlight the huge proportion of CAP of viral origin, the high number of co-infection by multiple viruses and the low number of bacterial CAP, notably in children under 5 years, and address the need to re-evaluate the indications of empiric antimicrobial treatment in this age group.

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1. Background

Community-acquired pneumonia (CAP) is the leading cause of death in children under five years of age in the world [1]. In developed countries, the systematic prescription of antimicrobial drugs to patients with CAP has led to a dramatic reduction in mortality linked to this pathology [2,3]. However, a bacterial origin of CAP has not been documented in a large proportion of cases despite extensive aetiological investigations. The current recommendations [4–6] encourage pediatricians to prescribe a probabilistic antimicrobial treatment, even when no bacterial infection is documented, which results in prolonged use of antibiotics and in the possible selection of resistant strains within the endogenous flora [7].

Until the beginning of the current century, the absence of documented bacterial infection was attributed to the difficulty in obtaining deep respiratory specimens that are not contaminated by bacteria from the local flora [8] together with the lower sensitivity of blood cultures in proving bacterial sepsis [9]. At this time, most of the epidemiological data from pediatric CAP relied

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on traditional bacteriological cultures. With the occurrence of new diagnostic tools and notably of multiplex PCR assays able to simultaneously detect a large panel of viruses and atypical bacteria, it now appears that a large proportion of CAP could be related to viral infection [10–12]. Many studies have evaluated these new tools but most of them were limited to subgroups of children notably to the young [13,14], to hospitalized children [11,13–17], or for selected pathogens [10,12,18,19].

2. Objective

The aim of the present study was to document the presence of a large variety of pathogens in respiratory specimens from children attending the Pediatric Emergency Department of the University hospital of Saint-Etienne, France, during a six-month period and presenting a CAP based on clinical and radiological evidence. The microbiological diagnostic approach combined bacterial cultures and biplex commercially available rt-PCR tests detecting a wide range of respiratory pathogens.

3. Study design

3.1. Clinical data

A single center epidemiological observational study was conducted over a six-month period (November 2012 to April 2013) on children aging from one month to 16.5 years and presenting with CAP at the Pediatric Emergency Department of the University hospital of Saint-Etienne, France. The study was submitted for approval to the local Ethics Committee. After oral information was given together with a form explaining the content of the research, a consent form was signed by a parent or legal tutor before inclusion of each patient.

A CAP case was defined [6] as a subject with fever of at least 38.5 °C, an age-corrected polypnea [20] and a chest radiograph showing images of acute pneumonia confirmed by a second examiner (a pediatric radiologist for ambulatory children or a pediatrician for hospitalized patients). A few subjects were excluded after this second reading, notably in the case of associated bronchiolitis.

The data collected at inclusion comprised the demographic characteristics of the child, their vaccine status, the smoking habits of parents, the date of the beginning of the current respiratory episode and the drugs, including antimicrobials, that they received during this period. According to the current guidelines [4–6], a pneumonia was defined as severe for this study if the patient presented at least one of the following criteria: respiratory rate above 70 per minute in infants less than 1 year of age and above 50 per minute for older children, a tachycardia adjusted to age, a capillary refill time >3 s and an oxygen saturation <92%.

A control visit was systematically carried out at days 2 and 5 either by phone call for ambulatory-treated children or after a physical examination in the service of hospitalization for children admitted to hospital.

3.2. Biological investigations

A number of biological parameters were recorded systematically, including C reactive protein (CRP), procalcitonin (PCT), white blood cell count and natremia.

Nasopharyngeal secretions obtained by sputum induction [21,22] were sampled at entry for all the participants. The following tests were performed at inclusion: standard detection of conventional bacteria by culture, detection of five different viruses (respiratory syncytial virus (RSV), influenza viruses A and B, parainfluenza viruses, metapneumovirus and adenovirus) by indirect immunofluorescence (IFI) assay and detection of atypical bacteria by PCR as previously described [23].

In parallel, blood cultures and pneumococcal antigenuria were tested if prescribed by the clinician, notably in the case of hospitalization.

In addition to the test listed above that were performed at the time of hospital attendance, a rtPCR assay was performed at the end of the study on an aliquot fraction kept frozen at −80 °C for the whole panel of nasopharyngeal aspirates, as previously described [24]. Briefly, 200 μl of aspirate was extracted on NUCLISENS® easyMAG™ (bioMérieux, Marcy l'Etoile, France). The Respiratory Multi Well System (MWS) r-gene™ (Chla/Myco pneumo, Influenza A/B, RSV/hMPV, AD/hBoV, HCoV/HPIV, and Rhino&EV/CC) from bioMérieux was used for molecular testing. It consists of a series of biplex assays detecting either a couple of pathogens or a single pathogen and a cell control (CC) attesting for the presence of cellular nucleic acids within the specimen. The following pathogens were tested: *Mycoplasma pneumoniae* and *Chlamydophila pneumoniae*, influenza viruses A and B, RSV and human metapneumovirus (hMPV), adenovirus (ADV) and bocavirus (BoV), parainfluenza viruses and coronaviruses, rhinovirus/enterovirus (hREV) and a cell control.

3.3. Statistical comparison of bacterial and non-bacterial cases

An univaried analysis was performed to compare the cases documented as probably related to a bacterial infection (threshold of 10^7 CFU/ml for conventional cultures [25,26] or the presence of atypical bacterium by PCR in nasopharyngeal aspirates), and the others. Comparisons adjusted for age, severity of pneumonia and mono/multiple infection were also performed. The chi-square test or the Fisher exact test was used to compare qualitative variables whereas the Student t test was used for quantitative variables. A P value of 0.05 was considered as statistically significant.

A multivariate analysis of factors independently associated with detection of bacterial was secondarily performed; the parameters included in the logistic regression model were those with $P < 0.10$ by univaried analysis.

4. Results

4.1. Clinical characteristics of included cases

Over the six-month period of the study, 95 children thought to have CAP were included; 10 of them were excluded secondarily, comprising 7 cases with non-CAP infection, 2 cases without nasopharyngeal aspirate and one case of CAP whose inclusion was not consented by the child's family. With reference to the total number of cases of CAP recorded over the same period in the Pediatric Emergency Department ($n = 97$), the representation rate was of 87.6% (85/97).

The demographic and clinical characteristics of the 85 included cases together with the mode of management (ambulatory or hospital) and the probabilistic antimicrobial treatment are listed in Table 1.

Apyrexia was observed in 85.9 and 98.8% of cases at day 2 and 5, respectively. From the 35 children hospitalized at entry, 33 (94.3%) and 5 (14.3%) were still hospitalized at day 2 and 5, respectively. Only one child needed intensive care within the Pediatric Intensive Care Unit. The antimicrobial treatment was modified in only three cases. Neither fatal cases nor immediate sequelae were observed during the study. Twenty-six cases (30.6%) were classified as severe CAP.

Table 1
Demographic and clinical characteristics of the 85 patients included in the study.

Characteristics (unit)	Numerical data
Median age (years)	2.8
Interquartile range (years)	1.5–5.7
Categories by age	
Infants (<2 years old) – No. (%)	30 (35.3)
Preschool children (2 – 5 years old) – No. (%)	32 (37.6)
Children (5 – 12 years old) – No. (%)	19 (22.3)
Teenagers (12 – 18 years old) – No. (%)	4 (4.7)
Gender – No. females (%)	39 (46)
Vaccinal status	
Up-to-date pneumococcal vaccine – No. (%)	61 (71.7)
Up-to-date *Haemophilus influenzae* B vaccine – No. (%)	68 (80)
Medical history	
Previous hospitalization – No. (%)	17 (20)
Pulmonary disease – No. (%)	26 (30.5)
- Bronchiolitis – No. (%)	9 (10.5)
- Asthma – No. (%)	9 (10.5)
- Pneumonia – No. (%)	4 (4.7)
Parental smoking habits	
No smoking parent – No. (%)	44 (51.7)
At least one smoking parent – No. (%)	28 (32.8)
Medication before emergency's consultation	
None – No. (%)	16 (18.8)
Acetaminophen – No. (%)	58 (68.2)
Non-steroidal anti-inflammatory drug – No. (%)	15 (17.6)
Antimicrobial treatment– No. (%)	18 (21.2)
Average duration of symptoms before emergency consultation (days)	4
Radiological localization of lesions	
Right upper lobe – No. (%)	12 (14.1)
Right lower lobe – No. (%)	21 (24.7)
Right middle lobe – No. (%)	13 (15.3)
Left upper lobe – No. (%)	8 (9.4)
Left lower lobe – No. (%)	24 (28.2)
Interstitial syndrome[a] – No. (%)	13 (15.3)
At least one criterion of severe pneumonia at entry	26 (30.6)
Hospitalization at entry – No. (%)	36 (42.4)
Antimicrobial treatment	
Amoxicillin – No. (%)	50 (58.8)
Co-amoxiclav – No. (%)	18 (21.2)
Erythromycin – No. (%)	8 (9.4)
Amoxicillin + erythromycin – No. (%)	4 (4.7)
None – No. (%)	4 (4.7)
Others – No. (%)	1 (1.2)

[a] Interstitial syndrome was associated with lobar lesions in 6 cases and was the only radiological sign in 7 cases.

The general biological characteristics of the included cases were as follows: the median count of leukocytes was 14.7 10^9 per liter (interquartile range 10.84–20.08), with 66.5% of neutrophils; the CRP median rate was of 64.5 mg/L (interquartile range: 19–163), the PCT median rate was of 0.87 μg/L (interquartile range: 0.17–5.14); an hyponatremia (<135 mEq/L) was observed in 23 cases (27%).

4.2. Microbiological results

By using IFI prospectively, only 26 of the 85 nasopharyngeal aspirates (30.6%) were found positive, including 17 RSV, 9 ADV and 4 influenzavirus B; all of these infections were confirmed by the retrospective rt-PCR assay. The pneumococcal antigenuria, available in 60 cases, was positive in 13 patients, 3 of them exhibiting a threshold of 10^7 CFU/ml by conventional culture. In the three other pneumococcal infections, no antigenuria was available. Blood culture, performed in 38 cases (44%), was found positive for *Streptococcus pneumoniae* in only one case.

By combining bacterial culture and retrospective rt-PCR assays, at least one pathogen was identified in 81 cases (95.3%). The number of detected pathogens was of 0, 1, 2, 3, 4 and 5 in 4 (4.7%), 30

Table 2
Distribution of the 85 cases of CAP according to the number of detected bacteria and viruses.

Number of bacteria	Number of viruses				
	0	1	2	3	5
0	4	28	22	2	1
1	2	10	10	2	–
2	2	1	1	–	–

(35.3%), 34 (40.0%), 13 (15.3%), 3 (3.5%) and 1 (1.2%) specimens, respectively (Table 2). From the 85 cases of CAP, 4 bacterial exclusive infections were observed (4.7%), 24 infections with at least one bacterium and one virus (28.2%) and 53 viral exclusive infections (62.4%). Coinfection by at least two viruses was observed in 37 cases (43.5%). Table 3 displays the distribution of the identified pathogens and specifies the various associations, the most common being ADV/RSV (10 cases), *Haemophilus influenzae*/ADV (8 cases) and hREV/BoV (8 cases).

The monthly distribution of microbiological infections is depicted in Fig. 1.

4.3. Statistical analysis

As shown in Table 4, none of the variables tested was statistically correlated to the presence of a bacterial pathogen by univaried analysis, with the exception of age that was higher in the case of documented bacterial infection (mean age of 5.45 vs 3.49 years; $P < 0.05$ by Student t test) and the presence of abdominal pain at clinical examination at entry ($P = 0.02$). Concerning biological parameters, no correlation was observed between bacterial and non-bacterial cases for the most of them, notably for CRP and PCT, with the exception of the white blood cell count that was higher in case of viral infection. Severity was statistically associated neither to the bacterial or non bacterial etiology of CAP, nor to a younger age, except in the subgroup of viral exclusive infection ($n = 53$), in which the mean age of severe cases was 2.0 years vs 3.8 years in mild and moderate cases ($P < 0.05$ by Student t test). Coinfection was not associated to a younger age or a more severe disease, even if the number of detected pathogens tended to be related to the severity of CAP (2.1 infected agents in severe cases vs 1.7 in non-severe cases, $P = 0.09$ by Student t test).

By multivariate analysis, none of the tested variables was independently associated with bacterial infection.

5. Discussion

The demographic characteristics of the included patients were coherent with those of the literature [6] in terms of age (median age of 2.8 years; interquartile range: 1.5–5.7 years) and sex ratio (1.18 to the benefit of males). Those patients with CAP were shown to exhibit different risk factors as illustrated by a lower vaccination coverage as compared to that of the French population, an elevated rate of previous hospitalization (17/85, 20.0%), a history of frequent respiratory disease (26/85; 30.6%) and a high level of smoking habits in parents (Table 1).

Approximately 4 children out of 5 reached hospital without having consulted another physician; most of them had already received an antipyretic treatment, mainly acetaminophen but also non-steroidal anti-inflammatory drugs (NSAID), despite the fact that the use of NSAID may be harmful [27].

At entry, 95.3% (81/85) of children had received an antimicrobial treatment. In most cases, the first choice for antimicrobial drug was amoxicillin, as currently recommended [4–6].

Despite the limited size of the present study and its restriction to a single center, its originality lies in the diversity of the included

Table 3
Detailed presentation of cases of community-acquired pneumonia exhibiting an infection with at least 2 pathogens. Gray boxes indicate the total number of positive cases for each pathogen. White boxes corresponds to the cases with co-infection.

Infectious agents	ADV	RSV	BoV	Hi	IVB	hMPV	HREV	Mc	Spn	PIV	IVA	Mp	Spy	Mn	hCoV
Adenovirus (ADV)	32														
Respiratory syncytial virus (RSV)	10	28													
Bocavirus (BoV)	4	5	18												
Haemophilus influenza (Hi)	8	6	0	11											
Influenzavirus (IVB)	3	1	1	1	11										
Human metapneumovirus (hMPV)	5	1	1	2	0	11									
Human rhinovirus or enterovirus (HREV)	2	3	8	1	0	1	11								
Moraxella catarrhalis (Mc)	2	1	4	0	2	0	2	9							
Streptococcus pneumonia (Spn)	2	0	1	1	1	1	1	1	6						
Parainfluenza virus (PIV)	1	1	2	0	0	0	0	0	0	5					
Influenzavirus A (IVA)	2	0	1	1	0	1	1	1	0	0	5				
Mycoplasma pneumonia (Mp)	2	1	0	0	0	0	0	0	0	0	0	4			
Streptococcus pyogenes (Spy)	0	0	0	0	0	0	0	1	0	0	0	0	1		
Moraxella nonliquefaciens (Mn)	1	1	0	1	0	0	0	0	0	0	0	0	0	1	
Coronavirus (CoV)	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
Total of coinfections (%)	26 (81.2)	19 (67.9)	18 (100)	11 (100)	6 (54.5)	10 (90.9)	6 (54.5)	9 (100)	5 (83.3)	4 (80)	4 (80)	3 (75)	1 (100)	1 (100)	1 (100)

population in terms of age and mode of management (ambulatory and hospitalization), together with the detection of a large range of pathogens including viruses and bacteria.

From a microbiological point of view, it is first useful to justify the definition of what level of detection constitutes a causative agent in children with CAP included in this study. Concerning bacterial loads, the threshold of 10^7 CFU/ml was retained as recommended by European experts when induced sputum specimen are used [25]. It helps the discarding of pneumococcal colonization with prevalence of up to 57–65% in children of less than 5 years old [28,29] from true infection. By contrast, "the asymptomatic carrier state of viruses is rather uncommon for most respiratory viruses [12,30], apart from the post active phase of respiratory virosis" [25], which justifies to consider the detection of viral genome in respiratory specimens as a marker of probable viral infection.

In the more exhaustive studies performed earlier, the percentage of elucidated cases from a microbiological point of view ranged from 72% to 86% [6,11,30] whereas it was of 95.3% in the present study detecting a wide spectrum of infectious agents with respiratory tropism. As reported earlier [30], viral infections were shown to be predominant in children under 5 years of age and only 32% of all CAP were found to be of bacterial origin.

Eighteen children received antimicrobial therapy before emergency consultation, which could be considered as a source of false-negative culture. However, all of them were always symptomatic at entry, which implies that a bacterium, if present, had a significant opportunity to be recovered by culture.

An interesting finding of this study is the large proportion of viral coinfection (43.5%), much higher than that previously reported [10,12,30], notably for bocavirus, metapneumovirus and adenovirus that were detected in association with at least one other virus in more than 80% of the CAP cases involving these agents (Table 2). It has been suggested that infection by several viruses could enhance the severity of CAP [12,30–32]. In the present study, a trend was observed in the association between the mean number of infectious agents and the severity of CAP as defined above ($P = 0.09$); a larger
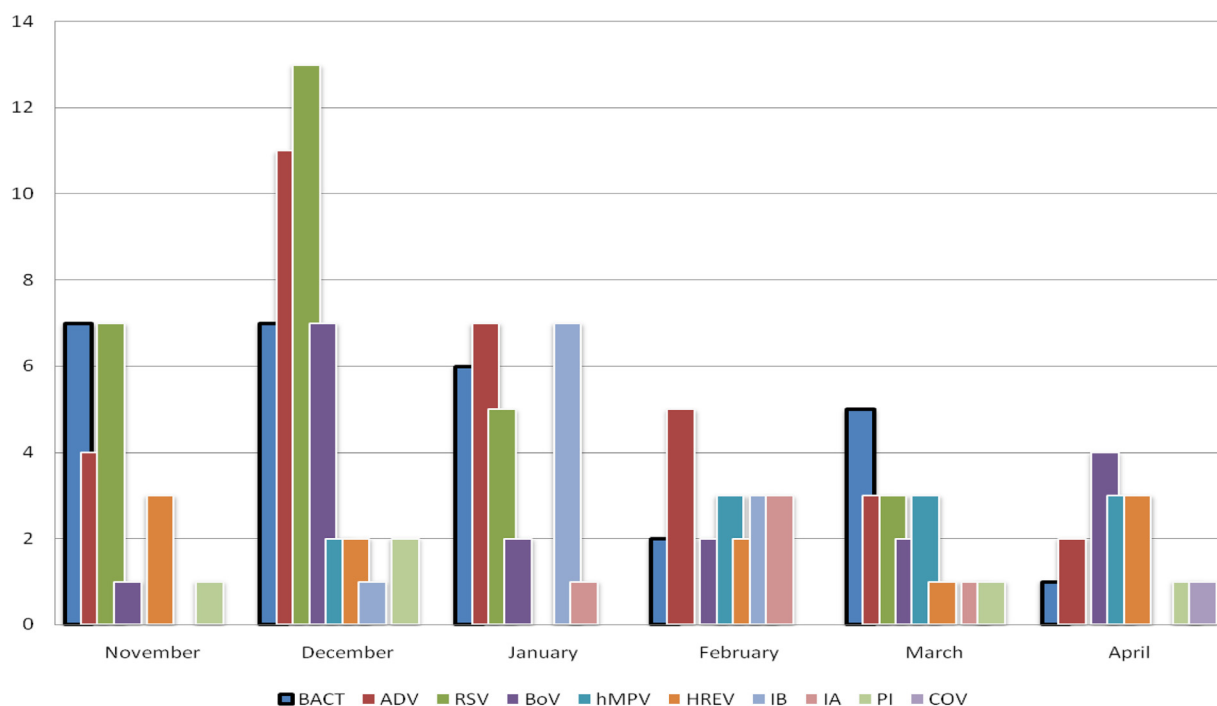

Fig. 1. Monthly distribution of microbiological infection. BACT: bacteria. ADV: adenovirus; RSV: respiratory syncytial virus; BoV: bocavirus; hMPV: human metapneumovirus; HREV: human rhinovirus and enterovirus; IB: influenzavirus B; IA: influenzavirus A; PI: parainfluenza virus; COV: coronavirus.

Table 4
Data from individuals with versus without bacterial infection.

Characteristics (unit)	Bacterial infection ($n = 28$)	Non bacterial infection ($n = 57$)	p[a]
Mean age (years)	5.45	3.49	0.0210
Gender (No. females)	13	26	1
Vaccinal status (No.)			
Up-to-date pneumococcal vaccine	17	44	0.3744
Up-to-date *Haemophilus influenzae* B vaccine	24	44	1
Medical history (No.)			
Previous hospitalization	7	10	0.5647
Pulmonary disease	7	19	0.4662
Parental smoking habits (No.)			
At least one smoking parent	8	20	0.6105
Medication before emergency's consultation (No.)			
None	6	11	1
Acetaminophen	19	41	0.6176
Non-steroidal anti-inflammatory drug	5	10	1
Corticosteroids	3	2	0.3526
Average duration of symptoms before emergency consultation (days)	4.3	3.9	0.4829
At least one criterion of severe pneumonia at entry (No.)	10	16	0.6170
Hospitalization at entry	9	26	0.2466
Still hospitalized at day 2	10[b]	23	0.8137
Clinical examination at entry (No.)			
Asthenia	21	38	0.4662
Chest pain	4	7	1
Abdominal pain	11	9	0.0279
Cough	22	45	1
Wheezing	5	9	1
Crackles	11	11	0.0656
Biology at entry			
White blood cells (G/L)	13.45	17.67	0.0181
Polynuclears neutrophils (%)	66.7	62.3	0.3607
Platelets (G/L)	293	350	0.0513
CRP (mg/L)	105	111	0.8366
Procalcitonin (µg/L)	6.3	4.9	0.5783
Natremia (mEq/L)	135.2	136.3	0.0809

[a] Fisher's exact test for categorical data and Student t-test for continuous values.
[b] By comparaison to the precedent line, a patient was hospitalized after coming back home.

effective size would have been needed to determine a statistically significant difference.

In terms of clinical evolution, neither death nor major complications were reported in this study, despite rates of 30.6% for severe pneumonia and 42.4% for hospitalization. There was no statistical difference in the severity of CAP between viral and bacterial infections. Almost all of the children received antibiotics, which was unnecessary in a large proportion of cases (62.4%) for which only viruses were detected. This finding raises the question of the systematic use of antimicrobials to treat childhood CAP, which is still recommended in different guidelines [4–6].

The present findings, together with those of others, allowed the identification of a subpopulation of children less than 5 years of age with mild or moderate symptoms for which a viral etiology of CAP is highly probable. This situation represents half of the cases recorded in this study: 62 children (72.9%) were less than five-years old, and 40 of them presented a mild or moderate CAP, which corresponds to 47.1% of all cases. The use of a rapid molecular test detecting a large set of viral and bacterial pathogens within 2 or 3 hours, such as that described in [33], would allow an improvement in the management of the antimicrobial treatment. In the case of positive result, it would be recommended to avoid the use of amoxicillin as a first-intent therapy (or to prescribe erythromycin in the case of detection of an agent of atypical pneumonia) and to reconsider the use of antimicrobial treatment 24–48 h later according to the clinical evolution and to the results of bacterial cultures. Conversely, the negativity of the rapid test would lead to the empiric prescription of amoxicillin, as currently recommended [4–6]. It is obvious that this attitude would be dedicated to CAP with mild or moderate symptoms and that CAP with severe presentation at entry should include a systematic probabilistic antimicrobial

therapy, whatever the results of PCR assay. The present results are indicative that this strategy could dramatically reduce the proportion of unnecessary antimicrobial treatments in mild or moderate child CAP. Wider studies are needed to prospectively evaluate the benefits of this approach in terms of patient recovery, prevention of antibiotic resistance and medical economy.

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