Increased incidence of *Mycoplasma pneumoniae* infection in England and Wales in 2010: multilocus variable number tandem repeat analysis typing and macrolide susceptibility

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An epidemic of *Mycoplasma pneumoniae* infection began in Denmark in late 2010. A similar increase in *M. pneumoniae* infections was noted in England and Wales in the same period, with a decline in early 2011. Multilocus variable number tandem repeat analysis typing and analysis of macrolide resistance markers indicate that at least nine known and two novel strain types were circulating in England and Wales during October 2010 to January 2011. There was no evidence of macrolide resistance.

After an epidemic of *Mycoplasma pneumoniae* infection in Denmark in late 2010 was reported, we found a similar increase in the number of *M. pneumoniae* infections in England and Wales in the same time period. By early 2011, the number of infections had fallen in England and Wales.

Background

M. pneumoniae is a common cause of pneumonia and is transmitted by aerosol or close contact. In England and Wales, the pathogen is found in all age groups, with higher prevalence in children aged 5–14 years [1]. Epidemic periods lasting on average 18 months have occurred at approximately four yearly intervals, as seen Denmark [2,3]. Epidemic periods follow the same pattern: sporadic infection occurs at a low level with seasonal peaks from December to February [1,2]. Recently, data were reported from Denmark indicating that a *M. pneumoniae* epidemic had started in October 2010 [3]. As previous epidemic periods in England and Wales have been synchronous with those in Denmark, we sought to determine whether an epidemic was also occurring in England and Wales. In these two countries of the United Kingdom, data submitted voluntarily from routine laboratory reports are collated by the Health Protection Agency (HPA) to give an indication of the number of patients testing positive by serological, molecular or culture tests for *M. pneumoniae* per week.

More recently, community surveillance data based on quantitative real-time polymerase chain reaction (qPCR) analysis have been used successfully to monitor *M. pneumoniae* infection in patients with respiratory symptoms – influenza-like illness, upper respiratory tract infection, lower respiratory tract infection, fever ($>38.5^{\circ}\text{C}$) or myalgia – attending general practitioner (GP) clinics (from 2005 to 2009) [1]. This was an extension to the virological community surveillance that is undertaken annually in England and Wales for a range of respiratory viruses including influenza virus, respiratory syncytial virus and human metapneumovirus [4].

To determine whether an *M. pneumoniae* epidemic was occurring in England and Wales, we reviewed the laboratory reports submitted to the HPA and, from October (week 40) 2010 to January (week 3) 2011, undertook qPCR-based community surveillance for *M. pneumoniae* infection in patients with respiratory symptoms attending GP clinics. Furthermore, to determine what strains of *M. pneumoniae* were circulating during this time, community surveillance samples and 10 additional respiratory samples (submitted to our laboratory by GPs and hospitals for routine testing) that were positive by qPCR were investigated to determine the type of infecting strain and whether there was any evidence of genetic markers for macrolide resistance. Resistance to macrolides is an increasing problem in Asia and has been found in the United States and some European countries [3].

Methods

We carried out qPCR analysis of 1,221 anonymised combined nose and throat swabs taken from patients with respiratory symptoms during the winter months of 2010/11: October (week 40) 2010 to January (week 3) 2011. Nucleic acid was extracted and stored as previously described before qPCR testing for the presence of *M. pneumoniae* P1 and community-acquired respiratory distress toxin genes [1,5,6].

Samples that were positive by qPCR were examined for *M. pneumoniae* type and macrolide resistance. We also examined the additional 10 respiratory samples submitted to our laboratory for *M. pneumoniae* detection in this period.

Multilocus variable number tandem repeat analysis (MLVA) typing by fragment analysis has previously been used to type *M. pneumoniae* strains [7]. In this study, we used the same MLVA typing method for analysing nucleic acid extracts of clinical samples; culture isolation of *M. pneumoniae* was not undertaken. Putative novel MLVA profiles were given numerical designations, from MLVA-0027 onwards, to follow on from the known 26 MLVA types (MLVA-A to MLVA-Z) [7].

The possible presence of mutations in *M. pneumoniae* previously associated with macrolide resistance was examined by amplification and sequencing of a 720 base pair (bp) fragment of the 23S rRNA gene using MpnMR2063F (5'-ATCTCTTGACTGCTCGGC-3') and MpnMR2617R (5'-TACAACTGGAGCATAAGAGGTG-3') primers [8].

Detection and analysis of *M. pneumoniae* in clinical samples

The number of *M. pneumoniae*-positive laboratory reports from regional laboratories and hospitals submitted to the HPA during the study period is shown in Figure 1: one report is received per patient and four-weekly moving averages are plotted. From week 40

of 2010 to week 3 of 2011, there were a total of 322 reports, the highest number since the previous peak of *M. pneumoniae* infections seen during the same sampling period in 2005 to 2006 ($n=455$). The mean number of reports received from 2006 to 2009 (from week 40 of one year to week 3 of next) was 234.

A total of 21 of 1,221 (1.7%; 95% CI: 1.1 to 2.6) community surveillance samples from week 40 of 2010 to week 3 of 2011 were *M. pneumoniae*-positive by qPCR. The percentage of positive cases per week is shown from 2005 to 2011 in the Table, showing an increase from October to December (weeks 40–49) 2010. Samples were more likely to be positive during this period in 2010 (18 of 629; 2.9%; 95% CI: 1.8 to 4.5) than in the following six weeks – December (week 50) 2010 to January (week 3) 2011 (3 of 592; 0.5%; 95% CI: 0.1 to 1.6; Fisher's exact test $p=0.002$). In November (weeks 44, 45 and 47) 2010, *M. pneumoniae* infections significantly increased in comparison with all previous weeks of sampling since 2005 (binomial probability test $p=0.09, 0.07, 0.005$, respectively).

The mean age of the patients was 19.8 years (standard deviation (SD) ± 19.4 years; range: 0–91 years). We detected no difference in age group affected by *M. pneumoniae* infection (Figure 2).

MLVA analysis of 10 of the 21 qPCR-positive community surveillance samples and the 10 additional concurrent respiratory samples showed a total of 11 distinct strain

FIGURE 1

Laboratory reports of *Mycoplasma pneumoniae* infection by date of report, England and Wales, January 1995–March 2011

qPCR: quantitative real-time polymerase chain reaction.

Four-weekly moving total of the number of reports collated by the Health Protection Agency Centre for Infections, including serological, molecular and culture test results. Report numbers per year are: 1,687 in 1995, 490 in 1996, 990 in 1997, 2,278 in 1998, 727 in 1999, 483 in 2000, 804 in 2001, 1,409 in 2002, 819 in 2003, 472 in 2004, 991 in 2005, 818 in 2006, 737 in 2007, 573 in 2008, 624 in 2009, 743 in 2010, 162 in 2011 (up to the first week of March), giving a total of 14,807 for all years, giving a total of 14,807 for all years. The arrowed line indicates the qPCR study period, October (week 40) 2010 to January (week 3) 2011, during which time 322 reports were received.

Source: [9].

types: nine of known types (MLVA-B, C, E, J, M, P, U, V and Z) and two putative novel types (termed MLVA-0027 profile 34672 and MLVA-0028 profile 64573). An MLVA profile could not be obtained for the other 11 qPCR-positive community surveillance samples, probably because of the low levels of *M. pneumoniae* nucleic acid in these samples.

The most prevalent strain type was MLVA-M (5 of 20), which had been found previously in France (in 1997, 1999, 2000 and 2006), Germany (in 1995 and 2000) and Japan (in 2000 to 2003) [7]. In our study, patients with this strain type had a cough (n=3), upper respiratory symptoms (n=1) or lobar pneumonia (n=1).

A full-length sequence of the 720 bp fragment of the 23S rRNA gene containing all four loci associated with macrolide resistance (2063, 2064, 2067 and 2618) was obtained from 14 of the 21 qPCR-positive community surveillance samples and the 10 additional respiratory samples. No mutations in these loci associated with macrolide resistance were identified in these samples. For the remaining seven qPCR-positive community surveillance samples, sequence information could not be obtained, presumably due to low levels of *M. pneumoniae* nucleic acid.

Discussion

The overall level of *M. pneumoniae* infection in the qPCR-based community surveillance of patients from October 2010 to January 2011 was low (1.7%) and was at a similar level to that found in the same months during 2005 to 2009 (1.7%) [1]. Detectable *M. pneumoniae* infection was found in all age groups; however, no significant difference in age group affected by *M. pneumoniae* infection was found over this time period, unlike the situation in 2005 to 2006. At that time, *M. pneumoniae* infections were mainly reported in children aged 5–14 years.

Samples from October to December (weeks 40–49) 2010 were more likely to be positive than those in the following six weeks. The increased incidence in this period in 2010 is consistent with a rise in the number of *M. pneumoniae* laboratory reports in the same period (Figure 1) and that seen in Denmark [3]. The increase in 2010 in the number of positive laboratory reports submitted to the HPA is four weeks later than that detected by qPCR, as the reports are mainly based on IgM serology, highlighting that data from laboratory reports collected by the HPA on *M. pneumoniae* infection in England and Wales lag a month behind actual infection in the population.

TABLE

Percentage of clinical community surveillance samples positive for *Mycoplasma pneumoniae*^a per week, England and Wales, October (week 40) to January (week 3) of 2005–2011^b

Week number	Percentage of samples positive for <i>M. pneumoniae</i>				
	2005/06	2006/07	2007/08	2008/09	2010/11
	% (95% CI)	% (95% CI)	% (95% CI)	% (95% CI)	% (95% CI)
40	0.0 (0.0–28.5)	16.7 (0.4–64.1)	0.0 (0.0–100)	0.0 (0.0–60.2)	3.0 (0.1–15.8)
41	0.0 (0.0–16.8)	0.0 (0.0–52.2)	0.0 (0.0–70.8)	0.0 (0.0–84.2)	1.4 (0.0–7.8)
42	4.8 (0.0–23.8)	0.0 (0.0–60.2)	0.0 (0.0–12.8)	0.0 (0.0–60.2)	1.3 (0.0–6.9)
43	3.0 (0.1–15.8)	0.0 (0.0–14.2)	0.0 (0.0–13.2)	11.1 (0.3–48.2)	1.3 (0.0–7.0)
44	3.3 (0.1–17.2)	0.0 (0.0–10.0)	0.0 (0.0–14.8)	0.0 (0.0–60.2)	3.9 (0.8–11.0)
45	7.4 (0.8–24.3)	2.2 (0.1–11.5)	0.0 (0.0–12.3)	11.1 (0.3–48.2)	4.5 (0.9–12.7)
46	4.0 (0.1–20.4)	0.0 (0.0–7.5)	0.0 (0.0–8.0)	0.0 (0.0–20.6)	3.0 (0.4–10.4)
47	0.0 (0.0–14.8)	5.0 (0.6–16.9)	2.4 (0.1–12.9)	0.0 (0.0–30.8)	8.3 (2.2–20.0)
48	11.5 (2.2–30.2)	0.0 (0.0–5.7)	0.0 (0.0–6.5)	0.0 (0.0–14.8)	0.0 (0.0–7.4)
49	2.9 (0.1–14.9)	0.0 (0.0–6.3)	0.0 (0.0–5.6)	3.6 (0.1–18.3)	3.0 (0.4–10.5)
50	10.0 (1.9–26.5)	2.1 (0.1–11.3)	1.5 (0.0–8.0)	0.0 (0.0–8.0)	0.0 (0.0–3.6)
51	5.3 (0.6–17.7)	2.6 (0.1–13.8)	1.1 (0.0–6.0)	0.0 (0.0–8.6)	0.0 (0.0–1.8)
52	0.0 (0.0–41.0)	0.0 (0.0–8.2)	0.0 (0.0–5.4)	0.0 (0.0–17.6)	0.0 (0.0–4.9)
1	6.3 (0.8–20.8)	0.0 (0.0–5.1)	0.0 (0.0–3.1)	0.0 (0.0–18.5)	0.0 (0.0–5.9)
2	5.9 (1.2–16.2)	1.0 (0.0–5.6)	2.1 (0.3–7.5)	0.0 (0.0–33.6)	2.1 (0.4–6.1)
3	0.0 (0.0–8.8)	3.8 (0.8–10.6)	0.0 (0.0–4.9)	0.0 (0.0–33.6)	0.0 (0.0–36.9)
All weeks	4.5 (2.6–6.8)	1.4 (0.7–2.6)	0.6 (0.2–1.4)	1.2 (0.2–3.5)	1.7 (1.0–2.6)

The number of positive samples and total number of samples per year are: 20 of 449 in 2005/06, 10 of 703 in 2006/07, 5 of 818 in 2007/08, 3 of 249 in 2008/09 and 21 of 1,221 in 2010/11, giving a total of 59 positive in 3,440 samples for all years.

^a Determined by quantitative real-time PCR.

^b Excludes October (week 40) 2009 to January (week 3) 2010 when sampling was not performed.

A total of 11 distinct MLVA types were identified during the study period, with MLVA-M being the most prevalent. Patients with this strain type did not all have the same symptoms or severity of infection and the sample number is too low to investigate the association of particular types with clinical severity. Two putative new profiles were obtained, in addition to nine known types. One of these, MLVA0027, was identified in two different samples. Confirmation of the novel MLVA types obtained will require isolation of the strains.

It is not known whether increases in incidence of *M. pneumoniae* infections are due to an increased incidence of an individual strain or a concurrent increased incidence of several strains. Speculation that a shift in P1 adhesin type may be the cause of epidemics has been disputed [7,10]. Evidence from our study does not support the hypothesis that a single strain type of *M. pneumoniae* was responsible for the observed increase in infection in England and Wales. Rather, a decline in immunity or increase

of the immunologically naive population may have triggered the four-year cycle of epidemic periods. It would be interesting to type *M. pneumoniae* strains from Denmark and other countries during the same period to determine how strains differ geographically during periods of increased infection.

Macrolide-resistant *M. pneumoniae* is an increasing problem in Asia and has been seen in Europe and the United States; however, resistance remains uncommon in European countries (such as Denmark, France and Germany) [3]. Macrolide resistance was not identified in any of the qPCR-positive samples from England and Wales analysed during the study period.

Our study shows that qPCR based surveillance of *M. pneumoniae* infections in the community is invaluable, allowing rapid detection of infection in the population and contributing timely data on infecting strain characteristics, diversity and antimicrobial resistance.

FIGURE 2

Percentage of clinical community surveillance samples positive for *Mycoplasma pneumoniae*^a by age group, England and Wales, October (week 40) 2005 to January (week 3) 2011^b

qPCR: quantitative real-time polymerase chain reaction.

The number of positive samples and total number of samples per year are: 20 of 412 in 2005/06, 9 of 638 in 2006/07, 5 of 769 in 2007/08, 2 of 239 in 2008/09, 21 of 239 in 2010/11, giving a total of 57 positive in 3,265 samples for all years. Information on the age was not available for all patients. The error bars indicate 95% confidence intervals.

^a Determined by qPCR.

^b Excludes October (week 40) 2009 to January (week 3) 2010 when sampling was not performed.

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