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Community- Acquired Pneumonia Related to Intracellular Pathogens

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Introduction

Community-acquired pneumonia (CAP) is associated with high rates of morbidity and mortality worldwide[1;2]; annual CAP incidence among adults in Europe has ranged from 1.5 to 1.7 per 1,000 populations. CAP varies from a mild outpatients illness to a more severe disease requiring hospitalization, and among hospitalized patients, approximately 10% required admission to an intensive care unit (ICU)[3].

Intracellular bacteria are common causes of CAP[4;5] (Figure 1). However, there is a wide variation in the reported incidence, depending in part on the difficulties with microbiological culture, because they grow poorly in standard culture media and culture requires expertise. The new development of molecular microbiological techniques should help to clarify the epidemiology and presentation of intracellular pathogens in CAP and help a shift to more targeted antimicrobial therapy[6]. The intracellular pathogens that are well-established as causes of pneumonia are: *Legionella pneumophila*, *Mycoplasma pneumoniae*, *Chlamydophila pneumoniae*, *Chlamydophila psittaci* and *Coxiella burnetii* [7].

Although extra-pulmonary manifestations are often associated with these pathogens in pneumonia, no clinical features allow them to be reliably distinguished from classical bacterial agents of pneumonia such as *Streptococcus pneumoniae* (pneumococcus)[8]mainly in patients who need hospitalization. Due to the fact that these bacteria are naturally resistant to ß-lactams, they should be promptly identified. Macrolide and quinolones remain the best empirical treatment of intracellular pathogens, because of their good antimicrobial activity and high intracellular concentrations. Although, antibiotic resistance in these intracellular pathogens does

not currently represent a clinical problem in the majority of settings, recent studies from Asia and some European countries report about isolation of strains of *M. pneumoniae* resistant to macrolides, suggest a need to monitor these pathogens to evaluate the clinical impact of resistance in CAP[9;10].

These microorganisms in approximately 1% to 7%migth cause severe CAP[11-13].

Epidemiology and Clinical features

Legionella pneumophila

Legionella pneumophila is a Gram-negative intracellular bacillus that causes the majority of clinical cases of severe pneumonia termed Legionnaires' disease (LD). *Legionella* is responsible for summer to autumn outbreaks, and is an important cause of community acquired pneumonia and hospital acquired pneumonia[6;14]. Pneumonia caused by *L. pneumophila* is indistinguishable by clinical and radiographic features from other pneumonias caused by "typical" pathogens such as pneumococcus.

In Europe and in the USA *L. pneumophila* serogroup 1 causes the vast majority of *Legionella* pneumonia, accounting for approximately 85%of cases [15]. However, *L. longbeachae* is the specie reported in approximately 25% of the pneumonia cases in countries such as Australia, Scotland, Thailand and New Zealand [16-18].

LD is rare in children; the majority of reported cases occur in people ≥50 years and cases are predominantly in male patients[19] (Figure 2). The principal risk factors for LD are: current smoker (compromises mucociliary clearance), current alcohol comsumer, diabetes, chronic obstructive disease and immunesuppression. Epidemiology of LD is well recorded because it is notifiable disease. In 2014, a total of

6, 941 cases of LD were reported by 28 EU Member States and Norway[20]. Five countries (France, Italy, Spain, Germany, and Portugal) accounted for 74% of all notified cases (Figure 3). Most cases were community acquired (74%), 18% were travel-associated, and 7% were linked to healthcare facilities. People over 50 years of age accounted for 80% of all cases. The male-to-female ratio was 2.6:1. The case-fatality ratio was 8% in 2014, similar to previous years. Most cases (87%) were confirmed by urinary antigen test, but an increasing proportion of cases are reported to have been diagnosed by PCR. *L. pneumophila* serogroup 1 was the most commonly identified pathogen, accounting for 81% of culture-confirmed cases[20].

Woodhead *et al*[21]. in a study published in 2002, suggested a frequency of *Legionella* spp. in ICU patients of 7.9%. Similar data were reported in 2011 from Spain, showing 8% of ICU patients with CAP had infection with *L. pneumophila* [12]. Nevertheless, the recent article by Jain et al[11]. concerning the etiology of pneumonia in the community (EPIC) study, report that *L. pneumophila* was present in 1% of the ICU patients with defined microbial etiology. In USA the CDC estimates that between 8,000 to 18,000 people are hospitalized with LD each year and approximately 5 to 30% of these patients die[22]. Welte et al[23]. in a review article included 46 CAP studies from 13 European countries, observed a frequency of *Legionella* spp. in 12% of the ICU CAP cases. Nowadays, with improved detection methods, including urinary antigen test, more cases are being identified and many more mild cases than in the past are being recognized.

LD can present as a severe pneumonia that may be accompanied by systemic symptoms such as fever, diarrhoea, myalgia, impaired renal and liver functions, and delirium. LD is also frequently associated with hyponatraemia, dry cough, elevated

lactate dehydrogenase and markedly elevated C-reactive protein levels and platelet count. Although none of these features are specific for LD, Haubitz clearly demonstrated that in the absence at least 2 of these factors, LD was excluded with 99% accuracy [24]. In patients with risk factor (smoking, chronic cardiovascular or respiratory disease, diabetes, alcohol abuse, and immunosuppression) LD sometimes is complicated with more severe disease (cavitation and pleural effusions)[25-29]. Jacobson et al[30]. reported in a study of *Legionella* pneumonia in cancer patients the presence of multilobar pneumonia in 82% of the patients, bilateral involvement in 80% and pleural effusion in 56% of the patients.

Mycoplasma pneumoniae

M. pneumoniae is a common cause of CAP in adults[12;15;31]. *M. pneumoniae* have a single triple- layered membrane and lacks a cell wall structure; this characteristic makes this microorganism insensitive to ß-lactams antibiotics.

When an individual has active infection with *M. pneumoniae* it is possible to recover viable bacteria from the nose, throat, trachea and sputum samples. The incidence of *M. pneumoniae* CAP is lower in elderly patients compared with young adults[32]. The study by Dumke et al[6]. showed that *M. pneumoniae* was more common in younger patients, more frequent in females and patients with fewer comorbid conditions than CAP from other causes (Figure 4). Pneumonia caused by *M. pneumoniae* presented with frequent extrapulmonary manifestations (encephalitis, optic neuritis, acute psychosis, stroke, cranial nerve palsies and aseptic meningitis)[33;34]. In CAP *M. pneumoniae* accounts for up to 37% of the patients treated as outpatients and 10% of patients that required hospitalization[35;36]. In a few cases, pneumonia caused by *M. pneumoniae* developed into refractory or severe pneumonia[37;38]. Miyashita *et al*[37].

reported in a study of severe *M. pneumoniae* in ICU patients, that 6% of the 227 patients with *Mycoplasma* infection were admitted to ICU because acute respiratory failure. In a review of 52 cases of fulminant *M. pneumoniae* pneumonia from Japan, the authors observed that the dominant population of fulminant *M. pneumoniae* pneumoniae was young adults without severe underlying diseases. The major clinical manifestation was respiratory failure with diffuse abnormal findings in radiological examinations.[38].

There are recent reports from Asia and some European countries describing the isolation of strains of *M. pneumoniae* resistant to macrolide[9;10]. The clinical relevance of macrolide resistance of *M. pneumoniae* in CAP cases is still under debate, but some studies suggest that severity of the disease and clinical manifestations in adults are similar in patients with susceptible strains who are receiving treatment[39;40]. More studies are needed to understand the clinical significance of macrolide-resistance *M. pneumoniae* in adults with CAP.

Chlamydophila pneumoniae* and *Chlamydophila psittaci

C. pneumoniae is endemic globally, and has a unique life cycle where this intracellular bacteria expresses different phenotypes that permits to survival for many years into a phase of rest that allow it to cause persistent infection.

The incubation period is around 21 days; approximately 50% of young adults and 75% of elderly persons have serologic evidence of a previous infection. The clinical course infection with *C. pneumoniae* may vary from sub clinical to mild and, more rarely, to severe pneumonia. Outcome of the patients is directly related to immune competence. Risk for infection and persistence are advanced age, current smoking,

male gender, glucocorticoid use, and residence in a long-term care facility. Up to 20% of CAP in adults is reported to be caused by *C. pneumoniae*[12;41]. The majority of this study is based on serology, and the relationship between positive *C. pneumoniae* serology and CAP aetiology is debated. Cillóniz et al[12] found only 3% are caused by *C. pneumoniae*. Chalmers et al.[42] found a maximum of 2.2% of CAP caused by C. pneumoniae. The study of Arnold *et al*[15],showed that the incidence of CAP due *C. pneumoniae* from 4,337 patients was: North America 8%, Europe 7%, Latin America 6% and Asia 5% . Respiratory infections caused by *C. pneumoniae* are often asymptomatic but in approximately 10% of adolescent and young adults develop a mild disease[43]. Sore throat with hoarseness is often severe and may precede pneumonia by up to a week and resolve before pneumonia onset, resulting in a biphasic illness.

Common symptoms of *C. psittaci* infection include abrupt onset of fever, chills, headache, myalgia, non-productive coughing and dyspnea. Radiographic findings may include lobar or interstitial infiltrates. In some cases there are complications such as pericarditis, endocarditis or myocarditis, hepatomegaly and splenomegaly[44]. Persons exposed to infected birds are at risk for infection with *C. psittaci*, and this disease is found worldwide with approximately 70% of the cases having a known source of infection as a result of exposure to pet birds[45].

Mortality rate related to *C. pneumoniae* infection is low. However, if the first upper respiratory signs associated with *C. pneumoniae* infection is not treated; severe disease or even death may result[44]. The diagnosis of psittacosis can be difficult, and many more cases may occur that are not correctly diagnosed or reported.

Coxiella burnetii

Infection in humans by *C. burnetii* often is asymptomatic or presents as an influenza-like illness or atypical pneumonia with patchy pulmonary infiltrates that are recognized as the most common form of presentation in acute Q fever[46].

C. burnetii is an obligate intracellular Gram-negative bacterium. Pneumonia caused by *C. burnetii* is generally mild but progression to acute respiratory distress syndrome (ARDS) can occur[8]. European countries such as the UK[47;48], France, Greece, Spain and the Netherlands[49;50] have reported important outbreaks. In the USA, surveillancestudies from 2000 to 2012, reported an incidence of 0.38 cases per million persons/ year, with a mortality rate of 2% and a 62% of rate of hospitalizations[51].

A German study published in 2014 reported a high prevalence of Q fever as CAP (3.5%) in the summer of 2005 in Germany[52]. In 2007, a major epidemic occurred in the general population in the Netherlands which resulted in approximately 2,300 reported cases. An epidemiologic explanation for this outbreak was a possible spatial link between dairy goat farms and human cases[53]. Wielders et al[54]. describe epidemiological and clinical features of 183 hospitalized acute Q fever patients. Pneumonia was development in 86% of patients, 4% were admitted to the ICU and the mortality rate in general was 6%.

It was reported that Q fever is most frequent in males. In Australia and France, males are 5-fold and 2.5-fold more likely than females to develop disease, respectively[55].

Co-infection with other pathogens

The study by Cillóniz et al[56]. included 362 adult patients with CAP admitted to the ICU, identified that 10% of the CAP cases with defined microbial etiology were caused by

intracellular pathogens. Co-infection of intracellular pathogens and other microorganism were observed in 30% of the cases caused by intracellular pathogens.

Pathogenesis

The principal characteristics of intracellular pathogens are that they are able to grow and reproduce inside the cells of their host. This characteristics help to these pathogens evade the host immune system. Antimicrobial treatment for these pathogens is very challenging because many antimicrobials are unable to access intracellular spaces and achieve the optimum therapeutic concentrations within the infected cells.

Legionella pneumophila

L. pneumophila growths into lung macrophages after aerosols are inhaled from contaminated water sources. A recent published letter reported a probable person-to–person transmission of LD; the authors described two cases of LD. The patients were relatives (mother and son) and the factors that suggest person- to-persons transmittion are: The severity of the respiratory symptoms, 8 hours of close contact between the two patients, the small area of the non-ventilated room were the contact took place[57].

L. pneumophila multiplies intracellulary until the cell ruptures, then infect other macrophages. *L. pneumophila* have virulence factors that potentiate the infection of macrophages and inhibit phagosomal fusion avoiding macrophage cell death pathways and promoting bacterial survival therefore allowing sufficient time for replication. When *L. pneumophila* establishes infection, this intracellular bacteria causes a fibrino-

purulent pneumonia. Hepatic involvement is commonly manifested by mildly increased serum transaminases in *Legionella* CAP.

Mycoplasma pneumoniae

This microorganism is transmitted by aerosols from person to person. In general the incubation period varies from 1 to 3 weeks. Approximately 3% - 4% of patients with *M. pneumoniae* pneumonia develop severe CAP associated a respiratory failure and acute respiratory distress syndrome (ARDS). The study of Nilsson et al[58]. showed that the severity of *M. pneumoniae* seems to depend on *M pneumoniae* bacterial load rather than *M pneumoniae* genotype (MP1 or MP2). Other studies associated the delayed administration of adequate antimicrobials with severe *M. pneumoniae* pneumonia[37;38]. Pathogenicity of *M. pneumoniae* depends on its attachment to respiratory epithelium and initiation of injury to the host. The study of Jiang et al.[59] showed the association between abnormalities in ciliated respiratory epithelium with severity of *M. pneumonia*e infection.

The principal virulence factors in *M. pneumoniae* are related to hydrogen peroxide production, lipoproteins and more recently reported CARDS toxin (ADP-ribosylating and vacuolating cytotoxin)[60] that exhibits similarities with pertussis toxin.

In addition to these virulence factors, the emergence of macrolide-resistant *M. pneumoniae* strains[40;61;62] (by point mutations in the 23S RNA gene[33]) and the frequency of extrapulmonary complications (neurological, cardiac, dermatological, musculoskeletal and haematological) reported make treatment more difficult[63]. It is known that this development is associated with an active immune response to the pathogen and excessive expression of cytokines such as IL- 18, which was

demonstrated in the case-control study of pneumonia caused by *M. pneumoniae* published by Tanaka et al[64]

Chlamydophila pneumoniae

C. pneumoniae has a specific tropism and exhibits cytotoxic activity towards the airway epithelium, in which it proliferates and destroys infected cells by lysis.

Co-infection with other respiratory pathogens may play an important role [65] in approximately 30% of adult patients with CAP.

C. pneumoniae need neutrophil granulocytes cells to live and multiply, however these cells have a short life span. The inhibition of neutrophil apoptosis by *C pneumoniae* appears to play a major role in the production of infection of these cells by *C. pneumoniae*.

Coxiella burnetii

The principal reservoirs of infection are farm and domestic animals, especially sheeps and goats. Infection of humans is mainly through inhaling contaminated aerosols, which occurs mainly during and several weeks after labour of goats and sheeps. This bacterium is resistant in the environment and it may survive for long time periods in dust and fecal particles. The period of incubation varies from 7 to 40 days with an average of 21 days [66;67].

The target cells for *C. burnetii* infection are monocytes and macrophages. *C. burnetii* lives inside phagosome with special acid characteristics that allow this pathogen to receive nutrients and to resist antibiotics[68]. It is known that lipopolysaccharide (LPS) is the main virulence factor of *C. burneiti* infection. Development of infection is related to host comorbidities and cell-mediate immunity.

Microbiological Diagnosis

Intracellular pathogens are difficult to culture and isolated. The general microbiological assays to diagnose these pathogens are: antigen detection, molecular assays such a PCR and serology. Sputum, bronchoalveolar lavage, broncoalveolar aspirate and pleural fluid are the principal samples for the diagnosis of these pathogens (Figure 5).

Culture

Culture of *M. pneumoniae* is the reference diagnostic test for this pathogen but is rarely performed. The principal limitation is that it is expensive and time-consuming (almost 10 days), requires specialized media, technical expertise and is not available except in reference laboratories. The sensitivity is 60% and specificity 100%.

Culture of respiratory samples in buffered charcoal yeast extract agar (BCYE) is the gold standard method for diagnosis of *L. pneumophila.* Isolation of the strains involved in the infection allows epidemiological typing, which provides valuable data for the control and prevention of further cases. Sensitivity of *Legionella* culture varies widely ranging from 50% - 80%[69;70]. In cases of severe CAP the sensitivity is higher because of the higher microbial load in respiratory samples.

C. burnetii is a pathogen with high infectivity, dangerous and difficult to isolate. Culture of this pathogen is performed on human erythroleukaemia cells (HEL cells) over 5 to 7 days, or it is possible to observe *C. burnetti* by microscopy after immunoflurescence staining[71].

Antigen Detection

Diagnosis of *L. pneumophila* is made most frequently through detection of *Legionella* antigen in urine (urinary antigen test), the sensitivity of this test is approximately 70% with specificity of 95%[72]. The principal problem with this assay is that it is only

diagnostic for *L. pneumophila* serogroup 1 can we identified, which is the most frequent serogroup involved in CAP cases. The antigen of *Legionella* can persist in urine for weeks and months following infection.

Also the direct inmunofluorescence assay in sputum samples, respiratory secretions or pleural fluid is an easy way to diagnose *Legionella*, with a sensitivity ranging from 33% to 68% and a specificity of 95%, but the principal obstacle is to collect a good quality sample.

Serology

Serology is the main diagnostic assays for *M. pneumoniae, C. pneumoniae, C. psittacii and C. burnetiii.* However the lack of specificity due to a possible cross reactivity with other microorganisms is a major limitation. Also, IgM and IgG may take 6 – 8 weeks to appear, Requiring collection of both acute and convalescent titers.

An acutely elevated IgM titer for *M pneumoniae* or *C. pneumoniae* in a patient with CAP is considered diagnostic. While a four- fold increase in IgG titer is indicative of past exposure or infection by *M pneumoniae* or *C pneumoniae*.

In the case of *C. burnetii*, persistently highly elevated IgG levels indicate chronic Q fever rather than acute infection.

Molecular diagnosis (PCR)

Molecular techniques such as PCR or multiplex PCR are of use for identification of intracellular pathogens (*M. pneumoniae*[73;74]*., C. pneumoniae, C. psittaci, C. burnetii*) in CAP. These new techniques can also help to identify the infectious strains of these problematic pathogens.

Although, serology is the main diagnostic test for *C pneumoniae and C psittaci*, this is not well standardized, and PCR is emerging as a rapid sensitive mode of detection[75].

PCR in blood samples or serum provides a rapid and definitive diagnosis of *C. pneumoniae*. Also culture of infected tissue is an option, however it requires special facilities. PCR in sputum samples has greater sensitivity (80%) and specificity (90%) for the detection of *Legionella* and also has the possibility to detect other species such as *L. longbeachae*[76].

Molecular assays will help in the early diagnosis of chronic and acute Q fever[77]. In the last few years several molecular assays were developed to detect the DNA of *C. burnetii* in clinical samples and in cell culture.

Antibiotic Treatment

The selection of empiric antibiotic treatment of CAP generally is based on possible risk factors for specific pathogens and the knowledge of local antimicrobial resistance. Therefore, in moderate to severe cases it is recommended that empiric antibiotic treatment should covering typical and atypical (intracellular) pathogens.

Legionella pneumophila

Azithromycin and fluoroquinolones are superior to erythromycin or clarithromycin for the treatment of *Legionella pneumonia* [78;79]. BTS guidelines recommended fluroquinolones for severe LD [32]. The addition of rifampicin is advised for immunocompromised patients or patients with severe pneumonia. Duration of the treatment is 8 – 14 days for non severe LD. Antibiotic therapy should be continued for 10 to 21 days in immunocompetent patients to decrease the rate of relapse[80].

Mycoplasma pneumoniae

Antimicrobial therapies with tetracycline, macrolide, or fluoroquinolone shorten the clinical course of clinical symptoms[80]. Macrolides and tetracyclines are bacteriostatic against *M. pneumoniae*, whereas fluoroquinolones possess bactericidal activity[81;82]

Azithromycin is recommended for 5 days while other macrolides such as clarithromycin and erythromycin, as well as tetracyclines and fluoroquinolones, usually require longer courses.

Corticosteroids may be beneficial in severe *M. pneumoniae* pneumonia[83-85], but experience with this therapy is limited to case reports and small series. The recent published study by Miyashita et al[83]. included 41 patients with refractory or severe *M. pneumoniae* pneumonia observed that the administration of steroids to patients with refractory and severe pneumonia resulted in the rapid improvement of symptoms and a decrease in serum LDH levels in all patients.

Chlamydophila pneumoniae* and *Chlamydophila psittaci:

Antibiotic treatmet for *C. pneumoniae* is very similar to *M. pneumoniae*. Therapy for two weeks with macrolides, tetracycline, doxycline or fluoroquinolone is recommended[80;86]. Azithromycin and clarithromycin are an alternative option to erythromycin for the tolerance. In case of patients with intolerance to macrolides or tetracyclines; fluroquinolones such as levofloxacin or moxifloxacin are good alternatives.

Coxiella burnetti:

Doxycycline is the treatment of choice for acute Q fever and two weeks of therapy is recommended for adults[8]. Doxycycline has been shown to result in a mean time to

defervescence of 2 to 3 days after the start of therapy[87]. Levofloxacin or a macrolide are an alternative therapy.

Current recommendations from the CDC is to treat pregnant women with acute Q fever with co-trimoxazole up until the final 6 weeks of pregnancy, and to give doxycycline and hydroxychloroquine postpartum for 12 months to women who develop a serologic profile of chronic Q fever (Phase 1 IgG titer ≥1:1024)[88;89]. Because there is a risk for the fetus Doxycycline is contraindicated during pregnancy.

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