Microbial Etiology of Acute Pneumonia in Hospitalized Patients*

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The purpose of this study was to determine the microbial etiology of pneumonia by using strict criteria among a group of hospitalized patients. Patients with acute community-acquired or hospital-acquired pneumonia were studied in a systematic and comprehensive manner for bacterial, viral, chlamydial, mycobacterial, and fungal pathogens. A total of 198 patients with 204 episodes of pneumonia were evaluated. Despite 100 percent follow-up of all surviving patients, a specific etiologic agent could be found in only 103 episodes. Among 154 episodes of community-acquired pneumonia, a diagnosis was made in 79; the most common pathogen was from the genus Legionella, followed by various Gram-negative enteric bacteria, Gram-positive cocci, influenza A virus, and Mycoplasma pneumoniae. The etiologic agent was found in 24 of the 50 patients with hospital-acquired pneumonia; no pathogen predominated. We conclude that even when elaborate diagnostic studies are done, including many invasive procedures, the etiology can be determined in only about half of the patients with acute pneumonia. The pathogens of pneumonia in this study are not markedly different between community-acquired and hospital-acquired infection.

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Pneumonia and influenza combined are the sixth leading cause of death in the United States, and pneumonia has the highest age-adjusted death rate (13.1 per 100,000) of any infectious disease.† The mortality rate for pneumonia among the elderly has not decreased substantially over the past 30 years despite the introduction of many new and potent antimicrobial agents.‡

Regarding microbial etiology, some diagnostic methods are flawed; others are expensive and technically difficult. Simple tools, such as Gram stain and culture of expectorated sputum, are not accurate in many instances.§ Other, more reliable diagnostic procedures may place the patient at added risk of a complication or may require sophisticated methods not readily available in some hospitals. Many well-known pulmonary pathogens, such as Mycoplasma pneumoniae, and respiratory viruses, such as influenza virus and adenovirus, are difficult to isolate or are not routinely cultured. New pulmonary pathogens, such as various species and serotypes of Legionella, have been described in the past decade; and microbes that may have importance as pulmonary pathogens in adults, such as Chlamydia trachomatis and Chlamydia pneumoniae (TWAR strain), have been investigated in recent years.¶,‖

For these reasons, we are stimulated to investigate the microbial etiology of acute pneumonia among adult patients hospitalized in a Veterans Administration medical facility by using a number of techniques and laboratory resources that would not be used in routine clinical practice. The results are reported here.

MATERIALS AND METHODS

Patient Selection

All patients hospitalized on the medical service of the John L. McClellan Veterans Administration Medical Center, Little Rock, Ark., during the calendar year 1985 (approximately 6,500 admissions) were evaluated both on admission and throughout their hospital course for the presence or development of pneumonia. All patients with clinical evidence of pneumonia were evaluated within 24 h for inclusion in the study. For selection, each patient was required to have a new or progressive pulmonary infiltrate on chest radiograph together with at least two of the following: fever (temperature ≥ 37.8°C), production of purulent sputum (by visual inspection), or leukocytosis (white blood cell [WBC] count > 10,000/μl). When first evaluated, a patient was excluded if found to have radiographic or laboratory evidence suggestive of tuberculosis, fungal infection, or pulmonary neoplasia. Once these criteria were met and informed consent had been obtained, the patient was entered in the study.

To ensure that all aspects of the protocol were followed and to monitor data obtained on an ongoing basis, the investigative team met weekly to review the data on every patient entered, as well as those evaluated but not entered. The investigative team was composed of a recruitment and evaluation group (principal investigator [J.H.B.], study coordinator [G.A.M.], pulmonologist

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CMV = cytomegalovirus; HSV = herpes simplex virus.
Acute Pneumonia in Hospitalized Patients (Bates et al)

months after entrance to the study when a third convalescent-stage serum sample was collected. Acute- and convalescent-stage serum specimens were sent to the Veterans Administration Serology Laboratory, Lexington, Ky, to be tested for complement-fixing antibody against the following agents: influenza virus types A and B, parainfluenza virus types 1 through 3, varicella-zoster virus, rubella virus, Coxiella burnetii, Chlamydia, M. pneumoniae, respiratory syncytial virus, CMV, adenovirus, and HSV. Agglutinating antibodies against Francisella tularensis and IgG and IgM antibodies against L. pneumophila and L. micdadei were determined in our laboratory. Antibodies to C. pneumoniae (TWAR) were determined in our laboratory by direct microimmunofluorescence. Antigen slides were prepared from yolk sacs of infected chicken embryos and were fixed in acetone.

Invasive Diagnostic Studies

When recommended by the attending physician, certain invasive methods were employed to obtain specimens for diagnostic studies. Protected-brush specimens and transthoracic needle aspirations were taken from patients who were critically ill and/or immunosuppressed. The brush specimens were obtained via fiberoptic bronchoscopy with use of the sterile protected brush according to the method of Wimmerley et al. Other specimens were obtained by thoracentesis. These specimens were examined as described for routine microbiology, Legionella, and viral studies, except that an additional inoculation was made into thioglycolate broth.

Autopsy

When an autopsy was performed within 2 h of death, lung tissue was obtained aseptically and cultured for aerobic and anaerobic bacteria, mycobacteria, and fungi. Lung tissue obtained more than 2 h after death was cultured for only mycobacteria and fungi. Routine histologic studies and Gram stain were performed on all lung specimens, and special stains were performed for mycobacteria, fungi, and Legionella.

Criteria for Determination of Microbial Etiology

Prior to initiating the study, the investigative team set out criteria for the determination of microbial etiology. Organisms obtained by culture of material obtained by transthoracic needle aspiration and thoracentesis were considered etiologic agents. For patients whose blood cultures were positive for Staphylococcus epidermidis, we followed diagnostic criteria set out by the Centers for Disease Control (two positive blood cultures obtained at separate sites and times). For all other patients, organisms obtained from a single positive blood culture were considered pathogens. When 1,000 or more aerobic colonies were obtained from the protected brush, the organism was designated as the etiologic agent. Bacteria isolated by culture of lung tissue obtained at autopsy were designated etiologic agents only if the tissue was obtained within 2 h of death. Viruses, other than HSV and CMV, isolated from throat or bronchial washings and protected-brush specimens obtained from the bronchi were designated etiologic agents.

All patients showing a fourfold or greater rise in antibody titer to a respiratory pathogen were considered to have pneumonia due to that agent except for antibodies to CMV and HSV. For C. pneumoniae, results were considered positive if the IgM titer was greater than or equal to 32 or showed a fourfold or greater rise or if the IgG titer showed a fourfold or greater rise. Pneumonia due to more than one agent was designated as an acute episode when these laboratory criteria were met concurrently for two or more respiratory pathogens.

Gram stain and culture for aerobic bacteria were performed on all fresh sputum specimens, but information from these studies was not used to designate a specific microbial cause of infection. Instead, these data were tabulated separately and compared with those obtained with the other diagnostic methods employed.

Routine Microbiologic Studies

When it was possible for the patient to raise sputum, a fresh sputum specimen was collected by the study nurse and was taken immediately to the Clinical Microbiology Laboratory where it was studied by a single, designated microbiology technician. Gram stains of sputum were evaluated with consideration of the number of WBCs present. Stains showing WBCs in most fields at a magnification of 1,000 were reported as having numerous WBCs present, and those with rare or no WBCs in most fields were reported as having few or no WBCs present. The number of epithelial cells present was not recorded.

Sputum was cultured aerobically on sheep's blood agar, Levine eosin-methylene blue agar with added lactose, and heart infusion agar with disks impregnated with bacitracin and X and V factors. At least one blood specimen was obtained within 24 h after entry into the study and was cultured aerobically and anaerobically.

Legionella Studies

Specimens cultured for Legionella were plated on buffered charcoal/yeast-extract differential agar with and without polymyxin B, vancomycin, and ampicillin and were studied according to published methods. Direct immunofluorescence testing was performed on all sputum and lower respiratory tract specimens for Legionella pneumophila, serogroups 1 through 6, and Legionella micdadei.

Viral Studies

Throat washings, bronchial washings, and protected-brush specimens were transported to the clinical virology laboratory for viral isolation in cell cultures. Viruses that could be routinely identified were influenza virus types A and B, parainfluenza, adenovirus, herpes simplex virus (HSV), and cytomegalovirus (CMV).

Chlamydial Studies

Sputum and throat washings were inoculated on McCoy cells in shell vials following standard procedures for isolation of C. trachomatis.

Serologic Studies

Acute-stage serum was collected from each patient within 24 h after admission to the study and again after seven days. All surviving patients were followed up as outpatients at approximately two days.
RESULTS

Patients

In all, 233 patients who experienced a total of 240 episodes of suspected pneumonia were evaluated and admitted to the study. Each met the criteria established for entry, but on retrospective review after days or weeks of follow-up, 36 episodes involving 35 patients were diagnosed as due to another disease, such as pulmonary edema, pulmonary infarction, atelectasis, or tumor. Thus, for final analysis 198 patients with 204 episodes of pneumonia were studied and followed up to recovery or death. Of those 198 patients, 31 had a WBC count less than 10,000/cu mm, 54 were afebrile or hypothermic on entry, and 45 did not have purulent sputum, although all had new or progressive pulmonary infiltrates and were thought to have acute pneumonia by consensus among three pulmonologists.

The patients ranged in age from 19 to 98 years. The mean age was 64, and the median was 64. Eleven patients were under 40, 130 were over 60, and 19 were 80 or older. There were three women in the study. One or more significant underlying diseases were present in all but 12 patients. Fifty-eight had malignant conditions, 51 had central nervous system disorders, 62 had chronic obstructive lung disease, 50 had cardiovascular disorders, 22 abused alcohol, 14 had chronic renal insufficiency, 9 had collagen vascular disease, 7 had had recent chemotherapy for cancer, and 2 were renal transplantation patients. Among the 151 patients with a total of 154 episodes of community-acquired pneumonia, 43 had received prior antibiotic therapy. Of the 50 patients with hospital-acquired pneumonia, 16 had previously received antibiotics. Three patients over the course of one year had both an episode of community-acquired pneumonia and an episode of hospital-acquired pneumonia. There were 38 deaths among the patients with community-acquired pneumonia and 20 deaths in the set with hospital-acquired pneumonia. Of the 58 deaths, 24 occurred within seven days after entry to the study. A postmortem examination was done on 23 patients.

Results of Diagnostic Procedures

Despite the availability of extensive clinical and laboratory data with 100 percent follow-up for surviving patients, a specific etiology according to the criteria we employed was established in only 103 of 204 (50 percent) episodes. Results obtained with the various diagnostic measures used are set out in Table 1. For a few patients, more than one test was positive for the same or a different organism. A blood culture was obtained in 194 episodes (in 10 a blood culture was not taken), and 32 of these (16 percent) produced a pathogen. A transthoracic needle aspiration of the lung was done in 15 instances, and five of the aspirates produced a pathogen. Three of 14 pleural fluid samples were positive. A protected-brush specimen was obtained in 61 episodes, and 23 (38 percent) of these specimens produced bacteria in sufficient number to meet or exceed the threshold criteria set for designation as a pathogen. Of the 84 patients who underwent needle aspiration of the lung or pleural space or from whom a sample was obtained by protected brush, 26 had prior antimicrobial therapy. Seven of 194 sputum samples were positive for Legionella at direct fluorescent antibody study.

Influenza virus type A was isolated from throat swabs from three patients. Antibody responses resulted in the diagnosis of influenza for an additional nine episodes. Cytomegalovirus was isolated from three patients—from lung tissue at autopsy in two patients and from a throat washing in the third patient. On review of lung tissue, no histologic evidence of CMV infection was present in either patient. In the third patient, in whom CMV was isolated from throat washings, another pathogen was demonstrated, and the CMV serum antibody titer was low (1:16). Thus, CMV was not considered to be a pathogen in any of these cases. Significant (greater than eightfold) CMV antibody titer changes (rise or fall) were recorded in an additional 21 episodes of pneumonia in the absence of isolation of CMV.

It is interesting that HSV type 1 was isolated from 30 patients and proved to be the agent isolated with the highest incidence in the study. Twenty-eight isolates were from throat swabs, and 2 were from sputum specimens. Eightfold changes in HSV antibody titers were found in 3 of the 30 virus-positive patients and in 5 additional episodes. Again, a careful review of each virus-positive patient ruled out HSV as the pathogen. The HSV isolates were recovered mostly from patients without significant underlying disease. One patient had had a kidney transplant seven years before and had good renal function and survived the episode of pneumonia. Four other patients had solid

Table 1—Summary of Diagnostic Methods Employed and Results Obtained

<table>
<thead>
<tr>
<th>Diagnostic Method</th>
<th>No. of Episodes Studied</th>
<th>No. of Diagnostic Studies (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood culture</td>
<td>194</td>
<td>32 (16)</td>
</tr>
<tr>
<td>Transthoracic needle aspiration</td>
<td>15</td>
<td>5 (33)</td>
</tr>
<tr>
<td>Thoracentesis</td>
<td>14</td>
<td>3 (21)</td>
</tr>
<tr>
<td>Protected-brush specimen</td>
<td>61</td>
<td>23 (38)</td>
</tr>
<tr>
<td>Direct fluorescent antibody (sputum)</td>
<td>194</td>
<td>7 (4)</td>
</tr>
<tr>
<td>Viral isolation</td>
<td>192</td>
<td>3 (2)</td>
</tr>
<tr>
<td>Chlamydial isolation</td>
<td>192</td>
<td>0</td>
</tr>
<tr>
<td>Serology</td>
<td>204</td>
<td>52 (25)</td>
</tr>
<tr>
<td>Autopsy</td>
<td>23</td>
<td>3 (13)</td>
</tr>
</tbody>
</table>

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tumors, but no patient had a hematologic malignant condition. The WBC count in all 30 patients was either normal or elevated on admission to the study, and none of the 30 patients had skin lesions suggesting herpes infection.

A total of 163 serum samples were obtained from the 204 episodes, and 52 provided a serologic diagnosis. In three patients the diagnosis was not made until the postmortem examination. For some patients, more than one test was positive for the same or a different organism.

Community-Acquired Pneumonia

There were 154 episodes of community-acquired pneumonia in 151 patients, and 38 patients died. An etiologic diagnosis could be established in 79 (51 percent) episodes, and a single agent was identified in 69 of these (Table 2).

Thirteen episodes were caused by Legionella organisms; six were due to L pneumophila, and seven were due to L micdadei. It is important to note that of the 13 patients with Legionnaire's disease, 8 were diagnosed only after the convalescent-stage serum samples were obtained 2 months after discharge. Both patients whose specimens were sputum-positive for L pneumophila by direct immunofluorescence testing also showed seroconversion, but the three patients who were sputum-positive by immunofluorescence for L micdadei did not show seroconversion.

Various Gram-negative bacteria were the cause of 12 episodes, with no organism predominating. In one instance, tularemia pneumonia was diagnosed, but only after testing of the convalescent-stage serum. Gram-positive cocci accounted for 22 episodes, with Streptococcus pneumoniae found in 8 instances, Staphylococcus aureus in 7, and Staphylococcus epidermidis in 3. There were six episodes of influenza virus type A, eight of C pneumoniae pneumonia, and three of Mycoplasma pneumoniae.

Despite our efforts to exclude patients with mycobacterial and fungal infections based on clinical assessment on entry to the study, three were found to have tuberculosis, and one patient had aspergillosis.

Thus, from these 69 confirmed episodes of community-acquired pneumonia due to a single pathogen, 19 had a positive blood culture, 13 had positive protected-brush specimens, 27 were diagnosed serologically, 5 were positive by direct fluorescent antibody study of sputum, 2 showed positive culture from needle aspirates of the lung, 2 had positive pleural fluid cultures, 3 were diagnosed at autopsy, and 1 each was diagnosed by transbronchial lung biopsy (nocardiosis) and sputum culture (tuberculosis). Four episodes produced positive results for the same organism by more than one test.

Ten patients met criteria for having disease due to more than one etiologic agent; they are described in Table 3. In three patients, more than one organism was cultured from a single specimen (eg, the transbronchial needle aspirate of the lung or the protected-brush specimen grew two organisms). In three others, one agent was identified by culture of blood, and one or more other agents were identified by serology. The etiologic agents in the final four cases were all identified by serologic methods only. Four of these ten patients were found to have positive results for C pneumoniae. In one patient, Staphylococcus aureus was isolated from blood, and the serology results were positive for L pneumophila and C pneumoniae. In another patient, S epidermidis was isolated from two separate blood cultures, and serology was positive for
Table 3—Episodes of Community-Acquired Pneumonia with Multiple Pathogens Identified

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Organism and Diagnostic Method*</th>
</tr>
</thead>
<tbody>
<tr>
<td>216</td>
<td>Streptococcus faecalis (TNA), Salmonella group B (TNA)</td>
</tr>
<tr>
<td>85</td>
<td>Staphylococcus aureus (PBS), Staphylococcus viridans (PBS)</td>
</tr>
<tr>
<td>134</td>
<td>S aureus (PBS), Proteus spp (PBS)</td>
</tr>
<tr>
<td>191</td>
<td>Streptococcus pneumoniae (blood culture, PBS), Francisella tularensis (serology)</td>
</tr>
<tr>
<td>221</td>
<td>S aureus (blood culture), Legionella pneumophila (serology), Chlamydia pneumoniae (serology)</td>
</tr>
<tr>
<td>151</td>
<td>C pneumoniae (serology), Staphylococcus epidermidis (blood culture)</td>
</tr>
<tr>
<td>203</td>
<td>Mycoplasma pneumoniae (serology), F tularensis (serology)</td>
</tr>
<tr>
<td>199</td>
<td>L pneumophila (serology), M pneumoniae (serology), F tularensis (serology)</td>
</tr>
<tr>
<td>3</td>
<td>C pneumoniae (serology), influenza A virus (serology)</td>
</tr>
<tr>
<td>88</td>
<td>C pneumoniae (serology), F tularensis (serology)</td>
</tr>
</tbody>
</table>

*Diagnostic method is noted in parentheses. PBS = protected-brush specimen; TNA = transthoracic needle aspirate.

C pneumoniae. In the remaining two cases, serology was positive for C pneumoniae and influenza A virus in one and for F tularensis and C pneumoniae in the other.

Hospital-Acquired Pneumonia

There were 50 patients with hospital-acquired pneumonia, and 20 (40 percent) died. An etiologic agent was found in 24 (48 percent) of the 50 patients, and a single pathogen was identified in 15 (Table 4). No particular pathogen predominated in this group. Nine patients with hospital-acquired pneumonia had a mixed infection (Table 5); Gram-negative bacteria were the predominant pathogens in this group. From one patient with a tracheoesophageal fistula, four pathogens were cultured from a single protected-brush specimen, each in sufficient number to exceed criteria to be designated as a pathogen.

Results from Postmortem Examinations

An autopsy was performed in 23 of 58 patients. A primary pathogen had been identified prior to death in 11 patients. Among these, histologic evidence for pneumonia was seen in all but one; this patient had influenza virus type A isolated from throat washings, but the postmortem examination revealed only pulmonary edema. No antemortem pathogen was determined in the remaining 11 patients, but histologic examination and culture of autopsy material provided a diagnosis in an additional three patients; two had tuberculosis, and one had aspergillosis. Gross and histologic examination failed to reveal evidence of pneumonia in two other patients, showing instead bronchogenic carcinoma and atelectasis in one and pulmonary fibrosis and pulmonary edema in the other. Thus, the autopsy provided important new diagnostic information in 5 of 11 (45 percent) patients who had been studied extensively prior to death.

Discussion

It is distressing that, despite extensive laboratory testing and many invasive procedures done to obtain material for study, we were able to establish the pathogen in only 50 percent of the patients studied. However, our results are not substantially lower than those in other studies, in which an etiologic agent was found in only 67 percent, 55 percent, and 49 percent of patients.11-14 There are several reasons why we found fewer pathogens than expected. Wishing to use very

Table 5—Episodes of Hospital-Acquired Pneumonia with Multiple Pathogens Identified

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Organism and Diagnostic Method*</th>
</tr>
</thead>
<tbody>
<tr>
<td>154</td>
<td>Escherichia coli (PBS), Proteus morganii (PBS), Klebsiella pneumoniae (PBS), Pseudomonas aeruginosa (PBS)</td>
</tr>
<tr>
<td>116</td>
<td>E coli (blood culture), K pneumoniae (blood culture), P aeruginosa (blood culture)</td>
</tr>
<tr>
<td>64</td>
<td>P aeruginosa (blood culture), Staphylococcus aureus (blood culture)</td>
</tr>
<tr>
<td>11</td>
<td>E coli (blood culture), influenza A virus (PBS)</td>
</tr>
<tr>
<td>50</td>
<td>Proteus mirabilis (blood culture), Legionella micdadei (DFA-S)</td>
</tr>
<tr>
<td>133</td>
<td>E coli (PBS), Enterobacter aerogenes (PBS)</td>
</tr>
<tr>
<td>32</td>
<td>S aureus (PBS, blood culture), Legionella pneumoniae (serology), Chlamydia pneumoniae (serology)</td>
</tr>
<tr>
<td>18</td>
<td>L pneumophila (DFA-S), influenza A virus (serology)</td>
</tr>
<tr>
<td>1</td>
<td>C pneumoniae (serology), influenza A virus (serology)</td>
</tr>
</tbody>
</table>

*Diagnostic method is noted in parentheses. DFA-S = direct fluorescent antibody study of sputum; PBS = protected-brush specimen.
strict criteria, we did not accept Gram stain and culture data from expectorated sputum. However, had we used sputum results also, a diagnosis would have been made in only an additional five patients. Prior antibiotic therapy had been given in 56 episodes, and this undoubtedly lowered the diagnostic yield. We did not search for all the known genera and serotypes of Legionella, since only a limited number of specific antisera are available for our use. No Legionella organisms were grown on culture, and we conclude that the media and/or methods used were flawed. Finally, we speculate that there are additional pathogens infecting the patients we studied that have not been described.

Legionella organisms are among the most common pathogens found in our patients, an observation in agreement with other reports noting these organisms to be among the four or five most frequently recognized causative agents of pneumonia.15-17 The high frequency of Legionella infection in the present study is attributed to the careful follow-up of each patient to ensure that convalescent-stage serum samples were obtained. Had we failed to obtain convalescent-stage serum specimens, 13 of 18 diagnoses of Legionella infection would have been missed.

The Gram-negative bacilli as a group combined to form a major cause of pneumonia in our study, even among those with community-acquired infection. The combination of S aureus and S epidermidis was more frequently found than was Streptococcus pneumoniae. Only two Haemophilus influenzae infections were observed among patients with community-acquired pneumonia.

The relatively infrequent occurrence of S pneumoniae, the rare infection with H influenzae, and the relatively common encounters with enteric Gram-negative bacilli are in sharp contrast with other published reports where a pneumococcal pathogen is commonly observed and Gram-negative enteric bacilli are infrequently seen.19-20 These contrasts may be explained in part by the differences in the patient population studied and in the criteria and methods used to determine the etiologic agent. When sputum studies are used for detection of aerobic bacterial pathogens or when polysaccharide capsular products are measured in sputum, the designation of infection due to S pneumoniae and H influenzae increases. When sputum studies are not used and when secretions from the lower respiratory tract are obtained without oropharyngeal contamination, fewer pneumococcal and H influenzae isolates are obtained and more Gram-negative enteric bacilli and S aureus strains are isolated.21,22 Until a larger number of patients are evaluated using invasive methods to obtain material for study, these contradictions will persist. Correct answers are urgently needed, since recommendations regarding the appropriate antibiotic therapy for these patients turn on these observations.

It is interesting that influenza virus was the only respiratory virus isolated in the study, with no isolations of adenovirus or parainfluenza viruses. All influenza A virus isolates and seroconversions were observed during the winter months at a time when influenza was occurring sporadically in the state. A major outbreak of influenza did not occur in the state during the study year. As would be expected, influenza virus was implicated in hospital-acquired pneumonia as well as in community-acquired disease.

Our experience with HSV and CMV reflects the problems encountered in attempting to establish these two viruses as pathogens in pneumonia, particularly in a comprehensive study such as ours.23,24 In two instances, CMV was isolated from lung tissue at autopsy; even in these cases, however, a review of the histopathologic findings failed to implicate this virus. Colonization of the peripheral airways by CMV without evidence of pneumonia has been recognized previously.

We encountered a number of patients who had significant increases in antibody titers to CMV in the absence of isolation of the virus, but this was not found for HSV. No particular meaning can be attached to these findings with the information at hand.

Roy and coworkers25 studied Mycoplasma pneumonia among families and observed that M pneumoniae infection was highest among children, although it occurred in adults, peaking in the 30- to 40-year age group. Clyde27 has reported also that Mycoplasma pneumonia is relatively uncommon among older adults. In the present series, seven of nine patients shown to have M pneumoniae infection were over age 60. In two of these, there was a concurrent diagnostic antibody increase to one or more additional respiratory pathogens. In one, the pathogen was F tularensis; in the other, the pathogens were F tularensis and L pneumophila. It is probable that one or more of these episodes represent a serologic cross-reaction or non-specific polyclonal stimulation.

Grady and Gilfillan28 noted that patients with Legionella infection commonly have antibody to M pneumoniae. However, Renner and coworkers29 reviewed antibody titers to M pneumoniae and L pneumophila type I in the sera of 1,000 patients with acute respiratory tract infection and found no significant cross-reactivity. Antibodies to both agents are commonly found in the same patient, but fourfold increases to both agents following infection with only one is most uncommon. Serologic cross reactions between Chlamydia and Legionella have been reported, while cross-reactivity between F tularensis and M pneumoniae has not been reported.29 Since these three patients met our arbitrary criteria for
having mixed infection, we have included them in this category. Such diagnostic dilemmas can be expected when a large battery of serologic tests is done in multiple patients with suspected respiratory infection.

Chlamydia trachomatis is well recognized as a cause of pneumonia in young infants and has been isolated from the lower respiratory tract of immunosuppressed adults with pneumonia. Community-acquired pneumonia in otherwise normal adults has been reported to be caused by C trachomatis, but these conclusions were based on serologic evidence only, with no reports of positive culture results in such patients. In the present study, we attempted to culture C trachomatis from the respiratory secretions in 192 episodes of pneumonia and found none positive. Had this organism been present, we believe that positive isolations would have occurred, since our laboratory regularly isolates this organism from patients with genitourinary tract infection. At present, the data supporting the position that C trachomatis can cause pneumonia in relatively healthy adults must be viewed with great skepticism.

On the other hand, C pneumoniae (TWAR) has recently emerged as a potential cause of adult respiratory disease. Implication of this organism has had to rely mainly on serologic studies, because isolation from clinical specimens has not been successful generally. With application of rather strict criteria, 12 cases of community-acquired pneumonia were associated with the TWAR strain; in eight of them this agent was the only pathogen detected. These data strengthen the evidence that TWAR is a significant pathogen for pneumonia in adults.

Two cases of hospital-acquired pneumonia were associated with TWAR. In one of these patients, S aureus was isolated from blood cultures, while the serology was positive for L pneumophila. In the other patient a serologic response to influenza was found. Other studies have indicated a high incidence of TWAR infection in hospital-acquired pneumonia. If more liberal criteria had been used, namely, IgG antibody titers greater than or equal to 1,024, an additional 17 patients would have been considered to have TWAR infections. For 8 of these 17 patients, another cause was established. Four had influenza A, two had M pneumoniae, and two had L pneumophila infection. Thus, TWAR appears to play an important role in pneumonia among patients in Veterans Administration facilities.

This study provided an opportunity to evaluate the use of protected-brush specimens for diagnosis of acute pneumonia. Sixty-one patients were subjected to this technique, and no complications occurred, although several patients were very ill and markedly hypoxemic. Twelve had previously received antibiotics. Twenty-three (38 percent) of the 61 specimens obtained in this way provided a microbial diagnosis. Five of the 12 specimens obtained after antibiotic therapy gave a specific diagnosis. In seven, the brush culture was sterile, and in 30 the culture grew only a few colonies of one or more bacteria. There were five instances in which both the blood culture and the protected-brush culture were positive in the same patient; in all five cases, the organism grown from the blood was the same as that grown in high concentration from the brush. These data support the value of the protected-brush culture technique.

Gram stain and culture of sputum for aerobic bacteria failed to produce useful information in most of our patients in whom an etiologic diagnosis of bacterial pneumonia was established by another method. Among 52 patients with bacterial pneumonia, 34 were able to produce sputum; of these, 13 had had prior antibiotic therapy. Specimens obtained in 11 of these 34 cases showed numerous WBCs and a single pathogen on Gram stain. From these 11 specimens, the correct pathogen was obtained in five.

An autopsy was performed on 23 of the 58 patients who died. A known pathogen was identified prior to death in 11 of these; among this group, histologic evidence for pneumonia was seen in ten. A specific etiologic antemortem diagnosis could not be made in the remaining 11; among these 11, histologic examination and culture of autopsy material produced a diagnosis in three patients (two had tuberculosis, and one had invasive aspergillosis), and in two others no evidence of pneumonia was found. This information underscores the importance of the autopsy on patients in whom no cause for the pneumonia is found even though extensive diagnostic studies have been done.

We believe that this study permits the following conclusions. Despite elaborate diagnostic studies, including many invasive procedures, only about half of the patients seen with an acute episode of pneumonia will have a specific microbial pathogenesis established when strict criteria are used. In this population of patients, the microbial pathogens of pneumonia are not strikingly different between community-acquired and hospital-acquired infection. The protected-brush method can provide specimens that give reliable diagnostic information in a substantial percentage of patients, and its use is encouraged in selected patients who are critically ill and/or immunosuppressed, because knowing the specific pathogenesis may be important for a favorable therapeutic outcome.

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