Increased detection of *Mycoplasma pneumoniae* infection in children in England and Wales, October 2011 to January 2012

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Community surveillance data, based on quantitative real-time polymerase chain reaction analysis, showed that one in seven children aged 5–14 years with respiratory signs tested positive for *Mycoplasma pneumoniae* in England and Wales from October 2011 to January 2012 – a higher proportion than that seen in previous years. Multilocus variable number tandem repeat analysis indicates that at least seven known and two novel strain types were circulating in England and Wales during this period.

Recent reports indicate that an increased number of *Mycoplasma pneumoniae* infections have been detected in seven European countries including Denmark, Norway and Finland [1-4]. To determine the number of patients infected with *M. pneumoniae* in England and Wales and to see if the number had increased, compared with previous winters, community surveillance data and laboratory reports submitted to the Health Protection Agency (HPA) from 10th October (week 42) 2011 to 20th January (week 3) 2012 were reviewed. Our study shows an increase in the number of children with *M. pneumoniae* infection by PCR-based surveillance in the community during the study period.

Further analysis was carried out to determine which strains of *M. pneumoniae* were present in this period in the community surveillance samples, in addition to analysis of genetic markers for macrolide resistance.

Background

M. pneumoniae is a respiratory pathogen that is a common cause of pneumonia and may cause other serious sequelae such as encephalitis. The pathogen is found in all age groups, with higher prevalence in children aged 5–14 years [2,5].

In England and Wales, epidemic periods lasting on average 18 months have occurred at approximately four-yearly intervals [6]. In addition, low-level sporadic infection occurs with seasonal peaks from December to February [5,6]. Since 2005, a community surveillance scheme for *M. pneumoniae* using quantitative real-time polymerase chain reaction (qPCR) analysis has been used to monitor *M. pneumoniae* infection in England and Wales [7]. Until 2010, this scheme was used for monitoring patients of all ages and from 2010 to date, for children aged under 16 years [7]. It is an extension of the virological community surveillance that is undertaken annually in England and Wales for a range of respiratory viruses including influenza virus, respiratory syncitial virus and human metapneumovirus [8]. Combined nasal and throat swabs were taken during the winter months (from October to March, 2005 to 2012, and throughout the recent influenza A(H1N1) pdm09 pandemic) from patients with respiratory symptoms including influenza-like illness, upper respiratory tract infection, lower respiratory tract infection, or fever or myalgia who attended general practitioner clinics [5]. Additional voluntarily submitted reports from regional laboratories and hospitals in England and Wales were collated by the Health Protection Agency (HPA) according to age and region to give an indication of the number of patients testing positive for *M. pneumoniae* by serological, molecular or culture tests each week.

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

Laboratory reports

The number of *M. pneumoniae*-positive laboratory reports submitted to the HPA during the study period (week 42 2011 to week 3 2012) varied from 11 to 36 per week, as shown in the four-weekly moving averages in [9]. From week 42 2011 to week 3 2012, a total of 353 reports were received, higher than the number in the same period in 2010 (week 42 2010 to week 3 2011), when 290 were received. Reports were received from all areas of England and Wales during this period (Table 1). The patients were of all ages, with the youngest

being less than one week old and the oldest 92 years of age (Table 2). This age profile of submitted *M. pneumoniae*-positive reports was very similar to that for all such reports received from week 1 1975 to week 3 2012.

Community surveillance

We carried out qPCR analysis on 144 anonymised combined nose and throat swabs taken as part of community surveillance from patients aged under 15 years with respiratory symptoms during October 2011 to January 2012 (a total of 144 swabs were taken during that time). Nucleic acid was extracted and stored as previously described before qPCR testing for the presence of the *M. pneumoniae* P1 gene [5,10].

A total of 13 of the samples (9.0%; 95% CI: 5.2–15.0) were *M. pneumoniae* positive. One in seven of the children aged 5–14 years (12/84) had detectable *M. pneumoniae*, whereas only one of the 60 children aged under 5 years was positive (Fisher's exact test p=0.008) (Figure 1).

The percentage of positive cases per week (from week 42 to week 3 of the following year) for children aged under 15 years is shown for 2005 to 2012 in Table 3. This shows an increase from November 2011 to January 2012 (week 46 2011 to week 1 2012). Samples were more likely to be positive during this period in 2011/12 (13/91; 12.5%; 95% CI: 7.3–20.4) than in the previous four weeks (weeks 42–45 2011) and the following two weeks (weeks 2–3 2012) (0/40; 0%; 95% CI: 0.0–10.4; Fisher's exact test p=0.02).

In November 2011 (week 46), December 2011 (weeks 50 and 51) and January 2012 (week 1), the number of *M. pneumoniae* infections significantly increased in comparison with all previous weeks of sampling since 2005 (binomial probability test p=0.00001, 0.0007, 0.05 and 0.01, respectively).

The mean age of the 144 patients was 6.5 years (standard deviation (SD)±4.4; range: 0–14) with the majority of *M. pneumoniae*-positive patients being over 5 years-old (n=12 of 84). The mean age of the positive patients was 8.7 years (SD±2.6). Only one *M. pneumoniae*-positive patient was less than 5 years old (aged 4 years).

Table 1

Percentage of *Mycoplasma pneumoniae*-positive samples from laboratory reports by region, England and Wales, 10 October (week 42) 2011–20 January (week 3) 2012 (n=353)

Region	Percentage of samples positive for *M. pneumoniae*	
	% (95% CI)	Number of positive samples
East Midlands	5.7 (3.7–8.7)	20
East	8.5 (6.0–11.9)	30
London	24.4 (20.2–19.1)	86
North East	6.0 (3.9–9.0)	21
North West	13.9 (10.6–17.9)	49
South East	4.0 (2.3–6.6)	14
South West	7.1 (4.8–10.3)	25
West Midlands	5.4 (3.4–8.3)	19
Wales	14.5 (11.1–18.5)	51
Yorkshire and Humberside	10.8 (7.9–14.5)	38

Table 2

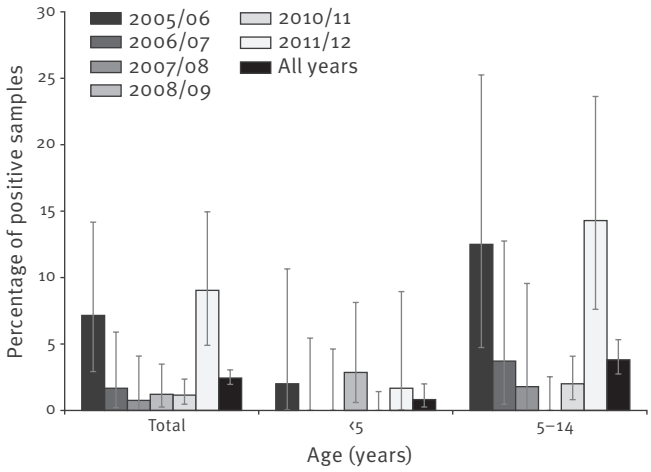
Percentage of *Mycoplasma pneumoniae*-positive samples from laboratory reports by age, England and Wales, 10 October (week 42) 2011–20 January (week 3) 2012 (n=353) and 1 January 1975 (week 1)–20 January (week 3) 2012 (n=38,221)[a]

Age in years	Percentage of samples positive for *M. pneumoniae*			
	Week 42 2011–week 3 2012		Week 1 1975–week 3 2012	
	% (95% CI)	Number of positive samples	% (95% CI)	Number of positive samples
<5	11.3 (8.4–15.1)	40	10.1 (7.3–13.4)	3,863
5–14	24.1 (19.9–28.8)	85	24.9 (20.1–29.5)	9,535
15–44	37.1 (32.2–42.3)	131	42.7 (37.8–47.7)	16,326
45–64	18.1 (14.4–22.5)	64	12.6 (9.6–16.3)	4,806
>65	9.4 (6.7–12.9)	33	5.1 (3.4–9.1)	1,957

[a] Information about age was not available for all reports.

Figure 1

Percentage of clinical community surveillance samples from patients aged under 15 years positive for *Mycoplasma pneumoniae* determined by qPCR, England and Wales, October 2005–January 2012[a] (total of 33 positive in 1,354 samples)



qPCR: quantitative real-time polymerase chain reaction.

The number of positive samples and total number of samples per year were 7 of 98 in 2005/06, 2 of 120 in 2006/07, 1 of 134 in 2007/08, 3 of 249 in 2008/09, 2009 not tested, 7 of 609 in 2010/11, 13 of 144 in 2011/12, giving a total of 33 positive in 1,354 samples for all years analysed. Error bars indicate the 95% CI for the percentages.

[a] Excludes October (week 42) 2009 to January (week 3) 2010 when sampling was not performed due to the influenza A(H1N1)pdm09 pandemic.

Of the 144 patients analysed, 62 were male and 79 female (sex was not specified for three patients). Of the 13 *M. pneumoniae*-positive patients, 5 were male and 8 female.

M. pneumoniae type and macrolide resistance

Samples that were positive by qPCR were examined for *M. pneumoniae* type and macrolide resistance. Multiocus variable number tandem repeat analysis (MLVA) typing by fragment analysis, which has previously been used to type *M. pneumoniae* strains [7,11], was used to analyse nucleic acid extracts of clinical samples in our study; culture isolation of *M. pneumoniae* was not undertaken. MLVA typing was also performed on nine additional *M. pneumoniae*-positive respiratory samples that were submitted to the laboratory during October 2011 to January 2012. Genetic diversity was calculated using Hunter and Gastons variation of Simpson's diversity index [12].

The presence of mutations previously associated with macrolide resistance was examined by amplification and sequencing of a 720-base pair (bp) fragment of the 23S rRNA gene using the primers MpnMR2063F (5'-ATCTCTTGACTGTCTCGGC-3') and MpnMR2617R (5'-TACAACTGGAGCATAAGAGGTG-3') [13].

MLVA analysis of eight of the 13 qPCR-positive community surveillance samples and the nine *M. pneumoniae*-positive respiratory samples that were submitted to the laboratory during October 2011 to January 2012 showed a total of nine distinct strain types: seven of known MLVA type (type E (n=1), type M (n=4), type P (n=2), type S (n=1), type T (n=1), type U (n=2)and type Z (n=3)) and two putative novel types (profile 4,4,5,7,3 (n=2) and 5,3,5,7,3 (n=1)) (Figure 2). A full MLVA profile could not be obtained for the other five qPCR-positive community surveillance samples, probably because of the low levels of *M. pneumoniae* nucleic acid in these samples.

The strain type most frequently found in the 17 samples was MLVA-M (n=4), which was also the most prevalent strain type in England and Wales in 2010 and has been found in France (in 1997, 1999, 2000 and 2006), Germany (in 1995 and 2000) and Japan (in 2000 to 2003) [5,11]. Comparison of the Hunter–Gaston diversity index (DI) indicated that both populations in October to January 2010/11 and 2011/12 were similarly diverse (2010 DI: 0.93; 95% CI: 0.88–0.98, 2011 DI: 0.91, 95% CI: 0.85–0.97).

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 12 of the 13 qPCR-positive community surveillance samples. No mutations in these loci associated with macrolide resistance were identified in

Table 3

Percentage of clinical community surveillance samples positive for *Mycoplasma pneumoniae* determined by qPCR per week for children aged under 15 years, England and Wales, October (week 42)–January (week 3) 2005–2012[a] (total of 33 positive in 1,354 samples)

Week Number	Percentage of samples positive for *M. pneumoniae*					
	2005/06	2006/07	2007/08	2008/09	2010/11	2011/12
	% (95% CI)	% (95% CI)	% (95% CI)	% (95% CI)	% (95% CI)	% (95% CI)
42	0.0 (0.0–45.9)	0.0 (0.0–97.5)	0.0 (0.0–33.6)	0.0 (0.0–60.2)	0.0 (0.0–28.5)	0.0 (0.0–97.5)
43	0.0 (0.0–84.2)	0.0 (0.0–97.5)	0.0 (0.0–70.8)	20.0 (0.4–71.6)	0.0 (0.0–52.2)	0.0 (0.0–45.9)
44	0.0 (0.0–45.9)	0.0 (0.0–45.9)	0.0 (0.0–84.2)	0.0 (0.0–36.9)	13.3 (1.5–40.5)	0.0 (0.0–36.9)
45	14.3 (0.3–57.9)	0.0 (0.0–60.2)	0.0 (0.0–60.2)	12.5 (0.3–52.7)	0.0 (0.0–30.9)	0.0 (0.0–36.9)
46	0.0 (0.0–60.2)	12.5 (0.3–52.7)	0.0 (0.0–28.5)	0.0 (0.0–23.2)	0.0 (0.0–23.1)	25.0 (5.7–52.4)
47	0.0 (0.0–41.0)	0.0 (0.0–52.2)	0.0 (0.0–41.0)	0.0 (0.0–17.6)	5.9 (0.7–19.7)	7.7 (0.2-36.0)
48	0.0 (0.0–60.2)	0.0 (0.0–21.8)	0.0 (0.0–19.5)	0.0 (0.0–16.1)	0.0 (0.0–9.7)	0.0 (0.0–52.2)
49	25.0 (2.5–65.1)	0.0 (0.0–24.7)	0.0 (0.0–28.5)	0.0 (0.0–16.8)	2.1 (0.1–11.1)	0.0 (0.0–30.8)
50	14.3 (2.6–36.3)	0.0 (0.0–30.8)	4.8 (0.1–23.8)	2.4 (0.1–12.6)	0.0 (0.0–4.4)	16.7 (4.0–37.4)
51	9.0 (0.2–41.3)	0.0 (0.0–20.6)	0.0 (0.0–17.6)	0.0 (0.0–10.0)	0.0 (0.0–2.4)	10.5 (1.2–33.1)
52	0.0 (0.0–70.8)	0.0 (0.0–33.6)	0.0 (0.0–97.5)	0.0 (0.0–11.2)	0.0 (0.0–6.3)	15.4 (0.0–60.2)
1	0.0 (0.0–70.8)	0.0 (0.0–36.9)	0.0 (0.0–18.5)	0.0 (0.0–16.1)	0.0 (0.0–8.4)	18.2 (2.3–45.4)
2	0.0 (0.0–41.0)	7.7 (0.2–36.0)	0.0 (0.3–70.8)	0.0 (0.0–26.5)	2.1.0 (0.3–7.4)	0.0 (0.0–33.6)
3	0.0 (0.0–70.8)	0.0 (0.0–28.5)	0.0 (0.0–36.9)	0.0 (0.0–33.6)	0.0 (0.0–36.9)	0.0 (0.0–36.9)
All weeks	4.5 (2.9–15.1)	1.7 (0.2–5.9)	0.7 (0.0–4.1)	1.2 (0.2–3.5)	1.2 (0.5–2.4)	9.0 (5.2–15.0)
Number of positive samples/total number of samples	**7/98**	**2/120**	**1/134**	**3/249**	**7/609**	**13/144**

qPCR: quantitative real-time polymerase chain reaction.

Shaded cells represent weeks when *M. pneumoniae* was detected.

[a] Excludes October (week 42) 2009 to January (week 3) 2010 when sampling was not performed due to the influenza A(H1N1)pdm09 pandemic.

these samples. For the remaining qPCR-positive community surveillance sample, sequence information could not be obtained, presumably due to low levels of *M. pneumoniae* nucleic acid.

Discussion

The level of *M. pneumoniae* infection in the qPCR-based community surveillance of children aged under 16 years from October 2011 to January 2012 was 9.0%, rising to 14.3% in the 5–14 year-olds. This is considerably higher than that in the same months from previous years from 2005 to 2011 (1.7%) [5]. Detectable *M. pneumoniae* infection was found by qPCR in children aged from 4 to 14 years and was absent from those aged under 4 years in the 2011/12 study period. As qPCR was not performed on specimens from adults, the level of adults with detectable *M. pneumoniae* DNA could not be ascertained. However, *M. pneumoniae*-positive laboratory reports collated from regional laboratories were received on adult patients during this period and the age profile was consistent with that of all reports received from 1975 to 2012.

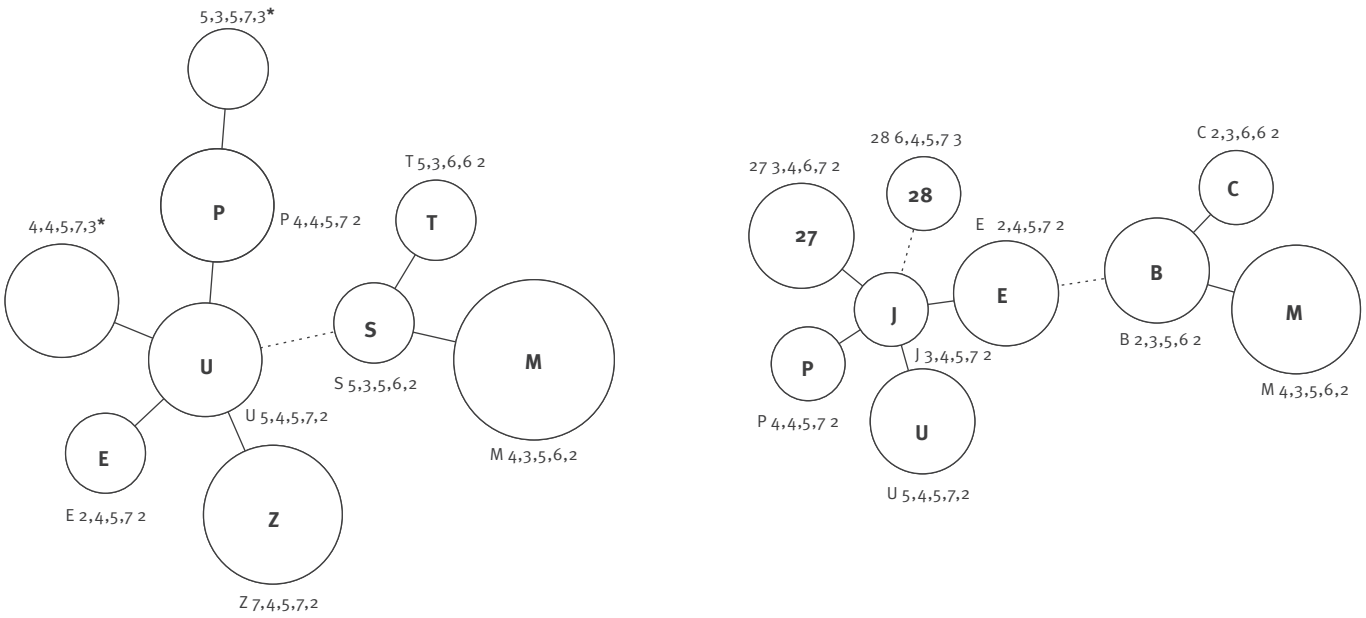
The last period showing a large peak of detectable *M. pneumoniae* infection by qPCR was winter 2005/06, in which the infection was detected in 6% of 5–14 year-olds attending general practitioners with respiratory signs. In the study period reported here (winter 2011/12), an even greater number of children of this age group were infected (14.3%), indicating at least one in seven children with respiratory signs attending general practitioners were infected with *M. pneumoniae*.

In a similar period in 2010/11 (week 42 2010 to week 3 2011), 11 differing MLVA types were detected in 15 clinical samples with MLVA-M being the most prevalent in England and Wales [7]. Within the study period reported here (week 42 2011 to week 3 2012), seven MLVA types were identified, four of which were MLVA-M. The sample number is too low to specify the exact diversity of the population or to investigate the association of particular types with clinical severity. Nonetheless, it is interesting that clonal strains were not detected. Two putative new profiles were obtained but confirmation of these apparently novel MLVA types will require isolation of the strains.

The typing method used here was originally described by DéGrange et al., in which stability of five isolates was determined over 10 passages, indicating that the *M. pneumoniae* MLVA type is relatively stable [11]. Clonal spread of *M. pneumoniae* does occur, however. In fact, Pereyre et al., recently described the detection of *M. pneumoniae* MLVA-type 3,4,5,7,2 in seven children attending a primary school in France [14]. In our study, patients were from a variety of locations in England and Wales and, similar to our findings last year [5], the data do not support the hypothesis that

Figure 2

Minimum spanning trees for *Mycoplasma pneumoniae* MLVA types detected in England and Wales, October–January 2011/12 (n=17) and 2010/11 (n=16)



MLVA: Multiocus variable number tandem repeat analysis.

Trees were derived from the five MLVA alleles [11]. Each circle represents a unique MLVA type. The size of each circle illustrates the proportion of isolates with that MLVA type (the smallest circle in each tree represents one isolate). Solid lines separate single locus variants and dotted lines separate double locus variants. The asterisks mark the two putative novel MLVA types.

a single strain type of *M. pneumoniae* was responsible for this observed increase in infection in England and Wales. MLVA typing discriminates well between *M. pneumoniae*-positive specimens. In fact, there is a high diversity of types in the population and it does not appear that a few clonal types dominate in circulation. It would be of value to have a consistent typing methodology for *M. pneumoniae* strains in use internationally, with a database of types similar to those for other bacterial species. It would also be interesting to type strains from other countries during the same time period to determine how strains differ geographically during periods of increased infection.

Macrolide resistance is becoming an increasing problem in other countries [15]; despite the low sample number, no resistance was detected in any of the qPCR-positive samples from England and Wales analysed during the study period.

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