Etiological diagnosis of pneumonia –
a critical view

Joaquim C. Rodrigues, Luiz V.F. Silva Filho, Andrew Bush

Abstract

Objectives: to search literature related to the etiological diagnosis of acute pneumonia in children.
Sources: systematic review of Medline and Lilacs databases.

Summary of the findings: the use of new diagnostic methods such as immunological techniques and polymerase chain reaction has proven invaluable for specific diagnosis and epidemiological investigation, showing adequate sensitivity, specificity and promptness of results, with the aim of guiding therapy properly. Review of epidemiological studies of community acquired pneumonia showed that Streptococcus pneumoniae is still one of the most significant etiologic agents in all age groups, in developing and industrialized countries. Resistance of this agent to penicillin and cephalosporins is increasing in all continents and is worrisome. Atypical agents such as Mycoplasma pneumoniae and Chlamydia pneumoniae are common in community acquired pneumonia, mainly in children older than 4 years, representing one third of the cases in industrial countries. However, their prevalence in developing countries remain to be determined. Respiratory syncytial virus is also a very common etiology of community acquired pneumonia and may cause severe infections, mainly in infants and younger children. The introduction of new conjugated vaccines for Streptococcus pneumoniae and Haemophilus influenzae type b resulted in significant reduction of morbidity and mortality of pneumonia in children.

Conclusions: a significant impact on morbidity and mortality of acute pneumonia in children is likely to occur if microbiological and antimicrobial control is continuously and dynamically performed, thus allowing for the development of new vaccines, particularly against the respiratory syncytial virus.


Introduction

Acute respiratory infections are an important cause of morbidity and mortality of children on a worldwide basis, especially in developing countries.1,2 Severe lower respiratory tract infections, chiefly those which affect children younger than five years, are the major determinants of mortality.2 The annual incidence of pneumonias in children younger than five years is 30 to 40 cases per 1,000 live births in Europe and in North America.3 In developing countries, childhood pneumonias are not only more common, but they are also more severe, causing greater mortality.3 The data published by the World Health Organization (WHO) show that, in the last decade, approximately one third of the world’s infant mortality (four to five million deaths a year) was caused by acute respiratory infections.2 The United Nations Children’s Fund (UNICEF) estimates that over three million children die from pneumonia every
Methods for the etiologic diagnosis of acute pneumonias

The etiologic diagnosis of acute community-acquired pneumonias still poses a great challenge, despite the major medical breakthroughs, given the wide variety of agents involved and the difficulty in obtaining representative material from the airways. Specific clinical signs, upper airway culture, laboratory rates of inflammation and peculiar radiological findings are poorly correlated with the etiologic agent. In addition, the difference between colonization and infection is still a hurdle to be overcome; and there are no reliable methods to be routinely used in the identification of some relevant etiologic agents of community-acquired pneumonias in children.

The methods for the etiologic diagnosis of acute community-acquired pneumonia may be divided into microbiological, immunological and DNA detection.

Microbiological methods

The classic microbiological method for pathogen culture has been widely used, but its validation basically depends on the site of origin of the cultured material, since the culture of upper airway samples does not satisfactorily show the colonization of the pneumonic focus in the lower airways. This lack of correlation between the cultures of the upper and lower airways often occurs for pathogens such as *S. pneumoniae* or *H. influenzae*, but there are some exceptions, as is the case of cystic fibrosis, in which there is a good correlation between sputum cultures and oropharyngeal smears and lung tissue specimens or bronchoalveolar lavage. The advantage in culturing bacterial pathogens is that we can assess the susceptibility of the pathogen to various antimicrobials, which contributes to therapeutic decision. Another limitation of the microbiological method concerns the difficulty in culturing pathogens such as *M. pneumoniae*, *C. pneumoniae* and *L. pneumophila*, which have slow growth or are too demanding as to the type of culture medium.

The most representative materials of pneumonic focus include lung biopsy specimens (obtained through thoracotomy or needle biopsy), pleural fluid, blood (showing the presence of bacteremia) and bronchoalveolar lavage, provided the criteria for quantitative culture and the adequate collection methodology are followed (protected lavage).

**Open lung biopsy**

Lung tissue cultures by means of open lung biopsy are obtained only in two special situations, as in the cases of severe community-acquired pneumonias with a bad outcome, despite empirical therapeutics, and severe pneumonias in immunocompromised patients or severe nosocomial pneumonias with etiologic agent of unknown origin. This type of biopsy is invasive, but has high positivity and is quite representative of the infectious pulmonary process.

**Transbronchial biopsy**

Transbronchial biopsy is an invasive procedure that may provide important information, just like lung biopsy, but whose complications are also significant. The downside of this method is that the amount of tissue collected for analysis is meager and that it is impossible to access more peripheral regions of the lung parenchyma.

**Lung aspiration biopsies**

Lung aspiration biopsies were carried out in some studies in the 1970s in developing countries and contributed towards knowing the etiology of pneumonias in children; however, the risk inherent to the procedure (pneumothorax, pneumomediastinum, subcutaneous emphysema, and hemoptysis) does not allow its routine use as diagnostic method. The culture of lung aspirate is positive in approximately 50 to 60% of the cases.

**Pleural fluid**

The presence of a parapneumonic exudate remarkably increases the chance of isolation of the etiologic agent in
culture, with positivity between 50 and 70%. In clinical practice, however, we have low positivity because of the frequent use of antibiotics before the treatment of pleural effusion.17

**Blood cultures**

Blood cultures are very reliable, but they have low positivity (from 10 to 35% in inpatients). Their major limitation lies in the low occurrence of bacteremia among patients with acute pneumonia.18

**Bronchoalveolar lavage**

The cultures of material obtained through bronchoalveolar lavage are quite useful for etiological investigation, especially in nosocomial pneumonias and in immunocompromised patients. The lavage specimen might become contaminated with microorganisms present in the upper airways,19 in such a way that the protected lavage has better specificity and should be the method of choice.20 In addition, the culture should be quantitative, and bacterial growth greater than 10^5 CFU/ml, or detection of less frequent pathogens, such as mycobacteria or opportunistic bacteria such as *P. carinii* should be valued.21

**Tracheal aspirate**

Tracheal aspirate cultures were assessed by several authors and their use in clinical practice in pediatrics is currently not recommended due to the risk of complications and because of the weak association with colonization of lower airways, especially in patients hospitalized for more than 24 hours.22

**Immunological methods**

Immunological methods may be very useful in diagnosis and can be divided into serological methods, detection of antigens and markers of inflammatory response.

**Serological methods**

The serology for different etiologic agents is a widely used method in epidemiological studies, but其 usefulness in every-day practice is limited. The necessity for two samples for seroconversion is one of the major disadvantages of using the serological method in clinical practice. In addition, the detection of serological response to capsular polysaccharides in young children is recognizably difficult.23 Some authors have found weak serological responses to antigens of *S. pneumoniae* (pneumolysin and polysaccharide C) in infants younger than six months.24 The detection of immune complexes containing antigens of *S. pneumoniae* in the acute phase has been regarded by some authors as a useful technique for the diagnosis of lower airway infection by pneumococci,25 however, a recent publication has shown that the sensitivity of this method is low.24

Serological methods are also useful for the diagnosis of infections by *Mycoplasma pneumoniae* and *Chlamydia pneumoniae*, and, currently, ELISA techniques for the identification of IgM against *M. pneumoniae* may eliminate the need for the collection of a second sample.26 The complement fixation technique is not appropriate for the diagnosis of infection by *C. pneumoniae*, since there is cross-reaction with other species of the genus, such as *C. trachomatis*. The most recommended technique in this case is microimmunofluorescence (MIF), as it is species-specific.26

**Detection of antigens**

The detection of bacterial antigens by immunological methods is a way to identify the etiology without relying on the viability of the pathogen, that is, it is not influenced by the previous use of antimicrobials. Another great advantage of the methods for the detection of antigens is their quick availability of results; depending on the technique that is chosen, the results are available in a few hours. Methods for the detection of antigens have been used in the last two decades in samples of CSF, pleural fluid and urine for the identification of bacteria such as *S. pneumoniae*, *H. influenzae* type b and *S. aureus*.27,28 Among the techniques for the detection of antigens, we have latex agglutination test, counterimmunoelectrophoresis (CIE) and Dot-ELISA. By assessing methods for the detection of antigens of *S. pneumoniae* and *H. influenzae* type b, in samples of urine and pleural fluid, Requejo et al.29 have found great positivity forlatex agglutination test and CIE in samples collected after the introduction of antibiotic therapy, even after seven days of therapy, showing 100% of positivity in pleural fluid samples. In a subsequent study, Requejo et al.30 have shown that the Dot-ELISA method is superior for the identification of antigens of *S. pneumoniae* in samples of pleural fluid of children with acute pneumonia, to culture methods, latex agglutination test and CIE.

In addition to bacterial antigens, the identification of viral antigens in the upper airways is a very useful strategy for the diagnosis of viral infections. Direct immunofluorescence for the detection of virus in swabs or nasal lavage samples has a minimum sensitivity of 85% for RSV, parainfluenza, influenza A and B and adenovirus. However, the high prevalence of infections by both viruses and bacteria should be considered in therapeutic decisions for patients with pneumonia.

**Markers of inflammatory response**

The impossibility of distinguishing between viral infections and bacterial infections based on clinical and radiological aspects of children with pneumonia has
encouraged the search for inflammatory markers that are able to help with the initial therapeutic decision. The limitations of leukocyte count in peripheral blood and of the erythrocyte sedimentation rate in the differentiation between viral and bacterial infections are well known.31

C-reactive protein is an inflammatory marker that has been successfully tested in several pediatric studies, as in the assessment of febrile children, with an unidentified focus of infection.32 Several studies have shown that children with bacterial infection may present normal values at hospital, and that some viruses, such as adenovirus and influenza, may evoke large inflammatory responses, resulting in high levels of C-reactive protein, suggestive of bacterial infection.33

Interleukin 6 (IL-6) is another inflammatory marker already assessed in respiratory infections, showing high levels in adults with pneumonia.34 Another study showed higher levels of IL-6 in infections by *S. pneumoniae* than those caused by *M. pneumoniae*.35 The production of IL-6, on the other hand, seems to be basically local,36 and the serum levels of IL-6 might not represent a good way to distinguish between viral and bacterial infections.36

Procalcitonin (PCT) is a recent inflammatory marker and has already been used in patients with bacterial sepsis,37 in the early diagnosis of bacterial infections in the neonatal period38 and in the distinction between viral and bacterial meningitis.39 The use of PCT in the differential diagnosis of bacterial and viral pneumonias in children has been assessed by a recent study, and compared with IL-6 and C-reactive protein. The value of the three markers as a method to distinguish between bacterial and viral infections was low, but PCT 2.0 ng/ml and C-reactive protein 150 mg/ml were associated with the presence of bacterial infection.36

Methods for DNA detection

Methods for DNA detection probably have the greatest potential for the etiological diagnosis of acute pneumonias. Their main use is in the diagnosis of pathogens whose isolation in culture media is more difficult or whose serology is not available. They can be grouped into probe hybridization methods and DNA amplification methods, whose most widely used technique is the polymerase chain reaction (PCR).

The probe hybridization methods can be carried out in the solid or liquid phase or directly on the specimen or tissue (in situ). Their performance is quite similar to the methods for the detection of antigens, with greater specificity, as a result of the interaction between the tube and the known parts of the genome sequence of the pathogen in question. There are commercially available hybridization methods for the identification of some viruses and bacteria, such as *M. pneumoniae*, *L. pneumophila* and *C. trachomatis*.

The DNA amplification methods offer the great advantage of increasing the sensitivity to the identification of viral, bacterial and fungal pathogens. They are rapid and complex methods, but their use is increasing. They do not depend on the pathogen’s viability, and can identify resistant species or genes. PCR methods have already been assessed for the identification of several respiratory viruses, cytomegalovirus, *S. pneumoniae*, *M. pneumoniae*, *C. pneumoniae*, *L. pneumophila*, *B. pertussis*, *M. tuberculosis* and oxacillin-resistant *S. aureus*, *P. carinii*, *C. albicans* and *Aspergillus sp*, among others.

Respiratory viruses, such as rhinovirus and coronavirus, are difficult to isolate in culture and no tests for the detection of antigens are available;40 the PCR is the only way to investigate these etiologies properly. Likewise, the PCR method can be quite useful in the etiologic diagnosis of “atypical” pneumonias, in the identification of *M. pneumoniae*,40 *C. pneumoniae*42 and *L. pneumophila*; however, it is only commercially available for the latter pathogen.43 *M. tuberculosis* is another respiratory pathogen for which there are commercially available DNA amplification methods for detection, which have been extensively tested.44,45 Several PCR methods have been assessed for the detection of *S. pneumoniae* in samples, such as total blood, plasma, pleural fluid, and lung aspirates. Genes well preserved in species, such as pneumolysin, autolysin, DNA polymerase I and PBPs, have been tested,44,46 but the results show a great discrepancy, and some studies have found positive PCR reactions among individuals of the control group and among individuals with nasopharyngeal colonization, but without pneumonia caused by this agent.48 A prospective study by Michelow et al.24 has shown high sensitivity and specificity of the PCR technique for the identification of *S. pneumoniae* in samples of blood and pleural fluid of children with acute pneumonia, suggesting that technical aspects of the PCR technique are the major obstacle to the standardization of commercial methods.

Etiology of acute community-acquired pneumonias (CAP) in Latin America and in developing countries

The set of microbiological studies about lung aspirates of children hospitalized due to acute pneumonias, in developing countries, showed that bacteria were identified as etiologic agent in approximately 60% of the cases.2 Shann reviewed 13 studies conducted in developing countries in children hospitalized with pneumonia and without previous antibiotic therapy, in which lung aspiration biopsy was made as a way to characterize the etiologic agents of this infection. Viruses were identified in 281 (23%) of 1,212 children with pneumonia, whereas bacteria were isolated in 640 (62%) of the children submitted to the procedure. The author emphasized that bacterial infection had been confirmed in 65% of the children who died of pneumonia.2
Based on these studies, the bacterial agents most frequently isolated in acute pneumonias were *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Staphylococcus aureus* and enterobacteria. The frequency of pneumococci and *Haemophilus influenzae* was more predominant.  

The previously mentioned data have not been confirmed in industrialized countries, due to the lack of microbiological studies of lung aspirates obtained from children in those countries.

In the early 1980s, the NAS Board on Science and Technology for International Development (BOSTID) implemented a program with the aim of analyzing the etiology of acute respiratory tract infections in developing countries. Etiologic studies were carried out in 12 geographically distinct countries in Africa, Asia, and Latin America. The major results and conclusions of these studies were the following: the incidence of acute respiratory infections and pneumonias and the mortality rate due to such causes are higher in children younger than 18 months; the mortality of hospitalized children ranged from 3.2% to 15.8%; the highest mortality rate was observed in the Philippines; viruses cause the largest number of episodes of acute respiratory infections; RSV is the most frequent agent; the most frequent bacteria are *Streptococcus pneumoniae* and *Haemophilus influenzae*; in some hospitalized patients, non-typeable *Haemophilus influenzae* accounted for one third of the episodes caused by *Haemophilus influenzae*. One of the studies, included in this program, carried out in Argentina, analyzed the etiology of lower respiratory tract infections in 1,003 children younger than five years, among which 406 had pneumonia. Viruses were identified in 19.2% of the cases, bacteria in 12.8% and mixed infection in 2.7%, by means of culture and immunofluorescence of the nasopharyngeal material for virus, and blood culture and pleural fluid culture for bacteria. RSV was the most frequently observed viral agent while *Streptococcus pneumoniae* was the most frequent bacterial agent. The mortality rate was 3.8% in patients hospitalized due to pneumonia or bronchiolitis. The same program was used in Uruguay, with a similar methodology. In this case, 204 children hospitalized with CAP, aged less than five years, were analyzed. (Table 1) The etiologic agent was identified in 41% of the cases (virus = 36%, bacteria = 13.2% and mixed etiology = 4.9%). RSV accounted for 82.4% of viral infections and *S. pneumoniae* and *H. influenzae* were the most frequent bacterial agents.

The etiologic studies of acute pneumonias in Brazilian children, by means of lung aspirate culture and microbiological analysis of the pleural fluid, similarly showed that *Streptococcus pneumoniae* was the predominant agent in all age groups, followed by *Haemophilus influenzae*. Recent studies in hospitalized children, due to lower respiratory tract disorders, have shown an important role of RSV, especially in infants.

Etiology of acute community-acquired pneumonias (CAP) in Europe

Pneumonias are frequent in Europe, with an annual incidence estimated at 2.2%. Death is uncommon in infants and in previously healthy children. For reference, see the chapters of three recent monographs by the European Respiratory Society, and also relevant sessions of the publication known as White Book by the European Respiratory Society (in press).

The incidence of pneumonia in the first two days of life is 1.79/1,000 live births. Differently from all pediatric ages, bacteria are the major cause of pneumonia in this group of patients. Bacterial pneumonias are part of a generalized septicemic process, often a complication of an early rupture of membranes and maternal chorioamnionitis, and appear right after birth. After the first 48 hours of life, premature birth and mechanical ventilation are important risk factors. Etiologic agents include *Streptococcus* group B (around 70%), and gram-negative bacilli (chiefly *Escherichia coli*, more rarely *Pseudomonas aeruginosa* or *Klebsiella sp*). *Chlamydia trachomatis* is acquired by infants by way of the vagina during delivery, and 10 to 20% of these infants will develop pneumonia in the first two months of life. The prevalence of *C. trachomatis* in hospitalized infants, was only 1% in one study. *Ureaplasma urealyticum* is also vertically transmitted and is a frequent cause (prevalence up to 25%) of pneumonia in critically ill newborns. In young infants, however, it represents only 4% of cases of pneumonia, especially in winter. Transplacental transmission of virus manifests itself as early-onset interstitial pneumonitis, and is a rare cause of isolated respiratory disease. A study showed the occurrence of radiologically defined pneumonia in 7.5% of febrile diseases in infants younger than three months.

Pneumonias represent 13% of infectious diseases in the first two years of life. The most common causes of pneumonia are respiratory viruses. Bronchiolitis is more common in infants, and RSV is involved in 50 to 70% of the cases, with epidemic outbreaks between October and March. Other viral causes, especially off the RSV season, include adenovirus, parainfluenza types 1 and 3, rhinovirus, enterovirus and influenza, especially type A. Evidence of previous infection by RSV is practically universal at the age of three years, and reinfections are common, because primary infection does not induce protective immunity. The parainfluenza virus type 3 causes diseases especially in spring (especially in infants younger than six months), and types 1 and 2, in fall. *Bordetella pertussis* is more common in infants; 50% of cases occur in infants younger than one year, and 80%, in children younger than five years. Predisposing factors for the occurrence of bacterial pneumonia include low socioeconomic level, large families, exposure to cigarette smoke, prematurity, and living in the urban area. Pneumococci and *Haemophilus influenzae* are important causes of pneumonia throughout
the school years. *Staphylococcal pneumonia* is more important in the first two years of life, while *Mycoplasma pneumoniae* is more common after this age; it accounts for approximately 5% of pneumonias in children between two and five years of age.

In school age years, the common bacterial causes of pneumonia include *Mycoplasma pneumoniae*, *Chlamydia pneumoniae* and pneumococci. *Mycoplasma* is the most common cause after preschool age, and small epidemics might occur in winter. *Chlamydia pneumoniae* has a peak incidence around the age of eight and nine years, and later, in old age. It accounts for 6 to 10% of pneumonias in hospitalized children. Subclinical infections are common, as well as reinfections. Relapses might occur if antimicrobial treatment is not prolonged. *Viral pneumonias* can also be observed in this age group, usually in winter epidemics by influenza A and B. Antigenic mutations in the influenza virus, which could cause repeated infections in the same individual, in successive seasons, are well documented. *B. pertussis* is also an etiologic agent in these patients. *Legionella sp* is a quite rare pathogen in this age group, in Europe.

### Table 1 - Comparison between recent etiologic studies with children with acute community-acquired pneumonia in Latin America, Europe and North America

<table>
<thead>
<tr>
<th>Agents</th>
<th>Latin America (Uruguay)</th>
<th>Europe*</th>
<th>Finland</th>
<th>United States</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hortal et al.112</td>
<td>Schaad et al.112</td>
<td>Juven et al.112</td>
<td>Wubbel et al.112</td>
</tr>
<tr>
<td></td>
<td>n = 204</td>
<td>n = 1.375</td>
<td>n = 254</td>
<td>n = 168</td>
</tr>
<tr>
<td></td>
<td>(5 years)</td>
<td>H and NH</td>
<td>H</td>
<td>NH</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(2 months-16 years)</td>
<td>(0.1 –16.7 years)</td>
<td>(6 months–16 years)</td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em></td>
<td>7.8</td>
<td>24</td>
<td>37</td>
<td>27</td>
</tr>
<tr>
<td><em>Type b Haemophilus influenzae</em></td>
<td>1.9</td>
<td></td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Non-typable <em>H. influenzae</em></td>
<td></td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Mycoplasma pneumoniae</em></td>
<td></td>
<td>12</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td><em>Chlamydia pneumoniae</em></td>
<td></td>
<td></td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td><em>Moraxella catarrhalis</em></td>
<td></td>
<td>5</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>RSV</td>
<td>30</td>
<td>20</td>
<td>29</td>
<td>7.7</td>
</tr>
<tr>
<td>Parainfluenza 1, 2,3</td>
<td>2.4</td>
<td>6</td>
<td>10</td>
<td>4.1</td>
</tr>
<tr>
<td>Rhinovirus</td>
<td>8</td>
<td></td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Adenovirus</td>
<td>1.4</td>
<td>5</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Influenza A and B</td>
<td>0.5</td>
<td>4</td>
<td>4</td>
<td>2.9</td>
</tr>
<tr>
<td>Other virus (CMV, enterovirus, herpes, EB virus)</td>
<td>2</td>
<td></td>
<td>7</td>
<td>1.7</td>
</tr>
<tr>
<td>Positivity (% identified)</td>
<td>41.2</td>
<td>76</td>
<td>85</td>
<td>43</td>
</tr>
<tr>
<td>Viral infection (%)</td>
<td>36.3</td>
<td>43</td>
<td>62</td>
<td>20</td>
</tr>
<tr>
<td>Bacterial infection (%)</td>
<td>13.2</td>
<td>51</td>
<td>53</td>
<td>35</td>
</tr>
<tr>
<td>Mixed infection (%)</td>
<td>2.9</td>
<td>18</td>
<td>30</td>
<td>9</td>
</tr>
<tr>
<td>Non identified (%)</td>
<td>58.8</td>
<td>24</td>
<td>15</td>
<td>57</td>
</tr>
</tbody>
</table>

**Table 1** - Comparison between recent etiologic studies with children with acute community-acquired pneumonia in Latin America, Europe and North America.

Large epidemiological studies

The etiologic agent was identified in 88% of children hospitalized due to pneumonia, in a large Finnish study: virus, in 60%, bacteria, in 62% and mixed infections, in 50% of the cases. The most common pathogens were pneumococci, *Mycoplasma pneumoniae* and RSV. Another prospective study carried out in that same country, in hospitalized children, showed isolated viral infections in 19%, bacterial infection in 15% and mixed infections in approximately 50% of the cases. In a review study of results obtained by different authors, the prevalence of viral infection was 29 to 100%, bacterial infection, from 9 to 77%, and mixed infection, from 2 to 37%. Possible etiologies could be identified in 37 to 88% of the cases.

Patterns of resistance to antimicrobials

These patterns basically depend on the practices of prescription of antimicrobials. Infections caused by penicillin-resistant pneumococci are relatively rare in children, but this possibility should be considered in areas of high prevalence, such as Spain, where over 40% of the
strains are resistant, in opposition to Scandinavia, where resistance of strains is lower than 5%. A new strain of community-acquired, methicillin-resistant Staphylococcus aureus, has been described, with different properties from those of hospital-acquired strains with this characteristic. The prevalence of macrolide-resistant microorganisms and cephalosporins has increased in regions with large use of macrolides. In general, the plan of antimicrobial therapy should not be based only on the identification of the most probable etiologic agent for a given age group and on the severity of the disease, but also on information about the local patterns of resistance to antimicrobials.

**Etiology of acute community-acquired pneumonias (CAP) in North America**

Wubbel et al. (Table 1) conducted an etiological assessment by using culture, PCR and serology in 168 children aged between six months and 16 years, with CAP, treated at an emergency center in Texas, from 1996 to 1997. The etiologic agent was identified in 43% of the patients, and Streptococcus pneumoniae was found in 27% of the cases, followed by Mycoplasma pneumoniae in 7%, and Chlamydia pneumoniae in 6%. There was serological evidence of previous exposure to C. pneumoniae in 11% of the children. The frequency of acute or previous infection by this agent increased with age, from 5% in children aged between 0-2 years, to 43% in those older than nine years. The viruses, in most cases RSV, were identified in 20% of the cases. In 40% of the patients with serological evidence of acute infection by S. pneumoniae, concomitant infection with a virus, or M. pneumoniae, or C. pneumoniae occurred.

The importance of M. pneumoniae and of C. pneumoniae in children with CAP was defined in two large prospective studies. Block et al. used culture, serology and PCR in 260 patients, aged between three and 12 years, and identified M. pneumoniae in 27%, and C. pneumoniae in 27% of the cases. The infection was serologically confirmed in 23% of the cases of documented infection by M. pneumoniae, and in 53% by C. pneumoniae. A reduction in the frequency of infection by both agents was observed in younger patients. The infection by Chlamydia pneumoniae, or Mycoplasma pneumoniae was detected, in this age group, in approximately half of the patients with CAP. Also, clarithromycin and erythromycin proved equally efficient in the treatment of the infection.

Later on, in a multicenter study, pretreatment, culture, PCR and serology were performed for M. pneumoniae and C. pneumoniae, in children between six months and 16 years, from different geographical regions of the United States. There was evidence of infection by these agents in 46% of the patients, of which 30% were by M. pneumoniae and 15% by C. pneumoniae. The frequency of these agents tended to increase with age. Therefore, M. pneumoniae and C. pneumoniae were respectively identified in 15% and 9% of the patients aged five years or less, and in 42% and 20% of the patients older than five years.

Table 1 summarizes the etiology of CAPs in more recent studies with children in different continents. For comparison purposes, we specified a representative study of Latin America, a set of nine studies of six European countries, a recent Finnish study and a North-American study.

**Etiology of acute community-acquired pneumonias (CAP) in hospitalized children**

There are few prospective studies about the etiological investigation of CAP in hospitalized children. Nohynek et al. used conventional diagnostic methods and serological methods in 135 Finnish children hospitalized due to pneumonia and found out that in 25% of the cases one bacterial agent was identified, one viral agent was detected in 25%, and that in 20% of the cases the infection was mixed. The etiological agent was identified in 70% of the cases. Non-typeable Haemophilus influenzae and Streptococcus pneumoniae were the most frequent bacterial agents, present in respectively 17% and 16% of the cases. RSV was the most frequent viral agent, and occurred in 28% of the patients. Mycoplasma pneumoniae was found in 9%, and Moraxella catarrhalis in 7% of the cases. This study showed that bacterial agents are more frequent in hospitalized children with acute pneumonia and that the frequency increases with age, even though Streptococcus pneumoniae occurs in all age groups. RSV and adenovirus are more frequent in infants and younger children.

Quite recently, Juven et al. have studied the etiology of CAP in 254 hospitalized Finnish children. Seventeen agents were investigated by means of nasopharyngeal aspirate, for virus, and by means of serology, for viruses and bacteria. One or more agents were identified in 85% of the patients; 62% had some evidence of viral infection, 53% of infections were caused by bacteria and 30% of them were mixed. The agents most frequently identified were Streptococcus pneumoniae (37%), RSV (29%) and rhinovirus (24%).

Vieira et al. have prospectively assessed the occurrence of viral infections in 239 hospitalized Brazilian children, with lower respiratory tract disease, by means of viral culture and immunofluorescence of nasopharyngeal material. The respiratory syncytial virus was identified in 41.8% of the children, adenovirus, in 4.6%, influenza, in 0.8%, and parainfluenza, in 0.4%. The bacterial agent, detected by blood cultures and pleural fluid culture, was identified in only 5.8% of the cases. RSV was associated with other viruses or bacteria in six cases. Most children with infection by RSV were younger than one year and had pneumonia or bronchiolitis. They concluded that RSV is an important agent in hospitalized children due to severe respiratory.
disease, with a peak frequency in fall up to the winter. In a previous publication, the same authors emphasize that RSV was associated with 84% of the cases of bronchiolitis and with approximately 47% of pneumonias.94

The coexistence of viral and bacterial infection was a relatively frequent finding, especially in infants younger than two years, in other etiologic studies in community-acquired pneumonias.1,88 It has been suggested that viruses may be the initial infectious agents, reducing local and/or systemic immunity and favoring bacterial infection.88 Therefore, the detection of virus in the upper airway is not conclusive as to its responsibility for the lower respiratory tract infection. The virus may be just the initial agent that favors or modifies the host’s response.

On the other hand, in infants hospitalized due to pneumonia, other agents may be implicated, such as cytomegalovirus, Chlamydia trachomatis and Pneumocystis carinii, 1,89-91 alone or simultaneously with RSV.89 Therefore, children with CAP, younger than two years, offer greater difficulty, due to the larger number of etiologic possibilities and to mixed infections, hindering the rational formulation of an empirical therapeutic strategy for these cases.

**Etiology of hospital-acquired pneumonias (HAP)**

The incidence of HAP ranges from 16 to 29% of hospitalized pediatric patients.92 HAPs represent 10 to 15% of all nosocomial infections.93 The mortality, in this situation, is very high, ranging between 20 and 70% of the cases, depending on the causative agent and on the host’s underlying disease.92 A multicenter study that assessed nosocomial infection in 62 intensive care units, in the United States, showed that HAP was the second most common cause of infection, accounting for 21% of nosocomial infections.94

The risk factors for HAP include hospitalization in intensive care unit, intubation, extensive burns, surgery, and chronic underlying disease.95 The agents related to hospital-acquired pneumonias are more virulent. The viruses, especially RSV, are common agents of nosocomial respiratory infection.96 Gram-negative bacteria are responsible for 50 to 90% of the cases, and Staphylococcus aureus, especially those resistant to oxacillin, may account for approximately 10 to 20% of the cases. Among gram-negative bacteria we have the group of enterobacteria (Escherichia coli, Klebsiella pneumoniae, Salmonella sp, Shigella sp, Enterobacter sp, Serratia sp, Proteus sp, Citrobacter sp, etc.) and Pseudomonas sp. Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa are the predominant gram-negative bacteria and are associated with high mortality.96 Staphylococcus aureus and Staphylococcus epidermidis are the most common gram-positive bacteria. Other agents include fungi (Candida and Aspergillus), cytomegalovirus and Pneumocystis carinii.96

**The problem of resistance of pneumococci to antimicrobials**

Several epidemiological studies conducted in different continents, countries, and regions, have reported a gradual increase in the prevalence of penicillin-resistant pneumococcal strains in the last few decades.97-99 Classically, the definition of resistance to penicillin is based on the minimum inhibitory concentration (MIC) of pneumococci. Strains with MIC 0.06 µg/ml are considered sensitive, those with MIC between 0.1 and 1 µg/ml are relatively resistant or immediately resistant, and strains with MIC 2 µg/ml are highly resistant.100 Recently, this classification has been reviewed by some authors, who consider, for pneumococcal pneumonias, upper limits for sensitive, immediately sensitive and highly resistant strains up to respectively 1 µg/mL, 1 to 2µg/l and greater than 4 µg/ml.101 These strains may be resistant to other antimicrobials (cephalosporins, chloramphenicol, erythromycin, clindamycin and sulfamethoxazole-trimethoprim), thus characterizing a multiresistant situation.100 The major resistance mechanism is the production of a penicillin-binding protein, which reduces the affinity with penicillins and causes a cross-resistance with other beta-lactam antibiotics, including third-generation cephalosporins and carbapenems.97

The data published by the Centers for Disease Control and Prevention (CDC), United States, show that the prevalence of multiresistant pneumococci has been increasing.98 A small number of serotypes is responsible for multiresistant strains, and the new conjugate vaccines protect against most resistant pneumococcal strains.98 In 1997, in the United States, 92% of pneumococcal strains with MIC > 2µg/ml were associated with serogroups 23, 6, 19, 9 and 14.102 The percentage of pneumococci with intermediate sensitivity or resistant to penicillin, obtained from children with systemic infection, has increased gradually. In addition, there has been a tendency towards increased resistance to ceftriaxone.99,103

The epidemiological data obtained from 360 pneumococcal strains isolated from Brazilian children with pneumonia and meningitis, between 1993 and 1996, showed that eight serotypes corresponded to approximately 80% of the isolated strains: serotypes 1, 5, 6A/B, 9V, 14, 19F, 19A and 23F.104 With regard to penicillin sensitivity, 78.6% of the strains were sensitive, 20% had intermediate resistance and only 1.4% were highly resistant.104

Friedland et al.105 have compared clinical characteristics in 78 South-African children with pneumococcal pneumonia, in which 32% of the isolated pneumococci were immediately resistant to penicillin. No difference was observed as to the outcome between children with sensitive pneumococci and those with pneumococci resistant to penicillin. Other studies have also shown that clinical characteristics and the evolution of pneumonias, with sensitive and resistant pneumococci do not differ and that in cases of resistance to penicillin, the therapy with beta-
lactam agents is efficient. More often than not, treatment is empirical and based upon the available epidemiological data. The materials indirectly related to pulmonary focus of infection, such as blood, urine, pleural fluid, have been used for the etiological analysis of acute pneumonias. Recently, new diagnostic methods, such as serology, quick detection of antigens by immunological methods (latex agglutination test, immunoenzyme assays) and PCR, have been developed and used in epidemiological studies and, in the near future, should be routinely used. The application of these methods, with etiologic purpose, is very important since it allows us to know about the disease in different populations and regions, with the aim of establishing better therapeutic schemes, and developing more appropriate vaccines in epidemiological terms. On the other hand, significant changes might occur to the predominance and relative proportion of different etiologic agents over time, as well as changes to antimicrobial sensitivity, as a result of the action of antimicrobials introduced at different moments and of the impact on the use of vaccines. It has been shown that with the use of several methods, the etiology of community-acquired pneumonias may be detected in most cases. However, the cost of these procedures is prohibitive. The treatment may therefore begin with the analysis of clinical, epidemiological and radiological data, without any specific diagnostic test, and if an inadequate response is found, the study of virus, Mycoplasma pneumoniae and Chlamydia pneumoniae, as well as the analysis, if possible, of sensitivity of pneumococci to antimicrobials, should be made. Although several factors such as age, nutritional status, underlying disease and environmental factors greatly influence the etiology of pneumonias in children, in community-acquired pneumonias, Streptococcus pneumoniae continues to be an important cause in all age groups, especially in infants and preschool children, both in industrialized and developing countries. Recently, Mycoplasma pneumoniae and Chlamydia pneumoniae have been recognized as important agents, especially among children older than four and five years. The studies carried out in Europe and in North America have shown that these agents may be responsible for up to one third of community-acquired pneumonias in children. Studies about the role of these agents in CAP are still necessary in developing countries.

Conclusions

The isolation of the etiologic agents of lower respiratory tract infections in clinical practice is relatively complex, since it is difficult to collect adequate and representative material from the focus of infection for microbiological analysis. More often than not, treatment is empirical and based upon the available epidemiological data. The materials indirectly related to pulmonary focus of infection, such as blood, urine, pleural fluid, have been used for the etiological analysis of acute pneumonias. Recently, new diagnostic methods, such as serology, quick detection of antigens by immunological methods (latex agglutination test, immunoenzyme assays) and PCR, have been developed and used in epidemiological studies and, in the near future, should be routinely used. The application of these methods, with etiologic purpose, is very important since it allows us to know about the disease in different populations and regions, with the aim of establishing better therapeutic schemes, and developing more appropriate vaccines in epidemiological terms. On the other hand, significant changes might occur to the predominance and relative proportion of different etiologic agents over time, as well as changes to antimicrobial sensitivity, as a result of the action of antimicrobials introduced at different moments and of the impact on the use of vaccines. It has been shown that with the use of several methods, the etiology of community-acquired pneumonias may be detected in most cases. However, the cost of these procedures is prohibitive. The treatment may therefore begin with the analysis of clinical, epidemiological and radiological data, without any specific diagnostic test, and if an inadequate response is found, the study of virus, Mycoplasma pneumoniae and Chlamydia pneumoniae, as well as the analysis, if possible, of sensitivity of pneumococci to antimicrobials, should be made. Although several factors such as age, nutritional status, underlying disease and environmental factors greatly influence the etiology of pneumonias in children, in community-acquired pneumonias, Streptococcus pneumoniae continues to be an important cause in all age groups, especially in infants and preschool children, both in industrialized and developing countries. Recently, Mycoplasma pneumoniae and Chlamydia pneumoniae have been recognized as important agents, especially among children older than four and five years. The studies carried out in Europe and in North America have shown that these agents may be responsible for up to one third of community-acquired pneumonias in children. Studies about the role of these agents in CAP are still necessary in developing countries.

References

8. The respiratory syncytial virus is an important agent of acute pneumonias, especially in infants treated at outpatient clinics or in those who are in hospital. In developing countries, vaccination against Haemophilus influenzae type b, formerly an important agent of pneumonias in children younger than three years, has had a great impact, reducing the frequency of pneumonias and other infections caused by this agent. Some bacteria, previously regarded as nonpathogenic to the respiratory tract, such as non-typeable Haemophilus influenzae and Moraxella catarrhalis, have occasionally been involved in acute pneumonias in children.
9. Staphylococcus aureus and enterobacteria are important etiologic agents in hospital-acquired pneumonias and in immunocompromised patients. Legionella pneumophila, although it is a more frequent agent of pneumonias in adults than in children, may sporadically cause pulmonary infection or small outbreaks.
10. In studies that assess the efficiency of vaccines, immunization of infants with the pneumococcal conjugate vaccine has reduced the incidence of invasive infection in 93% of the cases, and of pneumonia in 73%. The pneumococcal heptavalent vaccine is highly efficient in the prevention of invasive disease in children. The immunization of children with new conjugate vaccines against Haemophilus influenza type b and pneumococci in developing countries may have a considerable impact on the reduction of morbidity and mortality caused by acute pneumonias, rate of hospital admission and public health costs, as occurred in industrialized countries. The willingness of politicians and their health policies in terms of the use of new prophylactic resources by the population may be determinant factors for changing the current situation.


Etiological diagnosis of pneumonia... - Rodrigues JC et alii


