

# Aetiology of community acquired pneumonia in Valencia, Spain: a multicentre prospective study

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## Abstract

**A year long multicentre prospective study was carried out in the Valencia region of Spain, to determine the cause of community acquired pneumonia. The study was based on 510 of 833 patients with pneumonia. Of these, 462 were admitted to hospital, where 31 patients died. A cause was established in only 281 cases—208 of bacterial, 60 of viral, and 13 of mixed infection. The most common microorganisms were *Streptococcus pneumoniae* (14.5%), *Legionella* sp (14%), *Influenza virus* (8%), and *Mycoplasma pneumoniae* (4%). There was a higher incidence of *Legionella* sp than in other studies.**

The cause of community acquired pneumonia is often difficult to establish. The most effective methods are often invasive and cannot always be justified, whereas a serological diagnosis is too late to be of therapeutic use. These circumstances and the changing causative organisms of pneumonia<sup>1</sup> require a detailed knowledge of the microorganisms responsible in a particular environment. Many studies have shown that the microbiological cause of pneumonia is related to geographical location, though the influence of the identification methods used is also important.<sup>2-9</sup>

We determined the aetiology of pneumonia acquired in Valencia by studying emergency admissions to four hospitals.

## Methods

### PATIENTS

Eight hundred and thirty three patients over 15 years of age with a diagnosis of community acquired pneumonia were studied prospectively from March 1985 to February 1986. Emergency admissions to the Valencia University Clinic Hospital, La Fe Hospital (Valencia), Sagunto Hospital (Sagunto), and La Magdalena Hospital (Castellón) were studied.

The diagnosis of pneumonia was established on the basis of radiologically apparent disease of the lung parenchyma and clinical features of a lower respiratory tract infection. Criteria for hospital admission were based on risk factors<sup>10</sup> and were applied by the physicians in each participating centre.

After history taking, physical examination, and chest radiology, blood and where possible lower respiratory tract secretions were

obtained. Blood was collected for biochemical, haematological, and microbiological analysis (blood culture, detection of capsular antigens, and the first sample for detection of antibodies). Arterial blood gas tensions were determined when appropriate. Thoracentesis, transtracheal aspiration, percutaneous aspiration biopsy of the lung, or necropsy was performed to obtain samples in some cases.

All patients underwent clinical, radiological, and serological follow up after 21 and 42 days. All cases were followed to resolution or death.

### MICROBIOLOGICAL METHODS

Three blood cultures were performed before treatment. Tracheobronchial secretions were cultured after washing. Smears were stained by Gram's method and bacterial morphology was determined.<sup>11,12</sup> Semiquantitative counting was carried out on positive cultures.<sup>13</sup> Where legionellosis was suspected direct immunofluorescence tests for *Legionella pneumophila* serogroups 1-6 and *L. micdadei* were performed, and culture attempted in BCYE-alpha medium.<sup>14,15</sup>

Capsular antigens of *Streptococcus pneumoniae* and *Haemophilus influenzae* serotype b were sought in the first blood samples and respiratory secretions, after treatment of the sample with 0.1 M EDTA and heat,<sup>16</sup> by agglutination of latex particles sensitised with anti-*H. influenzae* serotype b (Wellcome)<sup>17</sup> and polyvalent anti-*S. pneumoniae* serum (Omniserum Serum Institutum), which had been previously adsorbed with other common streptococci of the oropharyngeal microflora.<sup>18</sup>

The serum samples obtained on days 1, 21, and 42 were assayed for antibody to influenza viruses A and B; parainfluenza viruses 1, 2, and 3; adenovirus; respiratory syncytial virus; cytomegalovirus; herpes simplex viruses 1 and 2; *Mycoplasma pneumoniae*; *Chlamydia psittaci*; and *Coxiella burnetii* by complement fixation. Antibodies to *Legionella* sp were determined by indirect immunofluorescence.<sup>19</sup>

### CRITERIA FOR AETIOLOGICAL DIAGNOSIS

Bacterial pneumonia was established by the detection of bacteria or their antigens in blood or respiratory samples. Pneumonia due to *Legionella*, *C. burnetii*, *C. psittaci*, *M. pneumoniae*, and viruses was diagnosed by a fourfold rise in titre in paired samples.

*S. pneumoniae* was accepted as the cause if it was cultured in blood or pleural fluid, if

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Table 1 Aetiology of pneumonia in relation to hospital admission and death

	No (%) of patients			No of deaths
	Inpatients	Outpatients	Total	
Number	462 (90.6)	48 (9.4)	510	31
(Mean age (y))	59.9‡	40.8	57.9	68.7)
Cause not established	209 (45.2)	20 (41.6)	229 (44.9)	10
Bacterial	190 (41.1)	18 (37.5)	208 (40.8)	18
<i>Streptococcus pneumoniae</i>	68 (14.7)	6 (12.5)	74 (14.5)	7
<i>Legionella</i> sp	64 (13.8)	6 (12.5)	70 (13.7)	7
<i>Mycoplasma pneumoniae</i>	16 (3.5)‡	6 (12.5)	22 (4.3)	
Enterobacteriaceae*	11	—	11 (2.1)	3§
<i>Haemophilus influenzae</i>	9	—	9 (1.8)	1
<i>Staphylococcus aureus</i>	8	—	8 (1.5)	
<i>Pseudomonas aeruginosa</i>	4	—	4 (0.8)	
<i>Coxiella burnetii</i>	3	—	3 (0.6)	
Other†	7	—	7 (1.4)	
Viral	50 (10.8)	10 (20.8)	60 (11.8)	
Influenza A	26 (5.6)	4 (8.3)	30 (5.9)	
Influenza B	10 (2.2)	3 (6.2)	13 (2.5)	
Parainfluenza 3	8 (1.7)	1 (2.1)	9 (1.8)	
Respiratory syncytial virus	5 (1.1)	1 (2.1)	6 (1.2)	
Adenovirus	1 (0.2)	1 (2.1)	2 (0.4)	
Mixed infection	13 (2.8)	—	13 (2.5)	3

\**Klebsiella pneumoniae* (7) and *Escherichia coli* (4).

†*S. viridans* (2), *Haemophilus parainfluenzae* (2), *Actinomyces naeslundii* (1), *Chlamydia psittaci* (1), non-fermenting Gram negative bacillus (1).

‡p < 0.001.

§*K. pneumoniae* (2), *E. coli* (1).

pneumococcal antigens were detected, or, in the absence of these, the presence of Gram positive encapsulated diplococci on the Gram stain and culture of *S. pneumoniae* or detection of capsular antigens in respiratory tract secretions (except in patients with chronic airways obstruction).<sup>4,20</sup> The diagnosis of pneumonia caused by Gram negative bacteria was based on established criteria.<sup>21</sup> The diagnosis of pneumonia caused by *H. influenzae* was based on the criteria used for pneumococcal pneumonia.

#### STATISTICAL ANALYSIS

Analysis was by  $\chi^2$  test and Fisher's exact test for qualitative variables. Student's *t* test was used for quantitative variables. Values of *p* below 0.05 were considered significant.

#### Results

Of the 833 patients with community acquired pneumonia diagnosed during the study, 323 (39%) were excluded for the following reasons: obstructive pneumonitis, tuberculosis, prior

treatment with antibiotics, and incomplete study data. The remaining 510 patients comprised 355 (70%) males and 155 (30%) females and were aged 15–92 (mean 58) years. Hospital admission was necessary for 462 patients (91%), the remaining 48 being treated as outpatients. There were differences in mean age and the incidence of pneumonia caused by *M. pneumoniae* (*p* < 0.001) between these groups (table 1).

Predisposing disease (table 2) was present in 393 patients (77%), chronic airways obstruction being the most frequent (169 cases). Smoking was reported by 236 (46%) of the patients.

There was no significant seasonal pattern, though the incidence was greater during winter and spring than in summer and autumn (288 v 222 cases).

A causal agent was determined in 281 cases (55%): 208 (41%) were due to a bacterial infection, 60 (12%) to a virus, and the remaining 13 cases (2.5%) to other microorganisms (table 1). Sputum or tracheobronchial secretions were obtained in 346 patients (67.5%). Of these specimens, 171 (49%) were adequate for microbiological study and 87 (51%) were diagnostic. Blood culture was diagnostic in 28 patients (5.5%) (table 3).

Table 2 Predisposing factors

	No	%
Smokers	236	46.3
Chronic airways obstruction	169	33.1
Diabetes mellitus	75	14.7
Cardiovascular disease	70	13.7
Previous pneumonia	66	12.9
Alcohol*	57	11.2
Bronchiectasis	29	5.7
Cerebrovascular accident	22	4.3
Neoplasia	14	2.8
Asthma	11	2.2
Drug abuse	7	1.4
Immunosuppression	5	1.0

\*Consumption > 100 g/day.

#### BACTERIAL PNEUMONIA

*S. pneumoniae* was responsible for 74 cases of bacterial pneumonia (14.5%), *Legionella* sp for 70 (14%), *M. pneumoniae* for 22 (4%), and *H. influenzae* for nine (2%) (tables 1 and 3).

Pneumococcal pneumonia was diagnosed by culture in 36 cases (7%), by capsular antigen detection in 27 (5%), and by culture with antigen detection in 11 cases (2%). Pneumonia caused by *Legionella* sp was diagnosed by indirect immunofluorescence in 67 of 70 cases.

Table 3 Diagnostic methods in bacterial pneumonia

Microorganisms	n	Culture			Capsular antigen			
		Respiratory specimen	Blood	Other	Respiratory specimen	Blood	DFA	SC
<i>Streptococcus pneumoniae</i>	74	31	20	3	31	10		
<i>Legionella</i> sp	70						3	68
<i>Mycoplasma pneumoniae</i>	22							22
Enterobacteriaceae	11	6	4	1				
<i>Haemophilus influenzae</i>	9	7			2	2		
<i>Staphylococcus aureus</i>	8	2	4	3				
<i>Pseudomonas aeruginosa</i>	4	3		2				
<i>Chlamydia burnetti</i>	3							3
Others	7	2		4				1
Total	208	51	28	13	33	12	3	94

DFA—direct immunofluorescence; SC—seroconversion.

Two patients were diagnosed by direct immunofluorescence and one by both direct and indirect immunofluorescence (tables 3 and 4).

#### VIRAL PNEUMONIA

The 60 cases of viral pneumonia were all diagnosed by seroconversion, influenza virus A being the main organism identified (table 1).

#### OTHER CAUSES

Thirteen (2.5%) cases of mixed cause pneumonia occurred, of which eight were infections with two or more bacteria and three were mixed bacterial and viral pneumonia. *S pneumoniae* was the cause of seven cases of mixed pneumonia and *Legionella* sp of three (table 5).

#### DEATHS DUE TO PNEUMONIA

Thirty one patients (6%), with a mean age of 68.7 years, died, all having been admitted to hospital. Seventeen deaths were a direct result of pneumonia, and 14 were due to coincidental factors. Ten deaths occurred with pneumonia of unknown cause, seven with *Legionella* sp, and seven with *S pneumoniae* (table 1).

#### Discussion

The cause of pneumonia was established in half the cases studied. Most cases were caused by bacteria; only 12% were caused by viruses and 2.5% by a mixed infection. We have used the term bacterial pneumonia to include bacterial and atypical pneumonia, as have others.<sup>22</sup> In the present study, as in previous studies,<sup>2,4,5,8,22,23</sup> typical bacterial pneumonias predominated, most cases being caused by *S pneumoniae*.

In our study *S pneumoniae* and *Legionella* sp were responsible for over half of all the cases of

bacterial pneumonia, followed by *M pneumoniae*, several species of Gram negative bacteria, and *Staphylococcus aureus*. A smaller proportion of cases of pneumonia was due to *S pneumoniae* and a higher proportion to *Legionella* sp than in other studies. The frequency of *S pneumoniae* in community acquired pneumonia has varied from 14% to 36%,<sup>3,6,8,22,23</sup> with occasional lower proportions.<sup>7,24</sup> Our diagnosis of pneumococcal pneumonia was based on isolation from blood and respiratory tract secretions and the detection of capsular antigens, whereas others have used detection of specific antibodies.<sup>6,22</sup> Blood culture was diagnostic in a quarter of cases of typical bacterial pneumonia, including those due to pneumococcus, which is a lower yield than in early studies and higher than in recent studies.<sup>2-5</sup>

The high percentage of *Legionella* sp infections we observed is similar to the proportions reported previously.<sup>4,22,25</sup> This organism probably replaces the *M pneumoniae* infections seen in other series. Many cases of pneumonia of unknown cause are considered to be bacterial and probably pneumococcal in origin, especially when such patients have been treated with antibiotics; the possible saprophytic behaviour of *S pneumoniae* means that its aetiological role can be accepted only when there are adequate guarantees, whereas *Legionella* sp is always considered to be a pathogen. Although the incidence of *Legionella* sp varies within a geographical area,<sup>4,8</sup> its importance as a cause of pneumonia in our study is clear.<sup>26-29</sup> Another probable reason for the high incidence of *Legionella* sp in our study is the methods of identification we used. Blood was collected at the start of the study and after 21 and 42 days for indirect immunofluorescence testing; this

Table 4 Diagnostic methods in pneumococcal pneumonia

	Culture*	Antigen	Both	Total
Respiratory specimens	14	18	10	42
Blood	12	6	1	19
Respiratory specimens and blood	7	3		10
Other	3			3
Total	36	27	11	74

\*With appreciable Gram staining of the respiratory secretions.

Table 5 Aetiology of mixed pneumonia

Aetiological agents	No of cases
<i>Streptococcus pneumoniae</i> + other bacteria	4
<i>S pneumoniae</i> + virus	2
<i>Legionella</i> sp + other bacteria	2
<i>S pneumoniae</i> + <i>Legionella</i> sp	1
Mixed (other bacteria)	1
Mixed (other bacteria + virus)	1
Mixed (viruses)	2
Total	13

yields more positive results as the interval between samples is increased.<sup>29</sup> A high prevalence of antibodies to *L pneumophila* 1 occurs in the healthy population in the Valencian community<sup>30</sup> and *L pneumophila* has been isolated in the water distribution system of this community.<sup>31 32</sup>

The incidence of *M pneumoniae* was similar to that found in some previous studies<sup>2 7</sup> and less than in others.<sup>3 5 6</sup> All of our patients acquired pneumonia in the community and most were aged under 50.<sup>3 5 7</sup> The incidence of pneumonia caused by other Gram negative bacteria, excluding *Legionella* sp, was similar to that observed in other studies.<sup>3 4</sup>

About 20% of cases of pneumonia with an identified cause were due to a virus. As in other studies,<sup>2 3 8</sup> half of all cases of viral pneumonia were caused by influenza virus A, the rest being due mainly to influenza virus B and parainfluenza virus 3.

In practice, the cause of community acquired pneumonia should be established. In many cases sputum culture and blood culture are sufficient.<sup>23</sup> More invasive techniques should be reserved for special cases, such as necrotising pneumonitis, nosocomial pneumonia, and pneumonia associated with immunosuppression. For identification of *S pneumoniae* sputum culture has been rated fairly low.<sup>33</sup> Minimising oropharyngeal contamination<sup>34</sup> and careful culture of sputum samples offers an 80% sensitivity and 72% specificity.<sup>35</sup> The detection of capsular antigens in respiratory secretions is faster than culture and avoids the problem of oropharyngeal contamination.<sup>36</sup> It is diagnostic if positivity coincides with the microorganism isolated in blood, sputum, or other body fluids.<sup>36 37</sup> The limitation of relying on antigen detection alone is that antigens may be detected in the respiratory secretions of patients with chronic bronchitis.<sup>20</sup> Mortality was similar to that reported previously<sup>3</sup> in that death was limited to patients admitted to hospital. Pneumonia of unknown aetiology or caused by *Legionella* sp and *S pneumoniae* with bacteraemia was associated with a risk of death.

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