Case Report

Outbreak of *Chlamydophila pneumoniae* infection in long-term care facilities and an affiliated hospital

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This paper reports an outbreak of *Chlamydophila pneumoniae* infection in long-term care facilities and an affiliated hospital. The outbreak involved rapid spread of infection, and was inconsistent with several outbreaks experienced among younger populations. In addition, there were differences in the incidences among facilities and the affiliated hospital in relation to mean age. Our findings indicate that it is possible that elderly residents may be more susceptible to acquiring this infection. Physicians and other health care providers in long-term care facilities should consider *C. pneumoniae* in the differential diagnosis of an outbreak of respiratory infection.

Introduction

*Chlamydophila pneumoniae*, an obligate intracellular human pathogen, has been proven to cause both epidemic and endemic respiratory tract infections in many areas of the world (Kuo et al., 1995). It is a significant cause of both lower and upper acute respiratory illnesses, and accounts for approximately 10% of cases of community-acquired pneumonia. Most respiratory infections caused by *C. pneumoniae* are mild or asymptomatic, although patients with underlying medical conditions occasionally become quite ill. Some studies have suggested a possible association of *C. pneumoniae* infection with acute exacerbations of asthma and chronic obstructive pulmonary disease (COPD). Seronepidemiological studies showing antibody prevalence rates with a range of 50 to 70% suggest that *C. pneumoniae* is widely distributed and that nearly everybody is infected with the agent at some time (Kuo et al., 1995).

Outbreaks of *C. pneumoniae* have occurred in closed populations including among families, university students, military trainees and in schools (Kuo et al., 1995; Yamazaki et al., 1990; Mordhorst et al., 1992; Blasi et al., 1994; Hagiwara et al., 1999; Kleemola et al., 1988; Ekman et al., 1993; Kishimoto et al., 1994; Soda et al., 1997). Such outbreaks among the younger population have been repeatedly observed. Outbreaks of *C. pneumoniae* among the elderly population have also been reported, and it has been suggested that the epidemiological and clinical findings of outbreaks in nursing homes differ from those in younger population settings (Troy et al., 1997). However, the available data for elderly population settings are limited. Recently, we encountered an outbreak of *C. pneumoniae* infection in a long-term care facility and an affiliated hospital. Here we report the epidemiological and clinical findings of outbreaks of *C. pneumoniae* infection in long-term care facilities and compare them with former outbreaks that we have experienced (Kishimoto et al., 1994; Soda et al., 1997).

Case report

The long-term care facility in question was a 100-bed facility in a three-story building with two different types of residents, those with senile dementia (first floor) and those with chronic mental disorders (second floor). These two floors housed 48 residents each. Most residents required slight to substantial assistance in their routine daily activities and personal care. The first floor is connected to other buildings including a general hospital operated by the same owner (46 patients admitted on the second floor). On the first floor were common areas including a large dining room, a lounge and a rehabilitation room shared by most residents and some patients. Most residents shared living areas with one to three other residents. However, 15 patients had been admitted to single rooms.

The index case, a 44-year-old man who had been housed in this facility for more than 10 years for congenital brain paralysis, complained of nasal discharge and low grade fever on 16 June 2002. His symptoms persisted for more than 1 week and new symptoms such as cough and sputum production appeared. He was treated with cefcapene pivoxil HCl (300 mg day⁻¹) for 3 days, but his symptoms still persisted. No micro-organisms were detected in the sputum by Gram’s stain and culture. A chest X-ray showed no infiltrative shadows in the lung fields. His laboratory data revealed mild elevation of the C-reactive protein value (1.21 mg dl⁻¹) and a normal white blood cell count (6200 mm⁻³). Clarithromycin (400 mg day⁻¹) was administered in...
place of cefcapene pivoxil HCl and his symptoms improved. This case was diagnosed as a case of C. pneumoniae infection, because seroconversion of C. pneumoniae-specific IgG and IgM antibodies measured by the microimmunofluorescence (MIF) test was observed among serum samples (day 14 after onset: IgM 1 : 32, IgG < 1 : 16; day 42 after onset: IgM 1 : 128, IgG 1 : 32).

This man always used common areas in the facility, such as the dining room and rehabilitation room. At that time, an increase in respiratory illness was noted in this facility and its affiliated hospital, but no increase in respiratory illness was observed in the community. A survey of this facility and the hospital was conducted.

### Methods

**Epidemiological and clinical investigation.** Respiratory illness surveillance was done by the medical doctors in the affiliated hospital until 30 September 2002, and microbiological laboratory tests were done both in our laboratory and the affiliated hospital. These respiratory illness surveillances were reviewed to determine the extent of the outbreak. In the case of those residents and patients who were hospitalized or died during the outbreak period, charts and hospital medical records were also reviewed for diagnosis, chest X-ray findings and laboratory results.

Paired serum samples were collected at intervals of at least 4 weeks after onset from 31 ill residents (11 residents with chronic mental disorders and 20 residents with senile dementia) and six patients with newly recognized respiratory illness. Single serum samples were collected from the remaining cases with respiratory illness and 46 non-cases (26 healthy residents and 20 patients with no reported respiratory symptoms).

Nasopharyngeal swab specimens and urine samples were obtained from all ill cases and 46 non-cases (26 healthy residents and 20 patients with no reported respiratory symptoms). However, more than 50% of the ill cases had received antibiotics such as clarithromycin, minocycline or levofloxacin at the beginning of surveillance. Swab specimens were stored at 4°C and transported to our laboratory packed in ice in an insulated container. We carried out laboratory tests within 4 h of collection.

**Microbiological laboratory tests.** The microbiological laboratory tests were performed as previously described (Miyashita et al., 2002). Serum samples were tested for antibodies to influenza A and B viruses, adenovirus, respiratory syncytial virus, cytomegalovirus and parainfluenza viruses (types 1, 2 and 3) by complement fixation test. Antibody to Mycoplasma pneumoniae was measured by the particle agglutination test (Serodia Myco II; Fujirebio) (Echevarria et al., 1990). Antibody to Legionella species was measured by the microplate agglutination test (Denka-seiken) as described previously (Yabuuchi et al., 1997). Heat-killed bacteria, including Legionella pneumophila (serogroups 1a, 1b, 2 to 6), Legionella bozemanae, Legionella dumoffii, Legionella gormanii and Legionella micdadei were prepared by incubating bacterial suspensions for 1 h at 100°C. Heat-killed bacterial suspensions (25 μl) were inoculated into each well of diluted serum samples. The plate was incubated at room temperature for 20 h, after which agglutination in the wells was determined.

The MIF test was used to measure antibodies to Chlamydia and Chlamyphilia species (Wang & Grayston, 1970). With regard to chlamydial serological testing, only use of the MIF test is recommended by the USA Centers for Disease Control and Prevention (CDC) and the Canadian Laboratory Centre for Disease Control (LCDC) because it is the only species-specific and sensitive antibody test (Dowell et al., 2001). Purified formalized elementary bodies of C. pneumoniae KKpn-15 and TW-183, Chlamydia trachomatis L2/434/Bu and Chlamyphilia psittaci Budgerigar-1 strains were fixed onto glass slides as distinct dots of antigen. Dilutions of sera were placed over the antigen dots and incubated. The presence of IgG and IgM antibodies against chlamydial species was detected using commercial FITC-conjugated goat anti-human IgG and IgM (Medical and Biological Laboratories). Rheumatoid factors were absorbed with Gulsorb (Gulf Laboratories) before IgM titrations.

Nasopharyngeal swab specimens were tested for influenza A and B viruses by direct enzyme immunoassay (ELISA) and for chlamydial species by isolation in cell culture and PCR. The swab specimens in a SPG transport medium were sonicated and briefly centrifuged (900 g for 10 min), and then the supernatant was overlaid on confluent monolayers of HEp-2 cells grown on round coverslips (14 mm in diameter) set in 24-well cell culture plastic plates. The plates were centrifuged at 1200 g for 60 min at room temperature. Next, 1 ml of a culture medium consisting of Eagle’s minimal essential medium (Nissui Pharmaceuticals), 10% heat-inactivated foetal calf serum (Gibco-BRL Life Technologies) and cycloheximide (Nakarai Tesque) at a final concentration of 1 μg ml⁻¹ was applied. Then the plates were incubated in 5% CO₂ at 35°C for 72 h and all specimens were passaged twice. Following incubation, a genus-specific FITC-conjugated mAb (Chlamydia FA Seiken; Denka Seiken) and C. pneumoniae species-specific mAbs were used to stain inclusions (Miyashita et al., 2002). Inclusion bodies formed in the cells were observed with a Nikon epifluorescence microscope at ×200 or ×400 magnification.

The C. pneumoniae-specific primers used for the nested PCR were from the DNA base sequence within the 53 kDa protein gene established in our laboratory (Fukano, 2004). The CDC-LCDC recommendations state that all new PCR assays should be compared to at least one of the four selected assays that meet the proposed validation criteria (Dowell et al., 2001). Data relating to the sensitivity, specificity and reliability of our 53 kDa nested PCR assay, as well as a comparison with the four most widely used conventional PCRs (cloned PstI fragment-single step, 16S rRNA gene-touchdown enzyme time-release, 16S rRNA gene-nested and ompA gene-nested touchdown assays) for detection of C. pneumoniae, were recently published (Fukano, 2004). This 53 kDa PCR assay was performed as previously described and was carried out without prior knowledge of the culture results. The cell-culture-grown C. pneumoniae strain KKp-15 was used as a positive control and chlamydial transport medium was used as a negative control in every run. After electrophoresis of amplification products on a 1.5% agarose gel at 100 V, bands were visualized by staining with ethidium bromide. The appearance of a 239 bp amplification product was taken as positive.

When sputum was available, a Gram’s stain and a quantitative culture were obtained. Sputum data were only evaluated when the Gram’s stain test revealed numerous leukocytes (>25 in a ×100 microscopic field) and few squamous epithelial cells (<10 in a ×100 microscopic field). Blood cultures were obtained from 40 cases. In addition to serology and/or culturing, the urinary antigen test (Binax NOW) was used for detection of Streptococcus pneumoniae and L. pneumophila.

### Criteria for determination of microbial aetiology.

The microbial aetiology was classified as `definitive`, `presumptive` or `unknown`. A definitive aetiology was defined if one of the following conditions was present: (1) blood culture yielding the presence of bacterial or fungal pathogen; (2) urinary antigen test results positive for L. pneumophila or S. pneumoniae; (3) nasopharyngeal antigen test results positive for influenza A and B viruses; (4) a fourfold increase in the antibody titre for viruses, M. pneumoniae (to ≥ 1 : 160), Legionella species (to ≥ 1 : 128) or Chlamydia and Chlamyphilia species (IgM or IgG); or (5) a single increase in the IgM titre for Chlamydia and Chlamyphilia species to
A mean age comparison was done by Student’s t test. A mean age comparison was done by Fisher’s exact test.

Results

The outbreak occurred between 16 June and 8 July 2002, with 48 (33.8%) of the 142 residents and patients reporting respiratory illness (Fig. 1). After 8 July, no respiratory illness was noted. The characteristics of the residents and patients with acute respiratory illness is shown in Table 1. Among the 48 residents and patients with chronic mental disorders, 15 (31.2%) had respiratory illness, including two cases of pneumonia, and there was one pneumonia-related death. Among the 48 residents with senile dementia, 25 (52.0%) had respiratory illness, including five cases of pneumonia, and there was one pneumonia-related death. Among the 46 cases in the general hospital, eight (17.3%) had respiratory illness, including two cases of pneumonia, but there were no deaths. The incidence in the facility for the residents with senile dementia was significantly higher than that in the general hospital (52.0% vs 17.3%, P = 0.0005). There was no significant difference in the incidence between the facility for the residents with chronic mental disorders and the general hospital (31.2% vs 17.3%, P = 0.1519). The mean age of the residents with senile dementia was significantly higher than that of those with chronic mental disorders (73.3 ± 11.1 vs 55.6 ± 10.0, P < 0.001) and the patients in the general hospital (73.3 ± 11.1 vs 46.1 ± 14.1, P < 0.0001). The mean age of the residents with chronic mental disorders was also significantly higher than that of the patients in the general hospital (55.6 ± 10.0 vs 46.1 ± 14.1, P = 0.0004).

The aetiologies of respiratory illness in 33 residents and patients in whom micro-organisms were detected are shown in Table 2. C. pneumoniae was detected by isolation in three cases and by PCR in 21 cases. All culture-positive specimens were also PCR positive. Serological evidence of acute C. pneumoniae infection was found in 16 cases. Nine cases were PCR positive and serologically positive, and 19 cases were positive with discrepancies in both the PCR and serology results. Seven cases were PCR negative and serologically positive, and 12 cases were PCR positive and serologically negative. All culture-positive cases were also serologically positive. C. pneumoniae was also detected by isolation in two non-cases and by PCR in six non-cases. Other respiratory tract pathogens such as bacteria were isolated from five individual cases from whom sputum specimens were collected for culture. No serological evidence of recent infection with other respiratory tract pathogens, including viruses, M. pneumoniae or Legionella spp., were found in any of the serum samples. Ninety-four urine samples and nasopharyngeal swab specimens were all negative for Legionella spp. and S. pneumoniae, and for influenza A and B viruses, respectively. However, the complement fixation tests for the detection of viral agents are widely recognized to be of limited sensitivity and we might have missed the diagnosis of some viral infections.

Discussion

Outbreaks of C. pneumoniae in closed communities such as families, schools and military garrisons have been observed (Kuo et al., 1995; Yamazaki et al., 1990; Mordhorst et al., 1992; Blasi et al., 1994; Hagiwara et al., 1999; Kleemola et al., 1988; Ekman et al., 1993). We have also encountered outbreaks of C. pneumoniae in nursery schools, kindergartens, elementary schools and junior high schools (Kishimoto et al., 1994; Soda et al., 1997). In these outbreaks, C. pneumoniae spreads slowly for about 4–5 months. The relatively long time needed for spread of the infection is similar to the slow spread observed in several previously reported outbreaks in closed environments. These prolonged case-to-case intervals indicate that C. pneumoniae has a long incubation period (Kishimoto et al., 1994; Soda et al., 1997).

In the present outbreak, however, rapid spread of the infection was observed. The same observation has been reported in nursing homes and families (Blasi et al., 1994; Hagiwara et al., 1999; Troy et al., 1997). Troy et al. (1997) suggested that the rapid spread of infection might be influenced by the close, compact living environment within nursing homes. Blasi et al. (1994) also indicated that the rapid spread might be explained by living habits in which families lived in small flats, with high person-to-person contact. In our cases, most residents shared a room with two
or three other residents, and several common areas including
the dining room and rehabilitation room were shared among
the residents. Our findings also suggested that rapid spread of
infection may occur in a close and compact living environ-
ment. In addition, early use of appropriate antibiotics may
shorten the duration of such an outbreak.

The incidence of the respiratory symptoms in the general
hospital was significantly lower than that in the long-term
care facility. There were significant differences in the mean
age, number of underlying diseases, and frequency of use of
common areas and buildings between these two groups. The
cases in the general hospital were younger, and they had a
lower number of underlying diseases and lower frequency of
use of common areas than the cases in the long-term care
facility. However, we could not find any differences in risk
factors between cases and non-cases. Our findings together
with those of Troy et al. (1997) indicate that it is possible that
elderly residents may be more susceptible to acquiring this
infection, because of impaired local and systemic host

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**Table 1. Characteristics of residents and patients with respiratory illness***

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. of patients or residents affected*</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Long-term care facility</td>
</tr>
<tr>
<td></td>
<td>Chronic mental disorders</td>
</tr>
<tr>
<td>Cases/residents or patients</td>
<td>15/48</td>
</tr>
<tr>
<td>Age (years)</td>
<td>58.4 ± 11.8</td>
</tr>
<tr>
<td>Male/female</td>
<td>8/7</td>
</tr>
<tr>
<td>Underlying disease</td>
<td>9</td>
</tr>
<tr>
<td>Current smoker</td>
<td>2</td>
</tr>
<tr>
<td>Mortality</td>
<td>0</td>
</tr>
<tr>
<td><strong>Type of respiratory illness</strong></td>
<td></td>
</tr>
<tr>
<td>Upper respiratory tract infection</td>
<td>9</td>
</tr>
<tr>
<td>Bronchitis</td>
<td>4</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>2</td>
</tr>
<tr>
<td><strong>C. pneumoniae detected by different methods</strong></td>
<td></td>
</tr>
<tr>
<td>Serology</td>
<td>7</td>
</tr>
<tr>
<td>Culture</td>
<td>1</td>
</tr>
<tr>
<td>PCR</td>
<td>7</td>
</tr>
</tbody>
</table>

*Data for age is presented as mean ± sd.

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**Table 2. Aetiology of respiratory illness in 33 residents and patients in whom microorganisms were detected**

<table>
<thead>
<tr>
<th>Aetiology</th>
<th>Definitive diagnosis</th>
<th>Presumptive diagnosis</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Chlamydia pneumoniae</em></td>
<td>16*</td>
<td>12</td>
<td>28</td>
</tr>
<tr>
<td><em>Mycoplasma pneumoniae</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Legionella spp.</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Viruses</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Bacteria†</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em></td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td><em>Hemophilus influenzae</em></td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

* Nine cases were also PCR positive and three cases were culture positive.
†Sputum was collected from 20 cases.
defences such as decreased mucociliary clearance and T-cell function, which also may have contributed to the rapid spread of infection.

One of the important findings reported in the nursing home outbreaks involved the smoking setting (Troy et al., 1997). It was reported that smokers had onset of illness earlier than non-smokers, suggesting that the smoking room may have played a role in airborne transmission early in the outbreak (Troy et al., 1997). In our cases, however, no differences were observed in the onset of illness between smokers and non-smokers. This may be because there were only a small number of smokers among our cases.

During this outbreak, a notice that staff members should visit the outpatient clinic if they experienced respiratory symptoms was issued by the hospital. Fifteen staff members visited the outpatient clinic while experiencing respiratory symptoms such as a cough, nasal discharge, a sore throat and/or hoarseness. None of the ill staff members had physician-diagnosed pneumonia, required hospitalization or died. Among these 15 cases with respiratory illness, C. pneumoniae was detected by PCR in five cases and by isolation in none of the cases. Serological evidence of acute C. pneumoniae infection was found in three cases (we measured only single serum samples because no paired samples were obtained from any of these cases). Two cases were PCR positive and serologically positive, and four cases were positive with discrepancies in both the PCR and serology results. Unfortunately, we could not determine the extent of C. pneumoniae infection accurately among staff members, because we did not know whether all symptomatic staff members visited the outpatient clinic.

Troy et al. (1997) encountered asymptomatic C. pneumoniae infection in eight of 72 (11%) healthy residents. In the present outbreak and former outbreaks experienced in schools, we confirmed the same observation. Our findings together with those of Troy et al. (1997) suggest that asymptomatic infections may play a role in the transmission of C. pneumoniae infection within long-term care facilities.

Acute respiratory tract infections are common among the elderly living in long-term care facilities, causing substantial morbidity and mortality. Influenza virus and respiratory syncytial virus are well-recognized causes of outbreaks in this setting. In our findings, outbreaks of C. pneumoniae infection occurred in closed communities among both younger populations and elderly populations. Therefore, physicians and other health care providers in long-term care facilities should consider C. pneumoniae in the differential diagnosis of an outbreak of respiratory infection.

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References
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