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Chlamydia pneumoniae and the lung

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ABSTRACT: Chlamydia pneumoniae is a frequently occurring respiratory pathogen affecting all age groups. It may cause 5–20% of community-acquired pneumonias in adults and children. The organism has also been implicated as an infectious trigger for asthma. Furthermore, new studies suggest that it may play a role in the pathogenesis of several chronic diseases including atherosclerosis.

However, despite the growing significance of C. pneumoniae as a pathogen, progress is hampered by the lack of standardized diagnostic methods including serology and polymerase chain reaction. This makes it practically impossible for the practitioner to make a specific microbiological diagnosis. The lack of standardized methods has also had an adverse effect on treatment trials. The dependence on serology for diagnosis in treatment studies has generated some questionable results.

Unless cultures are performed, microbiological efficacy cannot be assessed and it may never be possible to survey for or document the emergence of resistance.
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Chlamydia pneumoniae was first described as a respiratory tract pathogen by GRAYSTON et al. [1] in 1986. The genus Chlamydia comprises a group of obligate intracellular parasites that have a unique developmental cycle with morphologically distinct infectious and reproductive forms. All members of the genus have a Gram-negative envelope without peptidoglycan, share a genus-specific lipopolysaccharide (LPS) antigen and utilize host adenosine triphosphate for the synthesis of chlamydial protein. The genus now contains four species, C. psittaci, C. trachomatis, C. pneumoniae and C. pecorum.

Epidemiology of respiratory infection due to Chlamydia pneumoniae

C. pneumoniae appears to be a primary human pathogen. Attempts to identify zoonotic reservoirs have been unsuccessful, although C. pneumoniae infection has been described in several nonhuman species including koalas [2]. The mode of transmission remains uncertain but is probably via infected respiratory secretions. C. pneumoniae can survive in small aerosol droplets [3, 4]. There is a report of a laboratory accident in which transmission by aerosol is thought to have taken place [5]. Outbreaks of C. pneumoniae have occurred in enclosed populations such as among military recruits and residents of nursing homes [6–8]. Spread of infection has also been documented among family members in the same household [9]. Serological surveys demonstrate a rising prevalence of antibodies directed against C. pneumoniae with increasing age, from 10% at 5–10 yrs of age, reaching 30–45% by adolescence and often exceeding 80% in the elderly [1].

The proportion of community-acquired pneumonias in children and adults associated with C. pneumoniae infection has ranged 6–22%, varying with geographical location, age group examined and diagnostic methods used [10]. Most of these studies were based on serology alone.

The term "atypical" pneumonia has been used to differentiate infections caused by C. pneumoniae, Mycoplasma pneumoniae, Legionella and other related organisms from pneumonia caused by classic bacteria, the prototype being Streptococcus pneumoniae. However, recent studies have demonstrated that the clinical presentation of pneumonia due to atypical pathogens cannot readily be differentiated from that caused by "typical" bacteria [10]. This is further complicated by the observation that coinfections with atypical pathogens and other bacteria are frequent. Nonetheless, the term "atypical" can be useful as these organisms share a number of characteristics that separate them from "typical" bacteria. They are either obligate or facultative intracellular parasites that cannot be isolated using routine microbiological methods. The most commonly used method of diagnosis of these infections is serology, which basically offers a retrospective diagnosis. There are no US Food and Drug Administration-approved commercially available nonculture tests for C. pneumoniae and M. pneumoniae.

There is still considerable controversy regarding the significance of atypical pathogens, including C. pneumoniae, as agents of community-acquired pneumonia. Overall, atypical pathogens have been implicated as causing 2–30% of community-acquired pneumonia in adults and children [10]. The prevalences for individual pathogens can vary greatly from study to study depending upon the

populations studied and the diagnostic methods used. The results of several selected recent studies are summarized in table 1. A recently published population-based active surveillance study of community-acquired pneumonia requiring hospitalization in Ohio, USA [11] found that the major three atypical pathogens, *M. pneumoniae*, *C. pneumoniae* and *Legionella* species, together accounted for 10–38% depending on the serological criteria used for diagnosis. Individually, the prevalences were 32.5% for *M. pneumoniae*, 8.9% for *C. pneumoniae* and 3% for *Legionella* species compared to 12.6 and 6.6% for *S. pneumoniae* and *Haemophilus influenzae*, respectively. Serology was used for the diagnosis of *M. pneumoniae* and *C. pneumoniae* infection, and isolation of the organism from sputum or serology for *Legionella* species. In contrast, diagnosis of bacterial infection required isolation of the organism from blood or pleural fluid (definite infection) or from purulent sputum with supporting Gram-stain appearance (probable infection). A multicentre study in four Scandinavian countries [13] found, using serology exclusively, that 66% of the patients had a presumptive diagnosis of pneumonia caused by *M. pneumoniae*, chlamydiae or *Legionella* species. The prevalences for the individual pathogens alone causing infection were 17, 14 and 3% for *M. pneumoniae*, *C. pneumoniae* and *Legionella* species, respectively. Mixed infections were frequent; an additional 9% of patients had mixed infection with *M. pneumoniae* and *C. pneumoniae*, and 4% with *M. pneumoniae* and/or *C. pneumoniae* and *S. pneumoniae*. This study differed from the Ohio study in that outpatients as well as inpatients were included; thus the overall severity of infection may have been lower. However, this does not entirely explain the higher prevalence of atypical bacteria and lower prevalence of pneumococcal infection. A smaller US study from Baltimore, MD, USA, conducted during the same time period as the Ohio study, identified infection with an atypical organism in only 7.5% of the patients, over 50% of which were also infected with a second pathogen [14]. The diagnostic methods used included culture and polymerase chain reaction (PCR) in addition to serology. Conventional bacterial pathogens were identified in 36% of the patients.

Although initial serological studies suggested that infection with *C. pneumoniae* was uncommon in young children, subsequent studies utilizing culture found the prevalence to be similar to that observed in adults [15, 16]. As part of a multicentre pneumonia treatment study in children aged 3–12 yrs, BLOCK *et al*. [15] isolated *C. pneumoniae* from 34 of 260 (13.1%) children enrolled. Serological evidence of acute infection was found in 48 (18.5%), but only eight (23%) of the culture-positive children met the serological criteria for acute infection. In a subsequent multicentre study, HARRIS *et al*. [16] isolated *C. pneumoniae* from 7.4% of 420 children, aged 0.5–16 yrs, with community-acquired pneumonia. Only five (16%) of the 31 culture-positive children met the serological criteria for acute infection; most were seronegative. In both studies, the prevalence of culture-documented *C. pneumoniae* infection was the same in the children who were <6 yrs of age as in those who were >6 yrs of age. Coinfections with *C. pneumoniae* and other bacteria, especially *S. pneumoniae*, appear to be fairly frequent. Coinfection with *M. pneumoniae* was documented in 20% of children with culture-documented *C. pneumoniae* infection in the study of BLOCK *et al*. [15]. Clinically, the children who were infected with both organisms could not be differentiated from those who were infected with either organism alone. The only child in the study of BLOCK *et al*. [15], who had pneumococcal bactaeremia was also infected with *C. pneumoniae*. These patients frequently respond to treatment with β-lactam antibiotics, suggesting that *C. pneumoniae* may not be the primary cause of the pneumonia but might disrupt the normal clearance mechanisms, enabling other pathogens to invade. *C. pneumoniae* has been shown to have a cilia-static effect on ciliated bronchial epithelial cells *in vitro* [17].

Persistent nasopharyngeal (NP) infection with *C. pneumoniae* following acute respiratory infection has been documented in adults for periods of up to several years [18, 19]. However, background asymptomatic respiratory infection has been reported in 2–5% of subjectively healthy adults and children [20–23]. It is not known what role asymptomatic carriage plays in the epidemiology of *C. pneumoniae* infections.

Clinical presentation

Most pneumonia due to *C. pneumoniae* appears to be relatively mild and self-limiting and difficult to differentiate clinically from pneumonia due to other organisms such as *M. pneumoniae*. However exceptions can occur. Acute chest syndrome due to *C. pneumoniae* in children with sickle disease is frequently severe with significant hypoxia [24]. The chest radiograph may show interstitial or lobar infiltrates. Lobar consolidation and pleural effusions also occur. *C. pneumoniae* has also been isolated from empyema fluid [25] and can cause respiratory failure [26]. Laboratory findings are also nonspecific. The peripheral white blood cell count may be elevated to $\geq 20,000$ cells·mm^{-3} with a left shift, but is usually <15,000 cells·mm^{-3}.

Pathology

Data regarding histopathological findings in respiratory infection due to *C. pneumoniae* in humans are not available. However, studies have been performed in animals

Table 1. – Role of *Chlamydia pneumoniae* in community-acquired pneumonia in adults and children: results of selected studies

First author [Ref.]	Location	Subjects n	Age yrs	Prevalence of *C. pneumoniae*
MARSTON [11]*	Ohio, USA	2776	Adult	8.9
FILE [12]*	Multicentre, USA	456	Adult	22
NORRBY [13]*	Scandinavia	383	Adult	29
MUNDY [14]+	Baltimore, MD, USA	385	Adult	3.6
BLOCK [15]+	Multicentre, USA	260	3–12	13.1
HARRIS [16]+	Multicentre, USA	456	0.5–16	7.3

*: diagnosis based on serology alone; +: diagnosis based on culture and/or polymerase chain reaction.

and nonhuman primates. Experimental infection in cynologus monkeys after intranasal inoculation was clinically mild [27]. Chest radiographs 4–6 weeks after inoculation showed no infiltrates. In intranasally inoculated mice, an inflammatory response characterized by infiltrates can be observed in the lungs, with a response dominated predominantly by polymorphonuclear leukocytes in the acute phase and by mononuclear leukocytes in the chronic phase [28]. A characteristic feature is patchy distribution of inflammatory infiltrates interspersed among areas of normal or relatively normal histology. The organism can be seen in the areas of inflammatory reaction. The animals are usually not very ill and the infection resolves spontaneously. After intranasal infection, however, the infection appears to disseminate, probably haematogenously. *C. pneumoniae* can be isolated in culture from not only the lungs but also the spleen as well [28]. The organism has also been cultured from buffy coats of mice after inoculation, and identified in peripheral blood monocytes by PCR [28]. Dissemination following respiratory infection has been hypothesized as the mechanism whereby *C. pneumoniae* may gain access to the vascular system in humans and contribute to the pathogenesis of atherosclerosis. However, haematogenous dissemination of *C. pneumoniae* in the course of respiratory infection has not as yet been demonstrated in humans.

Other pulmonary diseases associated with *Chlamydia pneumoniae*

C. pneumoniae has also been implicated in other pulmonary conditions, specifically acute bronchitis, asthma and chronic obstructive pulmonary disease (COPD). A number of studies have been carried out, with contradictory results. Some of this probably relates to how infection with *C. pneumoniae* was defined. Most of the data were based on serology alone.

Acute bronchitis

Infection with *C. pneumoniae* has been implicated in acute bronchitis [1, 29], although its association with acute exacerbations of chronic bronchitis is less clear. The rate of *C. pneumoniae* infection in patients with acute bronchitis has been reported to range from 2–25% [30]. As with studies of community-acquired pneumonia, the rate of infection appears to vary depending on the diagnostic methods used. Most studies have been based on serology, using the microimmunofluorescence (MIF) assay. *C. pneumoniae* has been identified in sputa and throat swab specimens from patients with acute bronchitis by culture and PCR [29]. As seen with *C. pneumoniae* pneumonia, there is no characteristic clinical presentation. Patients are likely to exhibit productive cough, sore throats and hoarseness. In one study, the presence of sore throat and hoarseness, but not cough, was significantly more frequent in patients with *C. pneumoniae* infection [29]. Although it has been suggested that patients with acute bronchitis who test positive for *C. pneumoniae* (usually serologically), the results of several randomized treatment trials with antibiotics active against *C. pneumoniae* (erythromycin and doxycycline) showed no difference in response to therapy between those patients who tested positive and those testing negative [30].

Asthma and chronic obstructive pulmonary disease

C. pneumoniae may act as an infectious trigger, along with respiratory viruses such as respiratory syncytial virus (RSV), and parainfluenza and *M. pneumoniae*, for asthma [31]. This organism may be especially well suited for this role because of its ability to cause prolonged infection, often persisting for months. HAMMERSCHLAG *et al.* [18], reported refractory asthma in a patient with persistent *C. pneumoniae* infection, confirmed by culture, whose symptoms finally resolved after prolonged treatment with erythromycin. The patient was culture-positive for 11 months. Several studies have found association of serological evidence of *C. pneumoniae* infection, and asthma [31, 32]. EMRE *et al.* [22] isolated *C. pneumoniae* from the nasopharynx of 11% of children presenting with an acute episode of wheezing compared to 4.9% of a control group. Some of the children remained culture-positive for periods of up to 5 months despite treatment, and many were also receiving topical and systemic steroids. The majority of culture-positive children in this study were seronegative by MIF. Similar results in children were reported by CUNNINGHAM *et al.* [33] using PCR and serology. Seventy-five per cent of the children reported on by EMRE *et al.* [22] who were successfully treated with erythromycin and/or clarithromycin with eradication of the organism showed dramatic clinical improvement in their reactive airway disease. *In vitro* studies have shown that hydrocortisone enhances the growth of *C. pneumoniae in vitro*, but does not interfere with the antimicrobial activity of macrolides [34]. EMRE *et al.* [35] subsequently reported that specific anti-*C. pneumoniae* immunoglobulin (Ig)E could be detected by immunoblotting in 85% of the sera of children with culture-positive *C. pneumoniae* infection and wheezing, but was present in only 18–22% of culture-negative asthmatics or culture-positive children with pneumonia who were not wheezing. The presence or absence of IgE was not associated with the presence or absence of anti-*C. pneumoniae* IgG or IgA as determined by MIF. These findings suggest that type 1 allergy may be implicated in the pathogenesis of reactive airway disease associated with *C. pneumoniae* infection, similar to what appears to occur after viral infection (RSV and parainfluenza).

However, several subsequent studies did not find a significant association of *C. pneumoniae* infection and asthma in adults. COOK *et al.* [36] found antibody titres consistent with acute infection in 5.7% of patients with acute asthma and 5.7% of controls and serological evidence of past infection in 14.6% of patients with acute asthma and 12.7% of controls. LARSEN *et al.* [37] found that patients with asthma and healthy controls did not differ in prevalence of specific anti-*C. pneumoniae* IgE or previous *C. pneumoniae* infection as determined by the presence of anti-*C. pneumoniae* IgG. None of the patients or controls showed serological evidence of acute infection. *C. pneumoniae* neither induced nor enhanced the release of histamine from basophil leukocytes of patients or controls. These results are similar to experience with adults with asthma in Brooklyn, New York, where only 2% were found to be culture-positive compared to 3% of controls, and there were no differences in serological evidence of infection, including IgA and IgE, between cases and controls (S. Weiss, State University of New York,

USA. Unpublished data). GRAYSTON et al. [29] found that college students with *C. pneumoniae*-associated pneumonia or bronchitis did not wheeze any more frequently than patients with pneumonia or bronchitis who did not have *C. pneumoniae* infection. These data suggest that, if *C. pneumoniae* plays a role in exacerbations of asthma, it may be more significant in early childhood. Confirmation of an association may require well-controlled prospective studies utilizing culture and/or PCR as well as serology with assessment of the response to specific therapy including demonstration of eradication of the organism.

Serological evidence of *C. pneumoniae* infection has been reported in 7.3–>63% of patients with COPD [38, 39]. Identification of the organism in respiratory samples from these patients by culture or PCR has been infrequent, and most studies have relied on serology alone. BLASI et al. [39], reported that, despite serological evidence of infection being frequent in these patients, only 4% of exacerbations may be associated with *C. pneumoniae* infection.

Diagnosis

C. pneumoniae can be isolated from the NP and throat swabs, sputum and pleural fluid of patients with pneumonia, bronchitis and asthma. The nasopharynx appears to be the optimum site for isolation of the organism, especially in children. The relative yield from sputum is not known; however, many patients with *C. pneumoniae* infection may not have productive cough. This is especially true in children. Initial studies suggested that *C. pneumoniae* was very difficult to isolate in tissue culture as compared with *C. trachomatis*. Originally the same methods were used, HeLa or McCoy cells pretreated with diethylaminoethyl (DEAE) dextran. However, *C. pneumoniae* grows more readily in other cell lines derived from respiratory tissue, specifically HEp-2 and HL cells [40]. Culture with an initial inoculation and one passage should take 4–7 days.

Although PCR holds promise as a rapid diagnostic test, there are no standardized PCR or other nucleic acid amplification tests for detection of *C. pneumoniae*. The assays currently described in the literature are all in-house tests that employ different primers; the most frequently used have been those based on the *omp*1 gene, the 16 S ribosomal ribonucleic acid (rRNA) gene and a *C. pneumoniae*-specific deoxyribonucleic acid (DNA) fragment [41]. Some assays are nested, and different methods of detection are used. None have been extensively evaluated compared to culture in respiratory specimens; thus the performance of these assays can vary greatly between studies. A PCR-enzyme immunoassay using 16-S rRNA-based primers was compared to culture for detection of *C. pneumoniae* in NP specimens from 43 symptomatic and 58 asymptomatic individuals [42], PCR had a sensitivity compared to culture of 73% and a specificity of 99%. In contrast, other investigators using the same primers found that PCR was significantly more sensitive than culture for throat swabs, with 15 of 368 (4%) specimens from children with respiratory illness positive by PCR but only one (0.27%) culture-positive [41]. In contrast, it has been possible to isolate *C. pneumoniae* by culture from NP swabs of 4.9–5% of asymptomatic children, a 100-fold greater sensitivity than in the PCR used in the before-mentioned study, suggesting that the culture methods

used were suboptimal [21, 22]. Explanations for differences in performance between various studies include the presence of DNA polymerase inhibitors in clinical specimens and suboptimal culture methods [41].

Most investigators to date have relied on serological diagnosis, using the MIF test and the compliment fixation (CF) test. The CF test is genus-specific and has been largely used for diagnosis of lymphogranuloma venereum and psittacosis. Originally, GRAYSTON et al. [1] found that fewer than one-third of hospitalized patients with *C. pneumoniae* infection had the CF antibody. However, in a recent report of a small outbreak of *C. pneumoniae* infection among University of Washington (Seattle, WA, USA) students, all seven patients with pneumonia had CF titres of ≥1:64 [42]. GRAYSTON et al. [29] have proposed a set of criteria for serological diagnosis using the MIF infection test which are being used by many laboratories and clinicians. For acute infection, the patient should show a four-fold increase in the IgG titre, a single IgM titre of ≥1:16 or a single IgG titre of ≥1:512. Past or pre-existing infection is defined as an IgG titre of ≥1:16 and <1:512. On initial infection, the IgM response should appear ~3 weeks after the onset of illness and the IgG response at 6–8 weeks. On reinfection, the IgM response may be absent and the IgG response occurs earlier, within 1–2 weeks. Because of the relatively long period until the development of a serological response on primary infection, the antibody response may be missed if convalescent sera are obtained too soon, *i.e.*, <3 weeks after the onset of illness. The criteria for use of a single serum sample have not been correlated with the results of culture and are based mainly on data from adults. The antibody response in acute infection may take >3 months to develop. Acute culture-documented infection can also occur without seroconversion, especially in children; ≥70% of children with culture-documented infection were seronegative even after 3 months′ follow-up [15, 16]. Several studies have found poor correlation between MIF, serology, culture and PCR, even in adult populations [21, 42]. Background rates of seropositivity can be very high in some populations [21]. A study of asymptomatic *C. pneumoniae* infection among subjectively healthy adults in Brooklyn, New York, found 81% to have IgG or IgM titres of ≥1:16 and 17% showed evidence of "acute infection", an IgG titre of ≥1:512 and/or an IgM titre of ≥1:16 [21]. None of these individuals were culture- or PCR-positive. Some IgG may result from a heterotypic response to other chlamydial species as there are cross-reactions between the major outer membrane protein (MOMP) of the three species as well as cross-reactions due to the generic LPS antigen.

The MIF test is also not standardized; there can be a significant subjective component in performing the assay. A recent study [43] attempted to address the problem of interlaboratory variation in the performance of the MIF test by sending a panel of 22 acute and convalescent sera to 14 different laboratories. Some used an in-house MIF test and several used one of two commercially available kits. The overall agreement of all laboratories was 80%, using one laboratory as the "gold standard". The range was 50–100% depending on the isotype. Agreement for serodiagnostic criteria were 69% for negative, 68% for "chronic" and 87% for a four-fold increase in IgG titre.

The lack of reactivity in the MIF assay observed in children may be secondary to the lack of reactivity to the MOMP. When sera from culture-positive but MIF-negative children with respiratory infection were examined by immunoblotting, >89% had antibodies directed against a number of *C. pneumoniae* proteins but only 24% reacted with the MOMP [44]. The MOMP does not appear to be immunodominant in the immune response to *C. pneumoniae* infection, although it has been demonstrated to be immunodominant in *C. trachomatis* infection. Monoclonal antibodies directed against specific epitopes of the MOMP are neutralizing for *C. trachomatis* but not for *C. pneumoniae*. The MOMP is the major surface-exposed antigen of *C. trachomatis* and may be the major antigen in the MIF assay. Unfortunately, there was no reactivity to any *C. pneumoniae* protein or combination of proteins that readily differentiated infected from uninfected children [44]. When paired sera were examined, the band patterns remained the same for >70% of the children. In the remaining children, changes in the immunoblots of acute-phase sera compared with convalescent sera were unique to each patient. When the same sera were immunoblotted against recent clinical isolates of *C. pneumoniae*, there were differences in the intensity of reaction and the band patterns, indicating possible antigenic diversity between isolates. The recent publication [45] of the complete gene sequence of *C. pneumoniae* may facilitate the development of a more specific and sensitive serological assay.

Treatment

To date there have been few published data describing the response of respiratory infection due to *C. pneumoniae* to antibiotic therapy. *C. pneumoniae* is susceptible *in vitro* to macrolides, tetracyclines and fluoroquinolones (table 2) [46, 47]. Optimum dose and duration of therapy are uncertain. *In vitro* activity may not predict *in vivo* efficacy. Anecdotal data suggest that prolonged therapy (*i.e.*, ≥2 weeks) may be desirable since recrudescent symptoms have been described following 2-week courses of erythromycin and even after 30 days' tetracycline or doxycycline [1, 29]. Practically all pneumonia treatment studies evaluating new macrolides/azalides and quinolones presented or published to date have used serology alone for diagnosis, essentially limiting themselves to a clinical end point. In 1990, LIPSKY *et al.* [48] described four patients with bronchitis and pneumonia, treated with a 10-day course of ofloxacin, who were retrospectively identified as showing serological evidence of *C. pneumoniae* infection (four-fold rise in IgG/IgM titre, single IgM titre of ≥1:16 or IgG titre of ≥1:512). All reportedly demonstrated marked clinical improvement. Based on the minimal inhibitory concentrations (MICs) of ofloxacin (1–2 mg·mL^{-1}) against three laboratory strains, the authors concluded that ofloxacin was effective in these patients as the MICs were less than achievable serum levels. In a subsequent prospective pneumonia treatment study, PLOUFFE *et al.* [49] found a clinical response rate of 83% in those patients showing serological evidence of *C. pneumoniae* infection who were treated with ofloxacin compared to 75% of those who received standard therapy, which was a β-lactam antibiotic plus erythromycin or a tetracycline. Similarly, FILE *et al.* [12] reported a clinical

Table 2. – *In vitro* activity of various antibiotics against *Chlamydia pneumoniae*

	Minimal inhibitory concentration µg·mL^{-1}
Erythromycin	0.06–0.25
Doxycycline	0.06–0.25
Azithromycin	0.05–0.25
Clarithromycin	0.004–0.03
Telithromycin (HMR 3647)	0.031–2
Ciprofloxacin	1.0
Ofloxacin	0.5–2
Levofloxacin	0.25–1
Sparfloxacin	0.031–0.125
Trovafloxacin	0.5–1
Moxifloxacin	0.5–1
Gatifloxacin	0.125–0.25
Gemifloxacin	0.125–0.25
Sulphamethoxazole	>500

(Data from [46, 47].)

cure rate of 98% among patients showing serological evidence of *C. pneumoniae* infection who were treated with levofloxacin compared with 93% of those treated with ceftriaxone and/or cefuroxime axetil, plus erythromycin or doxycycline at the investigator's discretion. In the latter group, the response rate did not differ between those patients who had erythromycin or doxycycline added to their treatment regimen and those who were treated with a cephalosporin alone. There was also no difference in the response rate among those patients who showed "definite" serological evidence of infection, *i.e.* a four-fold rise in MIF IgG or IgM titre, compared to those who had "probable" infection, *i.e.* a single IgG titre of ≥1:512 or IgM titre of ≥1:32. The success of the cephalosporin regimens raises some questions about the specificity of the serological criteria as these antibiotics show no or poor activity against *Chlamydia* species *in vitro*.

Unfortunately, a number of subsequent treatment studies have claimed microbiological eradication, despite the fact that culture was not performed. A recent study by LEOPHONTE *et al.* [50] comparing two trovafloxacin regimens to amoxycillin clavulanate for the treatment of acute bronchitis found a similar clinical response rate for all three regimens and stated that *C. pneumoniae* and *M. pneumoniae* were successfully eradicated from 80–100% of the patients at the end of therapy. However, the investigators state in the methods section that "Atypical respiratory pathogens, which were identified by serologic testing (a 4-fold increase in antibody titre) were presumed eradicated if the patient's clinical response was cure or improvement".

There are only four published treatment studies of pneumonia that have utilized *C. pneumoniae* culture and assessed microbiological efficacy. BLOCK *et al.* [15] found that treatment with erythromycin suspension eradicated *C. pneumoniae* from the nasopharynx of 86% of culture-positive children with community-acquired pneumonia and clarithromycin suspension from 79%. All of these children improved clinically despite persistence of the organism. Persistence was not related to the development of antibiotic resistance as all isolates remained susceptible to erythromycin and clarithromycin during and after treatment [51]. Clarithromycin is 10–100-fold more active than erythromycin *in vitro* and has superior

pharmacokinetics and tissue penetration, and yet was not more effective than erythromycin in eradicating *C. pneumoniae* from the respiratory tract. Experience with azithromycin has been similar. In an open non-comparative multicentre pneumonia treatment study [52], adolescents and adults of ≥12 yrs of age were given 1.5 g azithromycin orally over 5 days. *C. pneumoniae* was eradicated from the nasopharynx of seven of the 10 (70%) culture-positive patients with community-acquired pneumonia after treatment. HARRIS *et al*. [16] reported that *C. pneumoniae* was eradicated after treatment from the nasopharynx of 19 of 23 (83%) evaluable children with community-acquired pneumonia, 0.5–16 yrs of age, who received azithromycin, and in four of four and seven of seven who received amoxycillin clavulanate and erythromycin, respectively (p=0.9, Chi-squared test).

The MICs and minimal bactericidal concentrations (MBC) of three of nine isolates obtained after treatment from two of seven persistently infected patients in both studies who were treated with azithromycin increased four-fold after treatment, although they were still within the range considered susceptible to the antibiotic. It is not clear whether this was an isolated event or suggestive of possible development of resistance. All patients improved clinically despite persistence of the organism. The results of two pneumonia treatment studies in adults which evaluated levofloxacin and moxifloxacin found eradication rates of 70–80% [53, 54]. The MICs and minimum bacterial concentrations of isolates of *C. pneumoniae* from the patients who were microbiological failures in respect of both drugs remained the same before and after treatment.

Although resistance of *Chlamydia pneumoniae* to quinolones has not as yet been reported, DESSUS-BABUS *et al*. [55] described two strains of *Chlamydia trachomatis* which became resistant to sparfloxacin and ofloxacin after four passages in subinhibitory concentrations of the drugs. Resistance appeared to be due to a point mutation in the *gyrA* quinolone resistance-determining region. Another possible explanation for persistence of *Chlamydia pneumoniae* in these studies was that the dose and duration of treatment were not optimal. KUTLIN *et al*. [56], using continuously infected HEp-2 cells, reported that 6 days' treatment with 4 μg·mL^{-1} ofloxacin or 0.05 μg·mL^{-1} azithromycin, which exceeds achievable serum levels, reduced the concentration of *Chlamydia pneumoniae* from 1×10^6 to 1×10^3 inclusion forming units·mL^{-1} but failed to completely eradicate the organism.

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