Review Article

Research progress in atypical pathogens of community acquired pneumonia

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ABSTRACT

Atypical pathogen, especially *Mycoplasma pneumoniae* is a common and important pathogen of community-acquired pneumonia. Physicians should pay more attention on them. Compared with bacteria, the clinical treatment of atypical pathogens is different, as beta-lactams are not effective for atypical pneumonia. Therefore, laboratory diagnostic methods and clinical biology research is particularly important for the diagnosis and treatment of atypical pneumonia. In order to provide more theoretical basis for clinical diagnosis of atypical pathogens infection, we performed a review of the research progress of prevalence, laboratory testing of atypical pathogens related infections.

Key words: Atypical pneumonia, community-acquired pneumonia, *Mycoplasma pneumoniae*

INTRODUCTION

The atypical respiratory pathogens *Mycoplasma pneumoniae*, *Chlamydia pneumoniae* and *Legionella pneumophila* are now recognised as a significant cause of acute respiratory-tract infections, but they remain colorless after Gram-staining and are difficult to identify by conventional bacterial culture tests. It is reported that patients with atypical pneumonia were more likely to have normal or reduced white blood cell counts.[1] However some published data showed that between atypical pneumonia and general bacterial pneumonia, there were no significant differences in the symptoms such as fever, cough, productive sputum, and the sign of lung rales.[2,3] Also, X-ray findings and the increase of white blood cell counts and percentage of neutrophils were similar between them. Especially, patients with pneumonia caused by *L. pneumophila* presented with the typical symptoms of *Streptococcus pneumonia* while patients with *S. pneumonia* also presented with the symptoms of atypical pneumonia.[4] However, beta-lactams are not effective for atypical pneumonia. Therefore, laboratory detection methods and clinical biology research on the diagnosis and treatment of atypical pathogens infection is particularly important.

ATYPICAL PATHOGENS

Atypical organisms such as *M. pneumoniae*, *C. pneumoniae*, and *L. pneumophila* are implicated in cases of community-acquired pneumonia (CAP). *M. pneumoniae* in 1962 was successfully isolated.[5] *M. pneumoniae* lacks a cell wall, but it can grow in artificial culture medium, and it is the most frequent pathogens found in patients with atypical CAP. *C. pneumoniae* found in 1986 is intracellular organisms and is a common cause of acute and chronic respiratory-tract infections.[6] *Legionella* was found in 1976 because it caused infection outbreak of America veterans. It has been confirmed more than 50 *Legionella* species, a total of 70 serotypes. *L. pneumophila* with 16 serotypes is intracellular organisms and closely related to human infection.[7]

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Access this article online	
Quick Response Code:	Website: www.caijournal.com
	DOI: 10.4103/2225-6482.141747

ATYPICAL PATHOGENS IN COMMUNITY-ACQUIRED PNEUMONIA

With the widespread use of antibiotics, the change of living environment and constantly updated diagnostic methods, we found atypical pathogen played an important role in CAP. A prospective study was performed on 665 consecutive adult patients with CAP at 12 centers in 7 Chinese cities between 2003 and 2004.[8] The results showed that atypical pathogens caused 32.4% cases of CAP, of which 20.7% *M. pneumoniae*, 6.6% *C. pneumoniae* and 5.1% *L. pneumophila*. Of 195 patients with a bacterial pathogen, an atypical pathogen was identified in 10.2% cases. Survey of Cao *et al*. on the etiology and clinical outcomes of CAP treated in an ambulatory setting showed that the most common pathogens were *M. pneumoniae* (29.4%) and then virus copathogens (2.5%).[9] The previous research data of "pathogens monitoring network among adults with CAP in Beijing" showed that of 410 patients with CAP, ≥4-fold increase of paired serum *M. pneumoniae* IgG antibody titer was observed in 18.8% of cases.[10] Atypical pathogens, rather than *S. pneumoniae*, become the most important pathogen of adult CAP.

LABORATORY DIAGNOSIS OF ATYPICAL PATHOGENS INFECTION

Laboratory diagnosis of *Mycoplasma pneumoniae* infection

At present, several methods are available for the definitive diagnosis of *M. pneumoniae* infections, including culture, serology, and real-time polymerase chain reaction (PCR) assay. Culture has been reported to be more specific and more sensitive for detection.[11] However, the culture of *M. pneumoniae* is time-consuming, requires 2-6 weeks for results, which is less helpful to early diagnosis of acute infection. Culture is essential for investigation of *M. pneumoniae* clinical isolates and molecular biology and drug sensitivity.[12] Serologic diagnosis is a common method by measuring serum antibody titers of acute and convalescent-phase serum samples. Sustained high IgM titer (≥1:160) of acute-phase serum samples or a four-fold or greater increase in IgG antibody titer of convalescent-phase serum samples than that of acute-phase serum samples can be regarded as a positive diagnosis.[13] A recent study showed that a four-fold or greater increase in titer of paired serum samples could be observed within 3-6 days.[10] Detection of *M. pneumoniae* by PCR as a rapid, sensitive, and specific method has been reported by many authors. Primary care hospitals rarely perform molecular tests, because it requires specialized equipment and highly trained personnel. With the continuous standardization of quality control, nucleic acid-based tests will become a mainstream technology of laboratory diagnosis of *M. pneumoniae* infection.

Laboratory diagnosis of *Chlamydia pneumoniae* infection

The general experience is that the culture of *C. pneumoniae* is not recommended as a standard of diagnosis, because it is difficult and time-consuming, as well as the sensitivity of isolation is low. Serology has so far been the most commonly used method for diagnosis of *C. pneumoniae* infections.

Incubation period of *C. pneumoniae* infection can last up to 1-3 months. IgM appears in the first infection in 3 weeks, IgG in 6-8 weeks. Therefore, serologic diagnosis frequently provides a retrospective diagnosis of *C. pneumoniae* infection. PCR has been very specific and more sensitive and is a valuable tool in early diagnosis of *C. pneumoniae* infection.

Laboratory diagnosis of *Legionella pneumophila* infection

Current diagnostic tests for *L. pneumophila* infection include culture, serological testing, antigen detection and nucleic acid amplification. Estimated sensitivities of sputum culture range from 15% to 90% and vary according to different comparison standards and by individual laboratories.[14] Hence culture diagnosis is rarely used in clinical practice, mainly for studies of bacteria biology.[7] Serological testing for *L. pneumophila* infection is a valuable epidemiological tool, but has little impact on clinical decision making because of the time delay before a result is available. A four-fold or greater increase in titer of paired serum samples is regarded to indicate current *L. pneumophila* infection. Detection of soluble *Legionella* antigen in urine specimens is a rapid method that provides an early diagnosis of *L. pneumophila* infection. For the detection of *L. pneumophila* serogroup 1 (accounting for about 70-80% of community-acquired *L. pneumophila* infection[15]), urinary antigen tests have sensitivities in the range of 80-90% and specificities approaching 98-100%.[16] *L. antigenuria* can be detected as early as 1 day after onset of symptoms and persists for days to weeks. The inadequacy of urinary antigen testing is a high price. Recently, DNA detection techniques have shown promise for the rapid diagnosis of *Legionella infection*. PCR has been successfully used to detect *Legionella* DNA in a range of environmental and clinical samples. The main gene targets used for detection of *Legionella* nucleic acid are 5S rRNA, 16S rRNA and mip gene. Further work is needed to establish a standard PCR method and procedures.

Comparison of different laboratory diagnosis methods

Mycoplasma pneumoniae is a common cause of CAP. The effect of laboratory diagnostic method has been an issue of great concern to clinicians. First, the standard laboratory diagnosis of *M. pneumoniae* infections presently relies on conventional serological methods. After infection by *M. pneumoniae*, IgM antibodies appear in 7 days of the illness, and high antibody titers can be maintained in

adolescents. Meanwhile, adults fail to respond with IgM as a result of re-infections. All these result in the low sensitivity of IgM assay, only 31.8% and 33.3% found in foreign studies.[17,18] In China, Qu *et al*. found the sensitivity of the IgM assay was only 7.4%, specificity was 94.9%, which may be relevant with a high rate of *M. pneumoniae* re-infection.[19] Second, previous studies reported that the culture was unacceptably insensitive for diagnosing *M. pneumoniae* infection. But, Qu *et al*. reported sensitivity and specificity in culture were 55.6% and 94.9%, respectively. In particular, positive likelihood ratio of 10.9% in culture meant that the culture were optimum diagnosis of acute *M. pneumoniae* infections in adults and adolescents.[19] It has been shown that PCR as a rapid, sensitive, and specific method may be more useful during the early stages of *M. pneumoniae* infection.[20] However, the sensitivity and specificity of PCR commercial kits in diagnosis of acute *M. pneumoniae* infection were only 40.7% and 88.8%, respectively. This may be relevant with detection ability of kits and the long-term asymptomatic infection caused by *M. pneumonia*.[21] Gnarpe *et al*. demonstrated that during the peak period of *M. pneumoniae* incidence, About 13.5% of healthy volunteers were found to harbour the bacterium in the throat and during a subsequent period of 11 months, the incidence of *M. pneumoniae* isolated decreased to 4.6% of volunteers.[22] Therefore, optimum diagnosis of acute *M. pneumoniae* infection relies on the use of specialized tests in combination of PCR and serological tests on the basis of clinical symptoms and signs.

TREATMENT AND DRUG RESISTANCE OF ATYPICAL PATHOGENS INFECTION

Macrolides and fluoroquinolones are generally considered to be the first-choice agents for the treatment of *M. pneumoniae* infection. Azithromycin and clarithromycin, a new macrolide antibiotics, may offer several advantages over erythromycin, including: Greater antimicrobial activity against certain organisms; longer elimination half-life, thus allowing less frequent administration; lower incidence of adverse gastrointestinal effects. Therefore, the compliance and tolerability of azithromycin and clarithromycin were superior to those of erythromycin.[23] In the 70s of the last century, resistance to macrolides was firstly reported in *M. pneumoniae* in a Japanese study.[24] Recently, macrolide-resistant *M. pneumoniae* have been spreading worldwide, with prevalences ranging from below 10% in Europe,[25-27] approximately 40% in America[28] and 8.2% in Japan.[29] The resistance rate of *M. pneumoniae* isolates from adult patients was 69%[12] in a 2009-2010 Chinese report and 71.7% in a 2013 Chinese report.[30] This indicates the prevalence of macrolide-resistant *M. pneumoniae* isolates in Chinese patients has increased sharply.

One report found there was a strong association between macrolide resistance in *M. pneumoniae* and point mutations in the 23S rRNA.[31] A2063G and A2064G mutations were responsible for the high-level marcolide resistance in *M. pneumonia*.[12] Previous results of 23S rRNA gene sequencing from a Beijing study indicated that all macrolide-resistant isolates harbored an A2063G mutation.[30,32] *M. pneumoniae* can be categorized into 2 genotypes, MP1 and MP2, based on the DNA sequence of the P1 adhesion protein, which is located in the cell membrane and is of vital importance for bacterial adhesion to epithelial cells. Previous studies found *M. pneumoniae* bacterial load and genotype were not associated with disease severity.[33] However, multiple-locus variable number tandem repeats analysis (MLVA) is a molecular typing method to be used to genotype several species of bacteria, showing nearly 30 subtypes of *M. pneumonia*.[34] "Pneumonia Monitoring Network in Beijing" research group using MLVA typing method to analyze the *M. pneumoniae* clinical isolates demonstrated that pneumonia severity index scores were significantly higher in patients with *M. pneumoniae* types U (5-4-5-7-2) and J (3-4-5-7-2) ($P < 0.001$), and total duration of cough were longer in them ($P = 0.011$). Moreover, the rate of macrolide nonresistance of isolates harboring Mpn13-14-15-16 as 3-5-6-2 was significantly higher than those with other variable number tandem repeats profiles.[32] In view of a few relevant studies, only 136 *M. pneumoniae* strains were covered in those reports. Therefore, it is difficult to accurately determine the influence of different genotypes of *M. pneumoniae* on clinical features of patients with infection.

In summary, atypical pathogens, especially *M. pneumoniae* is a common and important pathogen of CAP. Physicians should pay more attention to them and put forward higher requirements for various medical microbiology laboratories in pathogen detection. Meanwhile, with the deepening of relevant studies on pathogens epidemic, drug resistance and the association between genotypes and clinical features, more theoretical basis will be provided for clinical diagnosis of atypical pathogens infection.

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How to cite this article: Qu J, Cao B. Research progress in atypical pathogens of community acquired pneumonia. Community Acquir Infect 2014;1:11-4.

Source of Support: Nil, **Conflict of Interest:** None declared