Title: *Mycoplasma pneumoniae* infections across Europe and Israel (2011-2016).

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Abstract

Background

Mycoplasma pneumoniae is a leading cause of community acquired pneumonia with large epidemics occurring every 4 to 7 years. Infections are predominantly seen among paediatric populations.

Aim

To determine the diagnostic methods used to detect *M. pneumoniae*, the seasonality of detections, identification of epidemics, macrolide resistance data availability, patient age demographics for positive detections and the effect of geographical location on timing of epidemics.

Methods

A retrospective questionnaire was sent out to 18 countries across Europe and Israel requesting details on the number of *M. pneumoniae* positive samples from January 2011 to April 2016. Information requested included: methods of detection, date of detection, availability of macrolide resistance data and number of detections stratified by age group. The Moving Epidemic Method was used to determine epidemic periods across the countries for the five periods under investigation and effect of country latitude

Results

12/18 countries supplied data on *M. pneumoniae* infections accounting for 95,666 positive samples. Few laboratories have initiated routine macrolide resistance testing since 2013. Between 2011 – 2016 three epidemics were identified during 2011/12, 2014/15 and 2015/16. Three patterns emerged from the distribution of patient ages for *M. pneumoniae* positive samples. During epidemic years an association between country latitude and week number in which epidemic periods began was noted.

Conclusions

This study represents the largest multi-national epidemiological analysis of *M. pneumoniae* data. Association between epidemics and latitude was observed. Differences were noted in age distribution of positive cases, the methods used for detection and the lack of macrolide resistance monitoring.

Background

Mycoplasma pneumoniae is a major cause of respiratory infection in humans with macrolide antibiotics, such as azithromycin, used as the first-line treatment in many countries. The bacterium is transmitted from person to-person by respiratory droplets with the incubation period ranging from 4 days to 3 weeks (1). Prudent use of antibiotics has been urged for all cases of *M. pneumoniae* infection because of worldwide reports of macrolide resistance which have been reported as ranging from 0.2 % in Sweden to greater than 90% in China (2-5). *M. pneumoniae* infections show seasonal variation. In temperate climates the number of infections peak during the latter months of the years with epidemic periods on average every 4 to 7 years (6-8). The most recent survey in 2012 by Lenglet *et al.* indicated that some countries in the European Union and European Economic Area experienced an increase in *M. pneumoniae* cases in 2011 whereas others did not, indicating a universal geographic increase had not occurred (5). Little is understood about transmission of *M. pneumoniae* within populations and several factors have been postulated to account for dynamics including immunity level of the population, the bacterial population based on the P1 adhesin type, age and increased mixing of children in academic and nursery settings.

Testing methodologies include nucleic acid amplification test (NAAT), serology and culture with varying sensitivities and specificities. There is no international standard material for quality control detection in assays, although external quality control schema exist for some methodologies (NAAT). There are no internationally defined guidelines on the requirements for surveillance of *M. pneumoniae*, macrolide resistance testing and surveillance, reference system structure, routine testing and bacterial strain discrimination. However, a few countries such as France and the USA have introduced surveillance for specific regions and national surveillance is seen in countries such as Denmark and Japan which have maintained an active surveillance system for this pathogen for some time (9). Due Therefore, the laboratory confirmed cases and surveillance data regarding the number of cases and the reported cases of macrolide resistance are likely to be underestimates. This is further confounded due to both the challenge in diagnosis and the fact that an undefined proportion of patients will have mild disease or may be carriers within community settings, without active testing to confirm the infection. Further underestimation is likely to occur from patients receiving empirical treatment without diagnostics to confirm infection.

In response to an increase in infection seen in several countries in 2016, the ESCMID Study Group for Mycoplasma Infections (ESGMI), which is now recognised as the ESCMID Study Group for Mycoplasma and Chlamydia Infections (ESGMAC), established this study (10, 11). The purpose of the study was to determine case number per age group, concurrence of epidemic and annual peaks across geographies,

to gain a greater understanding of testing and surveillance systems in use for *M. pneumoniae* and to establish the extent of macrolide resistance testing, whilst establishing if macrolide resistance testing was in place. ESGMI conducted an email based survey of ESGMI members in countries in Europe and Israel to describe the existing laboratory confirmed case data for *M. pneumoniae* infection by age group from 03-01-2011 to 24-04-2016. Of the 18 countries that participated in the survey, 12 reported data for case numbers of *M. pneumoniae.*

Methods

Study type, data collection and analysis

A retrospective email survey based study was undertaken of laboratory confirmed documented detections of *M. pneumoniae* from national laboratory and surveillance institutions or, if not available, other regional laboratory and surveillance institutions. Countries invited to participate in the study were either active members of ESGMI or authors listed in the previous study by Lenglet *et al* (5). Participants were invited to join the study and provide the number of detections confirmed by nucleic acid amplification test (NAAT), serology, culture and total overall between weeks commencing 03-01-2011 to 24-04-2016 (positive results and if available negative results were also collated).

Additional information was requested including whether countries were actively monitoring macrolide resistance. Data from each participating country was collated and aggregated to give total number of detections per age group and 4 weekly moving averages of detections per country and overall where possible. We did not request data on the gender of patient from which the detections were made. Total weekly data were further subcategorised by age group (0-4y, 5-9y, 10-14y, 15-24y, 25-44y, 45-64y, 65+ or unknown).

Case definition

Cases of *M. pneumoniae* infection were defined by local practice. Due to local variation this study collated information on *M. pneumoniae* detections, not cases.

De-duplication and exclusion criteria.

Due to the heterogeneous nature of *M. pneumoniae* data collection from each country specific study-wide de-duplication criteria would not have been feasible; therefore, participants were asked to detail if data with duplicate samples from the same patient (eg. with NAAT and serology) was included as a single category and if possible to include as serology. Specific exclusion criteria were also not set for similar reasons stated above. Responses for de-duplication and exclusion criteria are listed in supplementary data (S1).

Characteristics of epidemics between January 2011 to April 2016.

To determine the characteristic properties of epidemics across the 12 countries which provided data on *M. pneumoniae* positivity the Moving Epidemic Method (MEM) was used. (12) An epidemic slope threshold of 2% was chosen and used to determine the pre-epidemic period, epidemic period and post-epidemic period for the 5 periods which coincide with annual peaks spanning week 19 of the first

year through to week 18 of the following year. These data were used to calculate the duration of epidemic for each country as well as the percentage of positive samples which were identified within this period. Data generated from the MEM model, such as week number in which epidemics began, was used to correlate the onset of epidemics with the geographical location of each country. Statistical analysis for generating p-values and calculation of r^2 were performed with GraphPad Prism 5.

Ethics statement

This study involved collation of surveillance data for epidemiological analysis and all data was retrospective and analysed for public health interest. Ethical approval was not required and no patient identifiers were included in the study.

Results

Data availability and diagnostic methods used for detection of *M. pneumoniae* across Europe and Israel

In the period between 3rd January 2011 and 24th April 2016 a total of 95,666 detections of *M. pneumoniae* were confirmed from 12 participating countries across Europe and Israel. Of the 18 countries approached, 12 provided information regarding *M. pneumoniae*. The method of *M. pneumoniae* detection varied between the 12 countries (Table 1). Two countries (Denmark and Israel) reported exclusively NAAT use, two countries (Greece and Ireland) reported serology exclusively, five countries (Great Britain, Germany, Hungary, Norway and Sweden) used a combination of NAAT and serology, one country (Slovenia) used NAAT in combination with culture, two countries (Belgium and France) used all three methods. No country relied on culture alone (Table 1).

Table 1. Availability of data for *M. pneumoniae* infections across Europe and Israel between 2011-2016

Country	Nucleic acid amplification test		Culture		Serology		Total number of positive samples	Total number of negative samples	Percentage of positive samples	Macrolide resistance monitoring
	Performed	Number of positive detections	Performed	Number of positive detections	Performed	Number of positive detections				
Belgium	Yes	894	Yes	49	Yes	12,047	21,094*	No data available	N/A	Only monitored when samples test positive at the National Reference Centre. No testing in Sentinel Laboratories
Denmark	Yes	20,081	No	N/A	No	N/A	20,081	264,770	7.0 %	No routine surveillance system in place. From 2010/11, 2011/12 and 2015/16 periods 809 samples were examined identifying 13 macrolide resistance associated mutations (1.5%). Samples are investigated on request form physicians
France	Yes	92	Yes	53**	Yes	298	390	7,463	5.0 %	Performed on all clinical specimens detected as *M. pneumoniae*-positive by NAAT since 2013 [9]
Germany	Yes	127	No	N/A	Yes	316	443	10,143	4.2 %	No comment
Great Britain	Yes	385	No	N/A	Yes	5,263	5,648	No data available	N/A	No national system All positives samples referred to PHE are tested.
Greece	No	N/A	No	N/A	Yes	140	140	1,498	8.5 %	No comment
Hungary	Yes	17	No	N/A	Yes	1,117	1,134	6,109	15.7 %	No comment
Ireland	No	N/A	No	N/A	Yes	535	535	2,853	15.8 %	No comment
Israel	Yes	848	No	N/A	No	N/A	848	5,309	13.8 %	No active monitoring
Norway	Yes	13,980	No	N/A	Yes	10,678	24,658	No data available	N/A	No comment
Slovenia	Yes	1,172	Yes	827	No	N/A	1,172	8,872	11.7 %	Only on request from physician
Sweden	Yes	9,499	No	N/A	Yes	10,024	19,523	169,501	10.3 %	No active monitoring
Sum of columns	N/A	47,095	N/A	876	N/A	40,418	95,666	476,518	N/A	

*Method of detection not known for sentinel laboratories ** Not included in the overall total for the purpose of deduplication. N/A = not applicable. No data was provided from Cyprus, Malta, Netherlands, Poland, Slovakia or Spain.

The greatest number of positive samples were reported using NAAT (47,095, 49%) followed by serology (40,418, 42%). Only 876 (1%) samples were reported positive by culture and 7,277 (8%) of tests were not specified. Norway contributed the greatest number of *M. pneumoniae* positive detections to the total figure (24,658, 26%) and Greece the lowest (140, 0.1%). De-duplication data was determined at a country level (S1).

Macrolide resistance data availability

With regards to active monitoring of macrolide resistance, five countries did not comment, Belgium noted that monitoring was only carried out on positive samples identified in the National Reference Laboratory, but not within sentinel laboratories, Slovenia noted that macrolide resistance determination was carried out only on request by the physician, Denmark investigated NAAT positive samples from three recent *M. pneumoniae* epidemic periods and found low level (1.5%) of macrolide resistance, and samples are investigated on request from physicians, France initiated a systematic monitoring on all NAAT-positive clinical specimens in 2013 using an in-house published method (13). In 2017, England and Wales introduced monitoring of all positive NAAT samples referred to the national reference laboratory. Two countries stated that no monitoring for resistance was in place (Table 1).

Total number of detections and seasonality

The distribution and seasonality of the 95,666 detections from the twelve countries across the study period was determined. To account for weekly bias in reporting, data was converted to four weekly moving averages. The greatest number of positive samples from the 4 weekly moving averages data was identified as 1758.5 positive detections during week 48 of 2011.

Detection of *M. pneumoniae* by NAAT (Figure 1b) correlated with the overall detections seen in Figure 1a. Detection of *M. pneumoniae* by culture gave the lowest number of positive samples accounting for 1% of the total positive samples. Detection using serology was the second most common method for detecting *M. pneumoniae* positive patients (Figure 1c). The 4 week rolling average for detection by culture (Figure 1d) was less than five positive samples for all reporting weeks with exception of Slovenia in the 2015 season where a maximum average of 51.8 positive samples were identified.

Identification of epidemic periods using the Moving Epidemic Model.

Analysis of detections during the annual periods was carried out using the Moving Epidemic Model (Figure 2). For the five annual periods described we noted 35747; 11089; 8510; 15312; 19439 detections for the periods 2011/12, 2012/13, 2013/14, 2014/15 and 2015/16, respectively. Three epidemics were detected between 2011/12, 2014/15, and 2015/16 in which 67%, 59% and 68% of each period's detections were identified during the calculated epidemic period, respectively. Epidemics had longer duration (19, 21 and 23 weeks, respectively) compared to numbers observed during annual seasonal peaks of infection (non-epidemic periods) 2012/13 (13 weeks with 30% of total detections) and 2013/2014 (15 weeks with 35% of total detections). For countries which provided the total number of negative samples the percentage of positive samples identified during the pre-epidemic period, epidemic period and post-epidemic was calculated for the epidemic periods of 2011/12, 2014/15 and 2015/16 (Table 2). In all cases there was a greater percentage of positive samples during the epidemic period than in the pre-epidemic period.

Table 2. Proportion of total samples positive for *M. pneumoniae* during the pre-epidemic, epidemic and post-epidemic periods for the three epidemic years of 2011/12, 2014/15 and 2015/16 for nine countries reporting both positive and negative sample data.

	2011/12					2014/15					2015/16				
	Pre-epidemic	Epidemic	Post-epidemic	Total across all periods	Detection per peak week during epidemic period	Pre-epidemic	Epidemic	Post-epidemic	Total across all periods	Detection per peak week during epidemic period	Pre-epidemic	Epidemic	Post-epidemic	Total across all periods	Detection per peak week during epidemic period
Denmark	920/7784 (12%)	6932/47522 (15%)	804/15760 (5%)	8656/71066 (12%)	185/879 (21%)	291/12171 (2%)	1206/23252 (5%)	204/7876 (3%)	1701/43299 (4%)	90/1238 (7%)	1120/18340 (6%)	5751/52827 (11%)	712/16139 (4%)	7583/87306 (9%)	546/3457 (16%)
France	71/862 (8%)	41/410 (10%)	27/583 (5%)	139/1855 (7%)	7/37 (19%)	32/786 (4%)	17/208 (8%)	19/381 (5%)	68/1375 (5%)	6/44 (14%)	28/690 (4%)	8/86 (9%)	22/506 (4%)	58/1282 (5%)	4/29 (14%)
Germany	38/833 (5%)	33/360 (9%)	37/669 (6%)	108/1862 (6%)	7/44 (16%)	8/447 (2%)	77/1373 (6%)	4/248 (2%)	89/2068 (4%)	7/39 (18%)	22/917 (2%)	13/293 (4%)	19/1639 (1%)	54/2849 (2%)	4/46 (9%)
Greece	N/A	18/110 (16%)	16/224 (7%)	34/334 (10%)	5/8 (63%)	N/A	9/27 (33%)	28/304 (9%)	37/331 (11%)	3/6 (50%)	0/15 (0%)	2/11 (18%)	6/179 (3%)	8/205 (2%)	2/3 (67%)
Hungary	15/154 (10%)	32/169 (19%)	105/942 (11%)	152/1265 (12%)	7/20 (35%)	75/442 (17%)	186/760 (24%)	54/367 (15%)	315/1569 (20%)	5/12 (42%)	96/570 (17%)	50/220 (23%)	59/544 (11%)	205/1334 (15%)	10/30 (33%)
Ireland	53/290 (18%)	77/261 (30%)	15/78 (19%)	145/629 (23%)	3/6 (50%)	28/263 (11%)	31/153 (20%)	17/144 (12%)	76/560 (14%)	4/10 (40%)	66/374 (18%)	39/153 (25%)	17/73 (23%)	122/600 (20%)	8/12 (67%)
Israel	84/572 (15%)	81/346 (23%)	N/A	165/918 (18%)	19/55 (35%)	57/499 (11%)	87/597 (15%)	N/A	144/1096 (13%)	5/17 (29%)	106/1052 (10%)	82/687 (12%)	3/36 (8%)	191/1775 (11%)	11/45 (24%)
Slovenia	32/389 (8%)	31/349 (9%)	12/413 (3%)	75/1151 (7%)	4/18 (22%)	166/775 (21%)	583/1866 (31%)	83/1037 (8%)	832/3678 (23%)	38/78 (49%)	N/A	29/469 (6%)	28/1447 (2%)	57/1916 (3%)	5/27 (19%)
Sweden	1389/7389 (19%)	5356/28226 (19%)	859/10034 (9%)	7604/45649 (17%)	186/686 (27%)	863/10734 (8%)	1447/17242 (8%)	501/8729 (6%)	2811/36705 (8%)	100/950 (11%)	500/7955 (6%)	1356/12414 (11%)	967/14066 (7%)	2823/34435 (8%)	98/731 (13%)
Total	2602/18273 (14%)	12601/77753 (16%)	1875/28703 (7%)	17078/124729 (14%)	423/1753 (24%)	1520/26117 (6%)	3643/45478 (8%)	910/19086 (5%)	6073/90681 (7%)	258/2394 (11%)	1938/29913 (6%)	7330/67160 (13%)	1833/34629 (5%)	11101/1317 02 (8%)	688/4380 (16%)

N/A = not applicable

Distribution of *M. pneumoniae* positivity by age

The distribution of *M. pneumoniae* positivity by age group was determined for each of the 12 participating countries (Figure 3). Even distribution across all age groups was not noted in the data from any country. Country specific age data revealed three distinct patterns. Four countries (Germany, Greece, Ireland and Slovenia) showed skewing of positive samples to younger patients (<10 y old), whereas two countries (Hungary and Sweden) showed skewing towards older patients (>25 y old). Five countries reported a bimodal distribution (Belgium, Denmark, Great Britain, France, and Israel). Data obtained from Norway was not sub-categorised by age. Data was not available from Ireland for ages 25 and above.

Correlation between latitude and onset of epidemic period.

When examining the epidemic period of 2011/12, 2014/15 and 2015/16, a clear association between the country latitude and beginning of the national epidemic period was observed (Figure 4). This was statistically significant for 2011/12 period ($p = <0.005$, $r^2 = 0.92$) and 2014/15 ($p = < 0.005$, $r^2 = 0.84$, but significance was not achieved during the 2015/16 period ($p = 0.1$, $r^2 = 0.38$). Furthermore this association was most apparent during the major epidemic period of 2011/12 in which the epidemic period was first noted in Norway (60.4^{o}N) during epidemic week 22 (calendar week 40 of 2011) and was then observed to start in Israel (31^{o}N) during epidemic week 43 (calendar week 9 of 2012). There was a lack of an association during the non-epidemic periods of 2012/13 and 2013/14 (data not shown).

Discussion

This study represents the largest data set to date of *M. pneumoniae* positive samples and associated data for which the method of *M. pneumoniae* detection in each country was determined. NAATs were the most common method used among the twelve countries. Although a variety of commercial and in-house methodologies were used in the detection of *M. pneumoniae*, a recent study of thirteen assays used across Europe, Israel and USA demonstrated comparable levels of detection of 20 *M. pneumoniae* genomes per reaction (14). Serological methods were also commonly used in the detection of *M. pneumoniae* infections. The presence of an IgM response may have the advantage to suggest recent acquisition of infection, but may be an unreliable marker due to documented long term persistence of antibodies (15). Culture dependent methods were used by three countries. Culture has the benefit of confirming the presence of viable *M. pneumoniae* and may permit phenotypic antimicrobial susceptibility testing, but due to considerable time required for growth (up to 4 weeks) does not provide results within a clinically relevant time period. Currently no single method is recommended for routine detection of *M. pneumoniae* and international guidelines or control materials do not exist. Real-time PCR and serology have previously shown agreement in ability to detect *M. pneumoniae* among samples (90% agreement) with only 7% of patients being PCR positive, with limited evidence of seroconversion (16). No single test can reliably detect all infections. Therefore a combined approach utilising both NAAT and serology may help to identify any potential false-negative results (17).

In addition to method of detection, we sought to identify if surveillance for macrolide resistance was routinely undertaken. Due to intrinsic resistance to many antibiotics, including all cell wall inhibitors, macrolide antibiotics, such as azithromycin and clarithromycin are the drug of choice or in cases of suspected infection among the immunocompromised, the bactericidal fluoroquinolones may be considered. Tetracyclines are also used in adults for treating possible macrolide-resistant *M. pneumoniae* infections. Routine macrolide resistance monitoring was not systematically in place. This may attribute to the under detection of resistance, or reflect the low levels of macrolide resistance reported across Europe (18-20), although high levels of resistance have been noted in areas such as Israel (30%) (21). However, macrolide resistance among *M. pneumoniae* appears to be endemic within countries such as China with studies routinely reporting macrolide resistance levels around 90 – 100% (22, 23). This high incidence of macrolide resistance adds weight to need for co-ordinated surveillance across Europe, increasing clinical knowledge of macrolide resistance rates and acquisition, as well as an understanding of the patient's recent travel history when considering therapy. The authors in this paper, including several international experts in *M. pneumoniae* recommend co-ordinated international surveillance for macrolide resistance, as this is the current treatment of choice in Europe.

Regardless of methodology used for detection of *M. pneumoniae*, clear seasonal trends were apparent between January 2011 and April 2016 with the peaks in infection occurring between the fourth quarter and first quarter of the following year. To calculate the epidemic period for each season the Moving Epidemic Method, as described by Vega *et al.*, was used (12). Three clear epidemics were noted in 2011/12, 2014/15 and 2015/16. *M. pneumoniae* epidemics have been suggested to occur every 4 to 7 years and in some cases lasting for longer than one annual season (8, 24). However, in this study with the largest data set so far available it was found that the interval between epidemic occurrence was 3 years from 2011/12 and 1 year to 2015/16. This latter epidemic period may reflect a secondary peak of cases within a 2 year epidemic span as previous epidemics have been shown to persist for some time (5, 24). Confirmation of circulating genotypes of *M. pneumoniae* from large geographical areas would be of interest to determine the microbiological nature of strains within these and epidemic periods.

For the major epidemic periods, we sought to determine any changes between the pre-epidemic, epidemic and post-epidemic periods in the reported number of detections in countries reporting both positive and negative data. The greatest rise was seen in Ireland in which the 18% of samples positive for *M. pneumoniae* in the pre-epidemic period, rose by 12% percent to 30% during the epidemic period. In a number of countries, such as Denmark, France, Slovenia and Sweden the prevalence in the post-epidemic period was lower than that of the pre-epidemic period. This lower prevalence in the post-epidemic period may reflect over-sampling as a bias as a result of higher prevalence during the epidemic. This may also reflect an increase in the population burden of infection prior to an epidemic. Additional analysis is required to understand if this is the case and if monitoring of levels can be used to predict imminent epidemics.

A curious observation was the pattern of positive detections stratified by age. *M. pneumoniae* cases are classically seen among children of 5 – 14 years with those under five experiencing milder disease (17). This trend was seen with countries such as Germany, Greece and Slovenia which show a skew to the younger age groups. For Greece this can be attributed to the acquisition of samples from a tertiary children's hospital in Athens. It should be noted that the skew to younger ages groups seen in Ireland may reflect the nature of only investigating patients up to the age of 25 years. The second pattern observed demonstrated a peak in infection among the 5-14 years age group, with an increase in the 25 – 44 years age group therefore giving a bimodal distribution. Finally a skew to the older age groups was seen in Hungary and Sweden. This observation is not likely to be an artefact of testing methodology in which the elderly may be more likely to have existing IgM levels, as both countries do not solely rely on serology. The reason for this increased detection in the elderly in two countries is

unknown and may simply reflect differences in local testing guidelines and routine practice for respiratory screening, or merely reflect age-based screening practice such as the fact that the majority of Hungarian samples were derived from an elder population.

The final analysis undertaken examined the association between the start of epidemic periods, as calculated by the MEM model and the geographical location of the country as determined by latitude. Geographical location is not thought to be of importance in progression of *M. pneumoniae* infection, but the data presented here examining the start of epidemic periods across 12 countries suggests that more Northerly countries experience the start of epidemic periods earlier than those in the South. This association was most clear during the 2011/12 epidemic period, but held true for both subsequent epidemic periods of 2014/15 and 2015/16. Previous national based studies have shown epidemics to be polyclonal in nature (25-30). Establishment of whether the microbiological nature of the epidemics across Europe are clonal or not may be beneficial and influence future sentinel surveillance design. The impact of climatic factors on *M. pneumoniae* infections has yet to be infected.

Limitations

A number of limitations are apparent; firstly, due to the variable reporting methods of each country specific case definitions were not considered and de-duplication methodologies were not imposed and were set at country specific level. Overall reported testing activity or testing-incidence was very different between countries, and conclusions on analysis across countries must be considered with caution. Some countries, such as Ireland, did not provide a complete data set due to only investigating *M. pneumoniae* in patients who were 24 years and under. Therefore the true number of positive individuals from this population is likely to be under estimated. Secondly, the data from some countries may not be fully representative of the whole nation. For example data from Germany and France was obtained from a single region within each country and therefore may not be representative of national coverage. Thirdly, the data examining the distribution of detections stratified by age group, should be interpreted with a level of caution. The age categories did not contain equal weighting of age groups, for example there were less age groups encompassed in the 0 – 4 years compared with older age groups such as 25 – 44 years group. Finally, this data did not take into account the subtypes of *M. pneumoniae* which have been described.

Possible clinical impact arising from this study

The comparative nature of this study has highlighted a number of interesting points with regards to trends in testing as well as epidemiology of *M. pneumoniae* infections. Firstly countries may be under

detecting cases due to limitation in age groups examined for the infection. The observation of a number of countries showing a skew towards older patients as well as bimodal distribution suggests that investigations for *M. pneumoniae,* although of significance in young children, should not be restrictive and that consistent testing guidelines are required. It would be beneficial to have an agreed international case definition for infection with *M. pneumoniae.*

This study also highlights the substantial lack of antimicrobial resistance testing and surveillance of *M. pneumoniae* and the potential reduced evidence base on resistance to guide therapy. Without active coordinated monitoring it will not be possible to track changes in resistance profiles and the emergence of high-level macrolide resistant clones. There is an absence of a structured European level surveillance and resistance monitoring for this infection, despite the extremely high levels of resistance in some global areas.

Finally the curious observation relating to association between Northern latitudes and earlier week initiation of epidemics may suggest the need for another more focussed study. It would also be interesting to assess the potential value of a rapid, real-time reporting system of *M. pneumoniae* infections across Europe. Such a system may aid in future epidemiological studies, resistance monitoring, and help to predict the *M. pneumoniae* epidemic season throughout the continent.

Conclusion

This study represents the largest collection of *M. pneumoniae* infection data to date. There is currently no standardised method for the detection of *M. pneumoniae* infection among patients and macrolide resistance screening is sporadic despite high levels in some areas globally. A wave of epidemics from the more northerly latitudes through to the south occurs during epidemic years, and the reason for this is not known. There is a need for testing guidelines and standardised international control material. The potential value of a co-ordinated international surveillance and macrolide resistance monitoring system needs to be further addressed.

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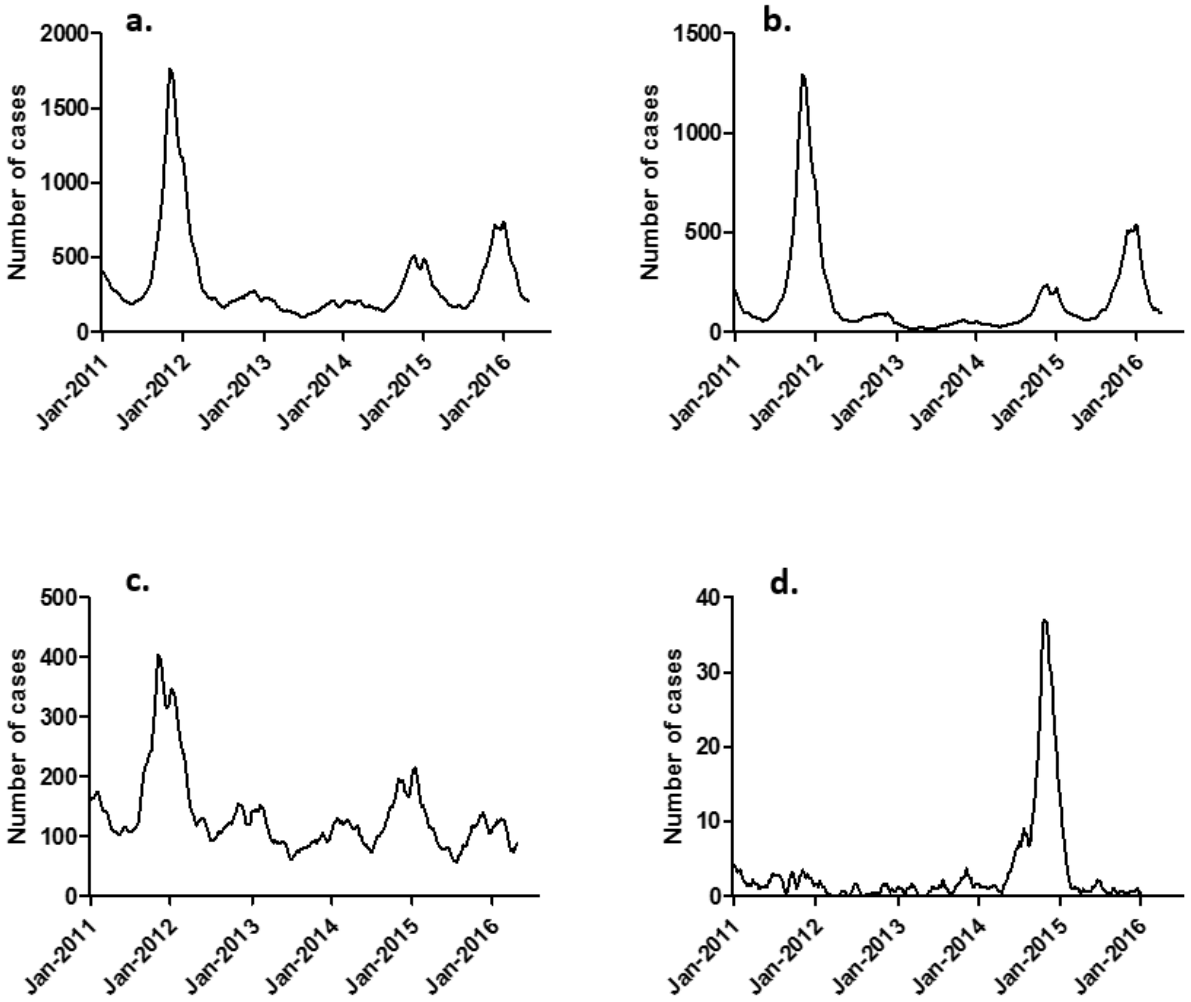

Figure 1. Four weekly moving average data on *M. pneumoniae* infections across participating countries in Europe and Israel between January 2011 to March 2016. (a) Total number of cases from 12 participating countries, (b) confirmations determined by NAAT from ten countries, (c) serology from nine countries and (d) culture from two countries.

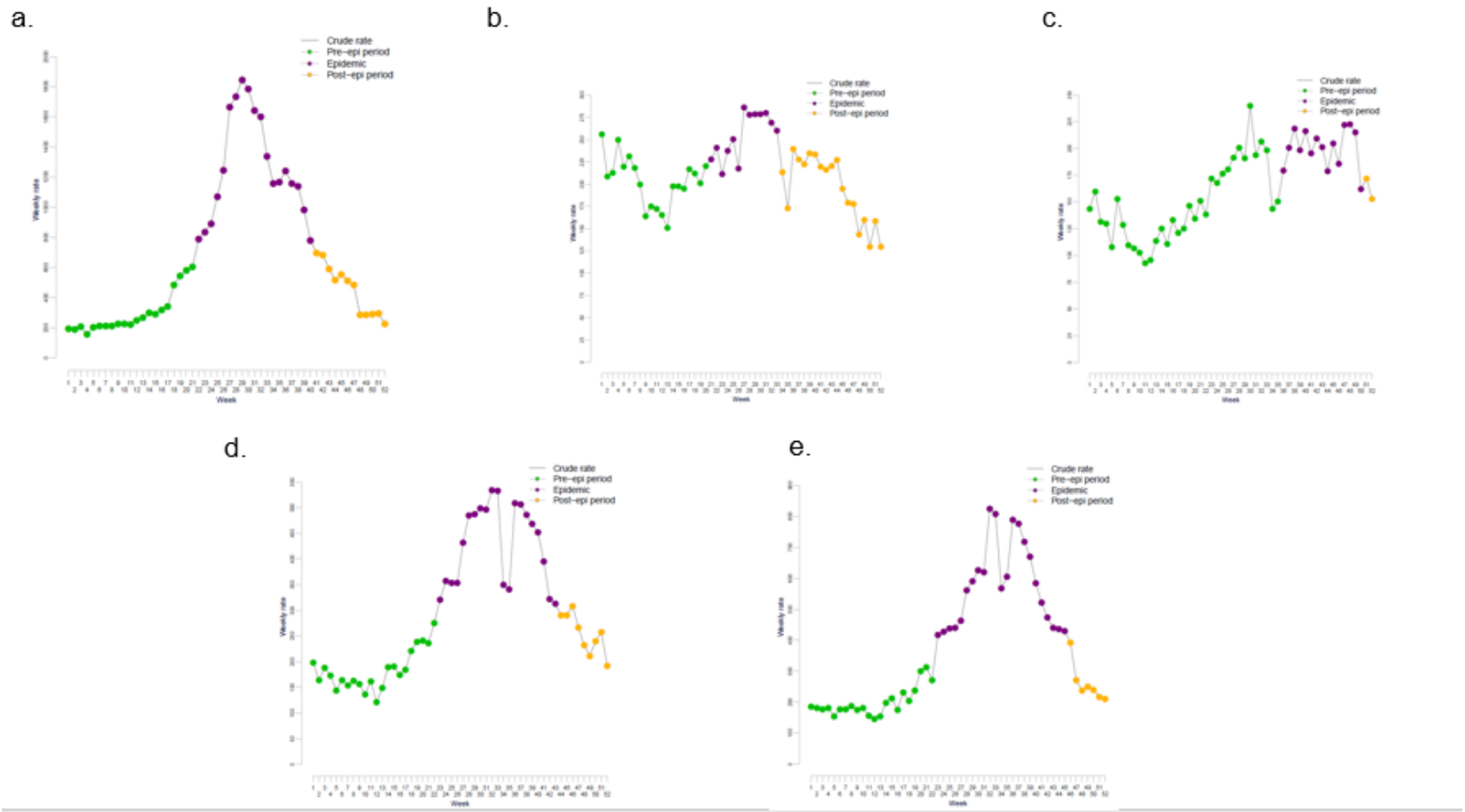

Figure 2. Analysis of *M. pneumoniae* in Europe and Israel between 2011 and 2016 using the Moving Epidemic Method (MEM). Week numbers represent epidemic week period (week 1 represents calendar week 19). Green dots represent pre-epidemic period, purple dots represent epidemic period and yellow dots represent post-epidemic period as calculated by the MEM. (a) week 19 2011 – week 18 2012 – identified as an epidemic year, (b) week 19 2012 – week 18 2013, (c) week 19 2013 – week 18 2014, (d) week 19 2014 – week 18 2015 – identified as an epidemic year and (e) week 19 – week 17 2016 – identified as an epidemic year.

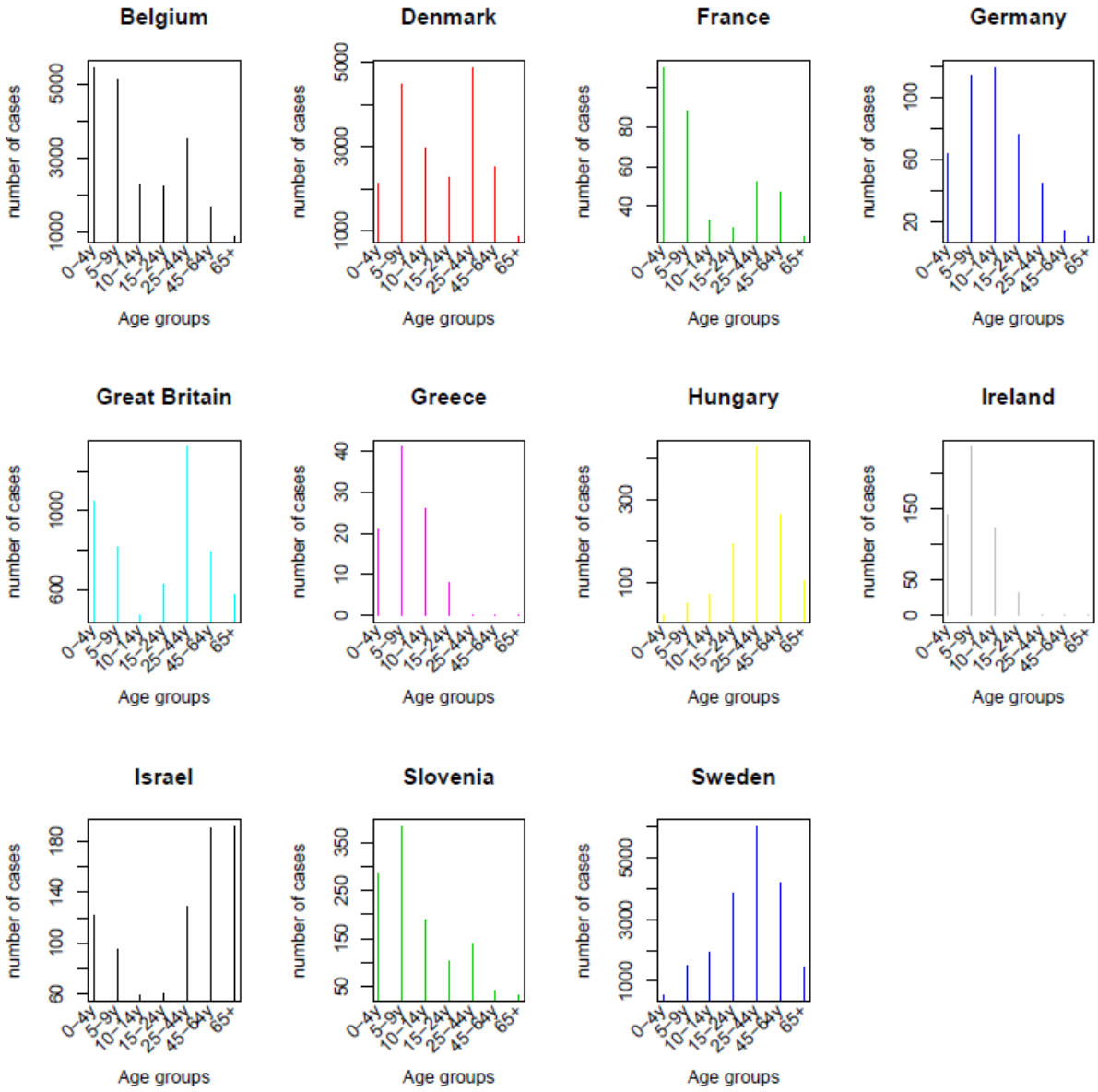

Figure 3. Number of *M. pneumoniae* cases per age group across eleven European countries and Israel. Age specific infection data was not available for Norway. Ireland did not test for *M. pneumoniae* in patients 25 years and older

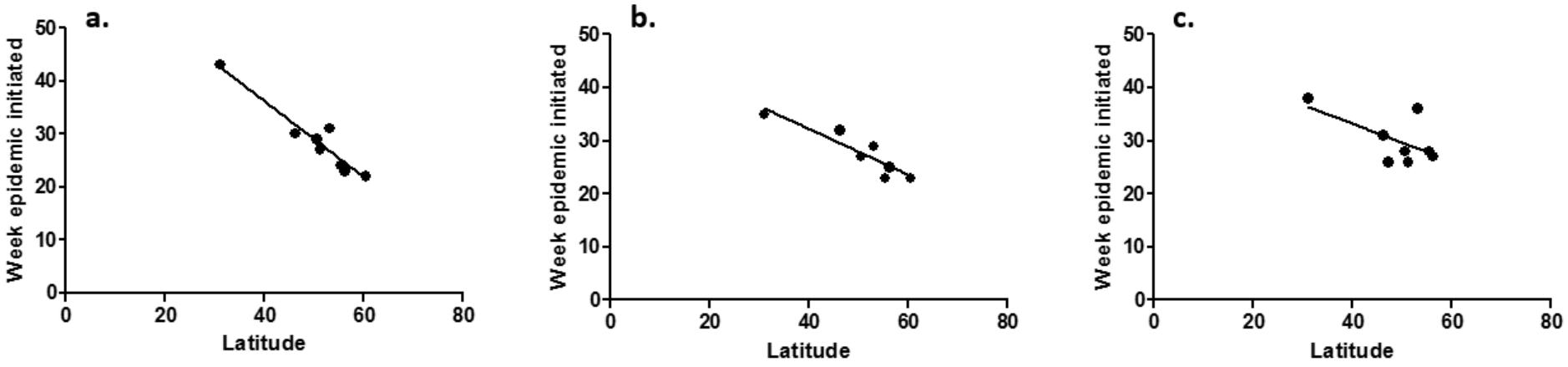

Figure 4. Correlation between country latitude and epidemic week number for three epidemic periods of (a) 2011/12, (b) 2014/15 and (c) 2015/16. Countries in which epidemic periods began prior to that predicted by the Moving Epidemic Method model were removed from the analysis. A statistically significant association between the week in which the country epidemic began and epidemic week number was seen in 2011/12 ($p = < 0.005$ $r^2 = 0.92$) and 2014/15 ($p = < 0.005$. $r^2 = 0.84$). No significant association was seen in 2015/16 ($p = 0.1$. $r^2 = 0.38$).

S1 – Exclusion and de-duplication criteria by country. This supplementary material is hosted by *Eurosurveillance* as supporting information alongside the article *'Mycoplasma pneumoniae* infections across Europe and Israel (2011 – 2016)' on behalf of the authors who remain responsible for the accuracy and appropriateness of the content. The same standards for ethics, copyright, attributions and permissions as for the article apply. *Eurosurveillance* is not responsible for the maintenance of any links or email addresses provided therein

Country	De-duplication	Exclusion
Belgium	Duplicate positive tests from 1 patient in a 90-day time window were grouped and appear in the dataset as 1 positive test.	The data of the sentinel network of microbiological laboratories include "Mycoplasma-like illness" syndromes cases confirmed by one of the following criteria: Detection of nucleic acid in a nasopharyngeal or oropharyngeal swab or in other deep respiratory specimens Detection of seroconversion of IgG or significant increase in acute and convalescent serum sample. Detection of *M. pneumoniae*-specific IgM antibodies.
Cyprus	Data not provided	Data not provided
Denmark	At individual level only the first mycoplasma positive test result within a season is included. If several negative mycoplasma tests are available per individual, only one negative mycoplasma test is included/counted per week. The first time an individual test positive for mycoplasma all subsequent negative and positive mycoplasma tests are excluded	At individual level only the first mycoplasma positive test result within a season is included. If several negative mycoplasma tests are available per individual, only one negative mycoplasma test is included/counted per week. The first time an individual test positive for mycoplasma all subsequent negative and positive mycoplasma tests are excluded
France	Duplicate samples from the same patient (eg. with NAAT and serology) was included as a single category : Included as serology	None given
Germany	None given	None given
Great Britain	Duplicate data from same patient only included if sample date >1 year between serology samples or >3 months between NAAT samples	Serology test results from samples other than blood, serum or plasma were excluded. Includes NAAT methods (coded on surveillance database as genomic/PCR/LCR detection) on respiratory samples or blood, serum or plasma only. All other methods and specimen types (including unknown) excluded.
Greece	None given	None given

Hungary	None given	None given
Ireland	None given	None given
Israel	Only NAAT	None
Malta	Data not provided	Data not provided
Netherlands	Data not provided	Data not provided
Norway	None given	None given
Poland	Data not provided	Data not provided
Slovakia	Data not provided	Data not provided
Slovenia	Duplicate samples from the same patient was included as a single case	Serology excluded
Spain	Data not provided	Data not provided
Sweden	Duplicate samples from the same patient has not been excluded.	Four of the laboratories could only deliver data from 2012 (three labs) and 2013 (one lab) and onward.