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New insights in the outbreak pattern of *Mycoplasma pneumoniae*

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ABSTRACT

Since a well-documented incidence peak in 2011/12 in European countries, infections due to the cell wall-less bacterium *Mycoplasma pneumoniae* have gained the increased attention of clinicians, microbiologists and health authorities. Despite the mild or asymptomatic clinical course of most *M. pneumoniae* infections, the microorganism is responsible for severe interstitial pneumonia and extra-pulmonary complications. Here, we report the time-dependence of 5545 notified cases of laboratory-confirmed *M. pneumoniae* disease in Saxony from 2001 until June 2014 as measured by serodiagnosis. In parallel, from 2003 until 2012 467 *M. pneumoniae*-positive respiratory samples or isolated strains were analysed by molecular typing based on sequence differences in the main P1 adhesin of *M. pneumoniae*. The epidemiological data showed a prolonged outbreak especially in the period 2011–2013. The typing of circulating strains during the outbreak did not support predominance of one of the two major P1 subtypes (mean proportion of subtype 1: 57%) or a change of one to the other subtype during the endemic situation before and during the outbreak period. From the last major outbreak in Europe, we conclude that the notification of *M. pneumoniae*-positive cases, which is legally required only in Saxony, should be expanded to the whole country, to optimise awareness of this human pathogen and to reflect upon antibiotic therapy.

1. Introduction

Community-acquired pneumonia (CAP) is an important cause of morbidity and mortality in humans (Torres et al., 2013; Welte et al., 2012). Among the causative agents of CAP in patients aged 18 to <65 years, *Mycoplasma pneumoniae* is the most frequent bacterial species after *Streptococcus pneumoniae* (Klapdor et al., 2012). According to the results of many international studies, between 3 and 20% of all cases of CAP can be attributed to the cell wall-less mycoplasma (Atkinson et al., 2008; Atkinson and Waites, 2014). Although older children and young adults are mainly affected, infections and diseases due to *M. pneumoniae* occur in all age groups (von Baum et al., 2009). Risk groups include not only children cared for in communal facilities like schools, but also the children's adult carers and also all persons living in close communities such as students or army personnel (Waites and Talkington, 2004). The epidemiology of pneumonia caused by *M. pneumoniae* is characterized by a constant proportion of 4–8% among all cases of CAP in endemic periods, and also by nation- or even world-wide incidence peaks every 3–7 years in which up to 40% of CAP cases are attributed to *M. pneumoniae*. The last outbreak in the 2011/12 was described in several European countries (Blystad et al., 2012; Chalker et al., 2012; Linde et al., 2012; Pereyre et al., 2013; Polkowska et al., 2012; Uldum et al., 2012). This frequent infectious agent has been underestimated because of the lack of an obligation to notify diagnosed infections due to *M. pneumoniae* in Germany (except in Saxony) and also due to the often-mild clinical course and deficiencies in diagnostic procedures (Loens et al., 2010). A recent report (Dumke et al., 2015) confirmed a major increase of up to 28% of *M. pneumoniae*-positive respiratory tract samples from German adult outpatients with symptoms of CAP in the last outbreak. Beside the respiratory symptoms, a broad range of extra-pulmonary complications has been described, especially during this period. Mainly dermatological and neurological disorders have followed the classical infection route and have been regarded as autoimmune phenomena (Meyer Sauteur et al., 2014; Narita, 2010).

Although *M. pneumoniae* is a frequent agent in human respiratory disease, the diagnostic repertoire, depending today mostly on indirect methods, i.e. PCR or serology, was integrated only recently in bacteriological laboratories. The absence of a bacterial cell wall and the limited resources for metabolic pathways (Kühner et al., 2009) render classical bacteriological culture and Gram staining methods challenging in routine bacteriological laboratories.

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The species *M. pneumoniae* of the genus Mycoplasma has a highly reduced genome of only 816 kbp (Citti and Blanchard, 2013). Because of this genome reduction mycoplasmas exhibit a more or less parasitic lifestyle. Obviously, the specificity of host–mycoplasma interaction restricts the range of species, which are affected. In consequence, the human respiratory tract is the only known habitat in which *M. pneumoniae* can multiply naturally. Known factors influencing the pathogenicity of *M. pneumoniae* are a complex of adhesion-related proteins mediating the targeted gliding and adherence of the bacteria to the respiratory epithelium of the host (Krause and Balish, 2004), the release of hydrogen peroxide (Hames et al., 2009) and a pertussis toxin-like CARDS toxin (Kannan and Baseman, 2006) causing the destruction of epithelial cells.

From a genetic point of view, *M. pneumoniae* is a remarkably conserved species showing a limited number of ORFs with sequence differences exceeding point mutations (Dumke et al., 2003). Recently, clinical isolates as well as strains in respiratory specimens can be typed by analysing differences in the sequence of the main P1 adhesin (Dumke et al., 2006) and by multi-locus variable-number tandem-repeat analysis (MLVA; Degrange et al., 2009; Dumke and Jacobs, 2011). MLVA has a higher discriminatory power to differentiate strains and is suitable for investigation of small-scale and more endemic outbreaks (Pereyre et al., 2012). In contrast, the P1 protein as an important virulence factor and major determinant of immune response (Razin and Jacobs, 1992) is strongly involved in host–pathogen interaction. Up to now, two subtypes have been described that show significant sequence differences in both of the two repetitive elements repMP4 and repMP2/3 (Dandekar et al., 2000) of the gene coding for the P1 adhesin of *M. pneumoniae*.

Here, we summarize the notified *M. pneumoniae*-positive cases since 2001 in Saxony and compare the time-dependent pattern of incidence of strains during the endemic periods with the outbreak strains by P1-typing.

2. Materials and methods

Respiratory samples were taken between 2003 and 2012 from adult and paediatric in- and outpatients with symptoms of CAP in Germany. In the majority of these patients (91%), suspected diagnosis of CAP based on defined criteria (www.capnetz.de). In all further cases, a suspicion of physicians led to extended laboratory diagnostics. Primary testing of samples was done with a real-time PCR based on amplification of repMP1-copies of *M. pneumoniae* (Dumke et al., 2007). Positive samples were culture-independently typed as described (Dumke et al., 2006).

The notification of *M. pneumoniae*-positive cases in Saxony is based on direct and indirect laboratory methods but mostly on serological parameters.

3. Results

Data from serological testing of patients with symptoms of community-acquired pneumonia in Saxony revealed increased numbers of notified *M. pneumoniae*-positive cases in 2003, 2006 and especially during the major outbreak in 2011 until 2013 (Fig. 1). In most years, the number of reported positive cases decreased in summer and increased in autumn/winter. A clear trend in an increase or decrease of distinct P1-type 1 or 2 or in the proportion the two P1-types was not observed. Between 2003 and 2012, 45% of all typed strains were type 1 and 55% were type 2. The percentage von type 1 strains varied between 21% (2008) and 62% (2009). Regarding the period of high incidence in 2011 and 2012, the proportion remained constant at 57% of type P-1 strains in both years.

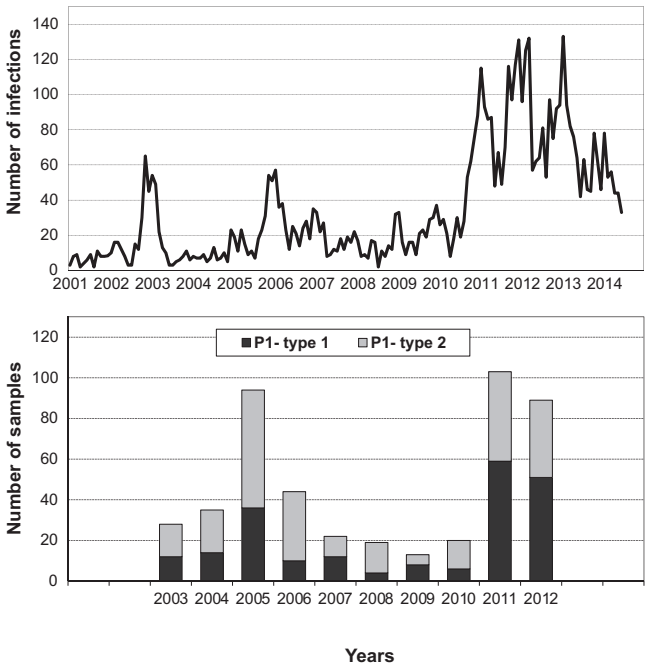

Fig. 1. Number of *M. pneumoniae* infections in the federal state of Saxony detected by Landesuntersuchungsanstalt Sachsen between 2001 and 2014 (upper diagram). Results of P1-typing of *M. pneumoniae*-positive respiratory tract samples from German patients with symptoms of community-acquired pneumonia (lower diagram).

4. Discussion

Long-term colonization of the patient with *M. pneumoniae* after antibiotic therapy (Nilsson et al., 2008) as well as the detection of *M. pneumoniae* in the upper respiratory tract of asymptomatic persons (Spuesens et al., 2013) are well-reported facts. This carrier status complicates interpretation of the detection of *M. pneumoniae* in respiratory samples, necessitating the inclusion of serodiagnostic acute parameters and of clinical symptoms. However, from an epidemiological point of view, both specific PCR and antibody detection confirmed a noteworthy outbreak of cases of community-acquired pneumonia caused by *M. pneumoniae* in the years 2011 to 2013 in Germany. This is in accordance with reports from different European countries, especially those with more elaborate disease notification reports. The sentinel alarm system of the public health authorities in the Scandinavian countries helped general practitioners in particular to be aware of the increased infections due to *M. pneumoniae*. This is of practical importance since the betalactam antibiotics are the first choice of various guideline recommendations for treatment of CAP but are ineffective in case of the cell wall-less *M. pneumoniae* so that alternative antibiotics should be considered in periods with high *M. pneumoniae* incidence.

Besides the possible influence of global climate changes on epidemiology (Onozuka and Chaves, 2014), it was hypothesized that the occurrence or the alternation of different P1-types of *M. pneumoniae* in the human population might be the cause of the unique epidemiological pattern of time-dependent outbreaks (Dumke et al., 2004). Up to now, two P1-types and several variants have been described (Spuesens et al., 2009). Variants are characterized by integration of small boxes of type-specific repMP elements into the repMP4 and/or repMP2/3 copy in the P1 gene. Parts of the repetitive elements have been regarded as a reservoir for recombination processes (Rocha and Blanchard, 2002) and might, in the case of the antigenic P1 protein, influence the host's immune response. The data presented here show that partial or complete changes in the proportion of the two P1-types in the