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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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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.

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.