

Mycoplasma pneumoniae: now in the focus of clinicians and epidemiologists

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Several northern European countries have experienced outbreaks of *Mycoplasma pneumoniae* infection in 2010 and 2011, as described in recent reports and in this issue. Such outbreaks appear with regular periodicity and have occupied clinicians and epidemiologists for many years.

Some 50 years ago, Chanock et al. [1] described an artificial medium that enabled the identification of the aetiological agent of an atypical pneumonia first reported 20 years earlier, which was first described as pleuropneumonia-like organisms (PPLO) and renamed as *Mycoplasma pneumoniae* [2]. More recently, genome analysis has revealed the bacterium's limited metabolism and biosynthesis of carbohydrates, proteins, nucleic acid and lipids, showing that the agent is well adapted to its only host, humans. We are, however, still unable to mimic the natural environment of *M. pneumoniae*: faster growth in culture media is needed for diagnostic purposes. It takes more than 10 days – in fact often up to three weeks – to grow *M. pneumoniae* from respiratory specimens taken from patients with an interstitial pneumonia. The organism can be cultured from samples taken in the acute phase of the infection, but because of the length of time needed, culture techniques have not been established in most bacteriological laboratories.

Lind et al. were the first in Europe to identify *M. pneumoniae* infection by detecting increases in *M. pneumoniae*-specific antibody titre, based at that time on cold agglutinin and complement fixation tests [3].

One striking aspect of *M. pneumoniae* infection is the periodicity of epidemics. The Danish seroepidemiological study of Lind et al., conducted over a 50-year period, showed between 1958 and 1973 an almost regular pattern of epidemics every four and a half years [3]. The authors suggested that herd immunity lasts about four years (range: 2–10) before people are again susceptible to infection with *M. pneumoniae*.

A prospective study of 4,532 outpatients in Germany aged at least 18 years with community-acquired pneumonia showed that *M. pneumoniae* was one of the major causative bacterial agents: 307 patients (6.8%) were *M. pneumoniae*-positive by real-time-PCR and/or positive for *M. pneumoniae*-specific IgM antibodies [4]. Some 72% of the patients with *M. pneumoniae* infection had only a mild pneumonia: this, combined with the number of days of hospitalisation required, might suggest a less severe pneumonia outcome in *M. pneumoniae* infections.

In many countries, clinicians had to treat patients with community-acquired pneumonia due to *M. pneumoniae* infection empirically during the whole acute phase because of the delay in the increase of antibody titres or because of the time needed for culture. Epidemiological studies were hampered for a long time because of these diagnostic difficulties. Consequently, *M. pneumoniae* was more or less ignored or in many countries 'a black box' in epidemiology because of the lack of diagnostic results. The situation changed, however, with the introduction of several molecular techniques, especially real-time PCR, into routine diagnosis [5]. Another advance has been the characterisation of different *M. pneumoniae* genotypes circulating in the human population. Clinical strains can be differentiated on basis of differences in the P1 adhesin gene or in the number of repetitive sequences at a given genomic locus using multilocus variable number tandem repeat analysis (MLVA) [6,7]. Both typing methods are not currently used routinely in epidemiological studies. However, typing will allow us to get more information about outbreaks of defined strains in different countries of Europe or even worldwide as well as information about changes in strains within a population. A long-term genotyping study from Japan [8] suggests that epidemics arise due to a change in the two main P1 types or even of because of further variants of P1 sequences, which were found recently [9,10].

MLVA allows greater discrimination between *M. pneumoniae* strains because of the very variable numbers

of repeats in the genome of different strains. It was used recently by Chalker et al. describing increased numbers of *M. pneumoniae* infections in England and Wales in 2011 and 2012 [11,12]. Outbreaks were seen in the years 1995, 1997/1998, 2002/2003, 2006 and a prepeak in 2010 before the outbreak in 2011. The peaking periods described showed all the characteristics of a *M. pneumoniae* epidemic, i.e. a broad 'shoulder', sometimes in two consecutive epidemic years with slightly fewer cases in summer than in later autumn and winter. Such a pattern was shown in Denmark for 2010 and 2011 [13].

Typing should answer the question, if such peaks could be attributed to different or to the same genotypes. Interestingly, Chalker et al. showed a small peak in 2010 before the outbreak in 2011. These findings suggest it will be necessary in the future to type more often strains from different countries and periods to answer the question of whether there is common epidemic spread of distinct genotypes in different countries of Europe. It is as yet unknown whether the recent epidemics in northern Europe [13-17] are caused by a common type strain.

Macrolide resistance has been described recently in Asia, with up to 90% of *M. pneumoniae* strains being resistant [18]. In the reports from the countries in northern Europe, no macrolide resistance was found in the tested strains except for Denmark, where 0.9% to 2.9% of strains were resistant. This is in accordance with data from France and Germany, where about 3% of strains were found to be resistant [19,20]. Particularly as a vaccine against *M. pneumoniae* is not yet available, macrolides – which are the only recommended therapy for children (whereas doxycycline and fluoroquinolones can be used for adults) – should be used carefully, as pointed out by Linde et al. in this issue [16]. It is not yet known whether the increased use of erythromycin in Norway at the end of 2011 [14] will induce more resistance. We should nevertheless be aware of possible macrolide resistance of *M. pneumoniae* during therapy even though this was not been seen in the paper by Uldum et al. [13]. The first two reports of emergence of macrolide-resistant *M. pneumoniae* during therapy were published last year by Cardinale et al. from Italy [21] and Averbuch et al. from Israel [22] in children with severe pneumonia. Such resistance may pose a major problem for clinicians, as certain antibiotics are not recommended for young children. In both cases, ciprofloxacin was given and the children were cured within a few days.

We now have the laboratory tools to detect *M. pneumoniae* within a day and also to identify possible macrolide resistance [20]. In order to aid clinicians, real-time PCR can be used, especially in the acute phase of infection, to diagnose *M. pneumoniae* in nasopharyngeal swabs or a provoked sputum [4]: this could become the gold standard for diagnosis. For more sophisticated studies, epidemiologists in Europe should come to an

agreement on standard sampling and a common typing method for *M. pneumoniae* strains.

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