Clinical outcomes and macrolide resistance in *Mycoplasma pneumoniae* infection in Scotland, UK

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*Mycoplasma pneumoniae* has a cyclical, epidemic pattern of infection and the most recent epidemic occurred in Europe in 2011. Macrolides are recommended for the treatment of *M. pneumoniae* respiratory tract infection, but macrolide resistance has been reported at low levels in Europe. The aim of the study was to examine the clinical impact of the recent *M. pneumoniae* epidemic in a hospital setting in Scotland and to determine whether macrolide-resistant strains are present. Data were analysed retrospectively for 307 patients with *M. pneumoniae* respiratory infection diagnosed in 2010 and 2011 in Edinburgh, UK. Genotypic macrolide resistance testing was also carried out in 32 patients in whom resistance was considered most likely, based on their clinical picture. We found that 175 patients (59 %) were admitted to hospital, 20 (7 %) were admitted to critical care and 97 (38 %) required oxygen. All 48 adult patients (100 %) were admitted to hospital, compared with 127 children (51 %). Adults were also more likely to require oxygen [odds ratio (OR) 4.964, *P*<0.001, 95 % confidence interval (CI) 2.129–11.803] and to be admitted to critical care (OR 4.909, *P*<0.001, 95 % CI 1.735–13.829), compared with children.

Macrolide resistance conferred by the 23S rRNA gene mutation was found in samples from 6 out of 32 patients (19 %) in the subset tested. The results suggest that the recent *M. pneumoniae* epidemic was associated with a significant burden of hospital admission locally. The study also describes the first case series of macrolide-resistant *M. pneumoniae* in the UK, indicating that macrolide resistance surveillance is warranted in preparation for the next epidemic.

**INTRODUCTION**

*Mycoplasma pneumoniae* can be found in up to 40 % of cases of community-acquired pneumonia and in individuals of all ages, although predominantly in children, adolescents and young adults (Waites & Talkington, 2004; Sánchez-Vargas & Gómez-Duarte, 2008). *M. pneumoniae* is endemic worldwide but epidemics occur every 4–7 years; in 2011, epidemics were reported across Europe (Lenglet *et al.*, 2012) and elsewhere (Nir-Paz *et al.*, 2012). In the most recent epidemic in Scotland, cases peaked in the winter of 2011 and the highest incidence was seen in children aged 0–4 years (Gadsby *et al.*, 2012).

Abbreviations: CI, confidence interval; OR, odds ratio.

*M. pneumoniae* was originally isolated from cases of atypical pneumonia and later demonstrated to cause a spectrum of illness severity, including pneumonia, following experimental inoculation of healthy adults (Chanock *et al.*, 1961). Significant lower respiratory tract illness and rare fatalities have been described in patients in whom *M. pneumoniae* was the sole aetiological agent detected (Templetón *et al.*, 2005; Atkinson *et al.*, 2008; Kannan *et al.*, 2012). However, asymptomatic or mild and self-limiting infection with *M. pneumoniae* has been commonly described. A recent PCR-based observational study demonstrated high rates of asymptomatic upper respiratory tract carriage of *M. pneumoniae* in children (Spuesens *et al.*, 2013).
Macrolides are currently recommended as the first-line treatment for *M. pneumoniae* infection in the UK (Harris *et al.*, 2011). However, macrolide resistance has been emerging in Asia in the last decade, with a prevalence of up to 69% (Cao *et al.*, 2010). Macrolide-resistant cases have now been reported from countries in the Western Hemisphere such as Israel (Averbuch *et al.*, 2011), Denmark (Uldum *et al.*, 2012), Italy (Chironna *et al.*, 2011) and Germany (Dumke *et al.*, 2010). To date, a single case of macrolide-resistant *M. pneumoniae* in the UK has been reported (Chalker *et al.*, 2012). With the advent of widespread molecular testing for diagnosis and the large increase in cases during an epidemic, it is important to reassess the clinical outcome of cases as well as to assess whether macrolide resistance is occurring in the UK. The aim of the study was to examine the local clinical impact of the recent *M. pneumoniae* epidemic and to determine whether macrolide-resistant strains are present in Scotland.

**METHODS**

**Clinical cases.** Cases of *M. pneumoniae* infection occurring in the National Health Service Lothian health board region of south-east Scotland were identified from the laboratory records of the Specialist Virology Centre, Royal Infirmary of Edinburgh, for the period 1 January 2010 to 31 December 2011. *M. pneumoniae* infection was defined as a positive real-time PCR result on a respiratory specimen. Medical and laboratory records were examined for clinical details and laboratory indices. Repeated samples from the same patient were included when gathering data, as this was relevant to potential antibiotic resistance; however, duplicate samples were excluded when it came to other analyses. The study was carried out as part of a clinical audit of routinely gathered clinical data for a local clinical governance programme and was approved by the Quality Improvement Team, Royal Hospital for Sick Children, Edinburgh. Handling and testing of anonymized specimens for macrolide resistance was carried out in accordance with ethical approval from the Lothian Regional Ethics Committee (08/S11/02/2).

**Detection of *M. pneumoniae* and respiratory viruses.** Total nucleic acid was extracted from respiratory tract specimens suspended in virus transport medium by the automated NucliSens easyMAG system (bioMérieux). A real-time PCR assay targeting the P1 adhesin gene was used to detect *M. pneumoniae*. The hydrolysis probe and primers were adapted from a published assay (Templeton *et al.*, 2003) using a new alignment of P1 gene sequences to take into account the availability of new sequence information. The assay, which has been extensively validated in house, reliably detects 500 gene copies ml⁻¹ (10 copies per reaction) and has excellent performance in internal and external quality assessment programmes such as the Quality Control for Molecular Diagnostics programme. Nucleic acid extracts were also tested for the following viruses as part of a routine multiplex real-time PCR screening approach using assays developed in house and/or adapted from published methods (Gadsby *et al.*, 2010): influenza A and B, para influenza 1, 2 and 3, respiratory syncytial virus, adeno virus, human metapneumovirus and rhinovirus. Specimens were also tested for enterovirus and human parechovirus where clinically indicated.

**Criteria for selection of specimens for resistance genotyping.** Specimens from patients anticipated to be at greater risk of macrolide-resistant *M. pneumoniae* were identified by the presence of one or more of the following criteria: (1) two or more *M. pneumoniae*-positive specimens from the same patient taken more than 2 days apart; (2) the patient remained symptomatic despite a course of appropriate antibiotics; (3) the patient was admitted to critical care (intensive care unit or high dependency unit); (4) a patient with cystic fibrosis, bronchiectasis, oncological or haematological disease, or who had received a transplant. Using these criteria, 44 samples from 32 patients were genotyped for macrolide resistance.

**Macrolide resistance genotyping.** Domain V of the *M. pneumoniae* 23S rRNA gene was amplified and sequenced in the selected subset of *M. pneumoniae*-positive specimens in order to determine the existence of mutations associated with macrolide resistance, as described previously (Wolff *et al.*, 2008).

**Statistical analysis.** The clinical impact was assessed by exploring three markers of disease severity: hospital admission, requirement for oxygen and admission to critical care. For each of these three outcomes, three categorical clinical and demographic variables were analysed: (1) adult age (≥ 18 years); (2) presence of a co-morbidity; and (3) co-infection with one or more respiratory viruses. Analysis was performed using a *χ²* test with the Yates’s correction applied for 2 × 2 tables. All statistical tests were performed with SPSS Statistics (version 19; IBM) and a statistical significance level of 0.05 was assumed throughout.

**RESULTS**

**Demographic and clinical data**

*M. pneumoniae* was detected in 333 respiratory specimens from 307 patients during the study period. Of these specimens, 327 (98%) were from patients in National Health Service Lothian hospitals, including 198 specimens (59%) from emergency departments or acute assessment units. The remaining six specimens came from primary care clinics within the Edinburgh region. Eighty *M. pneumoniae* infections were detected in 2010 and 253 were detected in 2011. A total of 179 cases (54%) were detected between October and December 2011. There were significantly more cases in males than females; 176 (57%) males versus 131 (43%) females (*χ²* = 6.596, *P* = 0.010, *n* = 307). The median patient age was 6 years (range 0–78), with 123 patients (40%) below school age (aged 0–4 years) (Table 1).

The most common clinical features on presentation to hospital were cough (72%) and fever (61%), although there was a wide range of presentations and most patients had two or more symptoms (Table 2). A co-morbidity was present in 87 cases (29%) for whom data were available, with 13 patients (4%) having more than one condition (Table 3). Eighty-one patients (26%) tested positive for one or more viruses, with 11 patients (4%) positive for two viruses. Rhinovirus, respiratory syncytial virus and influenza A virus were the most common viral co-infections (Table 4).

**Indicators of disease severity**

During the study period there were two deaths in patients with poor prognosis due to significant underlying neurological disease, but there were no deaths attributable to *M. pneumoniae*-positive specimens from the same patient taken more than 2 days apart; (2) the patient remained symptomatic despite a course of appropriate antibiotics; (3) the patient was admitted to critical care (intensive care unit or high dependency unit); (4) a patient with cystic fibrosis, bronchiectasis, oncological or haematological disease, or who had received a transplant. Using these criteria, 44 samples from 32 patients were genotyped for macrolide resistance.

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pneumoniae infection. From the data available, 175 patients (59%) were admitted to hospital, 20 (7%) were admitted to critical care and 97 (38%) required oxygen. Length of stay in hospital ranged from less than 1 day to 4 months, with 130 patients (75%) admitted to hospital for 2 or more days. Of the patients with admission data available, all 48 adult patients (100%) were admitted to hospital, compared with 127 out of 247 children (51%). Adults were also more likely to require oxygen \([\text{odds ratio (OR)} 4.964, P=0.001, 95\% \text{ confidence interval (CI) 2.129–11.803}]\) and to be admitted to critical care (OR 4.909, \(P=0.001, 95\% \text{ CI 1.735–13.829}\)), compared with children. Patients with a co-morbidity were more likely to be admitted to hospital (OR 1.882, \(P=0.032, 95\% \text{ CI 1.052–3.384}\)) than those without a co-morbidity, but there was no significant association of viral co-infection with any of the three indicators of disease severity.

Antibiotic prescription

Data on the antibiotics prescribed in the community by the patient’s general practitioner were available in 86 cases; of these, three patients (4%) were empirically given antibiotics that covered \(M.\ pneumoniae\) infection, two were given erythromycin and one was given clarithromycin. On presentation to hospital, 144 patients (47%) were empirically given an antibiotic covering \(M.\ pneumoniae\) infection, 139 were given clarithromycin and five were given erythromycin or azithromycin. A further 81 (26%) were

Table 1. Age group of patients diagnosed with \(M.\ pneumoniae\) respiratory tract infection \((n=307)\)

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>Patients with (M.\ pneumoniae) infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–4</td>
<td>123 (40.1)</td>
</tr>
<tr>
<td>5–9</td>
<td>94 (70.7)</td>
</tr>
<tr>
<td>10–14</td>
<td>36 (82.4)</td>
</tr>
<tr>
<td>15–19</td>
<td>8 (85.0)</td>
</tr>
<tr>
<td>20–24</td>
<td>6 (87.0)</td>
</tr>
<tr>
<td>25–29</td>
<td>6 (88.9)</td>
</tr>
<tr>
<td>30–34</td>
<td>10 (92.2)</td>
</tr>
<tr>
<td>35–39</td>
<td>5 (93.8)</td>
</tr>
<tr>
<td>40–44</td>
<td>6 (95.8)</td>
</tr>
<tr>
<td>45–49</td>
<td>5 (97.4)</td>
</tr>
<tr>
<td>50–54</td>
<td>2 (98.0)</td>
</tr>
<tr>
<td>55–59</td>
<td>3 (99.0)</td>
</tr>
<tr>
<td>60–64</td>
<td>0 (99.0)</td>
</tr>
<tr>
<td>≥65</td>
<td>3 (100.0)</td>
</tr>
</tbody>
</table>

\(P<0.001, 95\% \text{ confidence interval (CI) 2.129–11.803}\) and to be admitted to critical care (OR 4.909, \(P=0.001, 95\% \text{ CI 1.735–13.829}\)), compared with children. Patients with a co-morbidity were more likely to be admitted to hospital (OR 1.882, \(P=0.032, 95\% \text{ CI 1.052–3.384}\)) than those without a co-morbidity, but there was no significant association of viral co-infection with any of the three indicators of disease severity.

Table 2. Presenting symptoms in patients diagnosed with \(M.\ pneumoniae\) respiratory tract infection \((n=279)\)

Information was not recorded for 28 patients.

<table>
<thead>
<tr>
<th>Symptoms on presentation</th>
<th>No. patients (%) with (M.\ pneumoniae) infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cough</td>
<td>201 (72)</td>
</tr>
<tr>
<td>Fever</td>
<td>169 (61)</td>
</tr>
<tr>
<td>Breathlessness</td>
<td>81 (29)</td>
</tr>
<tr>
<td>Coryza</td>
<td>57 (20)</td>
</tr>
<tr>
<td>Nausea/vomiting</td>
<td>39 (14)</td>
</tr>
<tr>
<td>Lethargy</td>
<td>34 (12)</td>
</tr>
<tr>
<td>Loss of appetite</td>
<td>26 (9)</td>
</tr>
<tr>
<td>Wheeze</td>
<td>21 (8)</td>
</tr>
<tr>
<td>Sputum production</td>
<td>18 (7)</td>
</tr>
<tr>
<td>Headache</td>
<td>16 (6)</td>
</tr>
<tr>
<td>Sore throat</td>
<td>16 (6)</td>
</tr>
<tr>
<td>Rash</td>
<td>13 (5)</td>
</tr>
<tr>
<td>Chest pain</td>
<td>11 (4)</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>9 (3)</td>
</tr>
<tr>
<td>Myalgia</td>
<td>8 (3)</td>
</tr>
<tr>
<td>Other*</td>
<td>61 (22)</td>
</tr>
</tbody>
</table>

*Includes rigors, haemoptysis, neck pain/stiffness, diarrhoea, night sweats, febrile convulsion, photophobia and sleep disturbance.

Table 3. Co-morbidity in patients diagnosed with \(M.\ pneumoniae\) respiratory tract infection \((n=87)\)

<table>
<thead>
<tr>
<th>Co-morbidity</th>
<th>No. patients (%) with co-morbidity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asthma</td>
<td>21 (24)</td>
</tr>
<tr>
<td>Trisomy 21</td>
<td>9 (10)</td>
</tr>
<tr>
<td>Haematological disease</td>
<td>9 (10)</td>
</tr>
<tr>
<td>Recurrent chest infections</td>
<td>9 (10)</td>
</tr>
<tr>
<td>History of prematurity</td>
<td>8 (9)</td>
</tr>
<tr>
<td>Cystic fibrosis</td>
<td>7 (8)</td>
</tr>
<tr>
<td>Cardiac disease/defect</td>
<td>6 (7)</td>
</tr>
<tr>
<td>Epilepsy</td>
<td>5 (6)</td>
</tr>
<tr>
<td>Kidney disease</td>
<td>3 (3)</td>
</tr>
<tr>
<td>Oncological</td>
<td>3 (3)</td>
</tr>
<tr>
<td>Other*</td>
<td>23 (26)</td>
</tr>
</tbody>
</table>

*Includes cerebral palsy, diabetes, history of transplant, tracheoesophageal fistula, human immunodeficiency virus and quadriplegia.

Table 4. Viral co-infections detected by PCR in patients diagnosed with \(M.\ pneumoniae\) respiratory tract infection \((n=81)\)

<table>
<thead>
<tr>
<th>Viral co-infection</th>
<th>No. patients (%) with viral co-infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhinovirus</td>
<td>39 (48)</td>
</tr>
<tr>
<td>Respiratory syncytial virus</td>
<td>23 (28)</td>
</tr>
<tr>
<td>Influenza A</td>
<td>14 (17)</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>8 (10)</td>
</tr>
<tr>
<td>Parainfluenza virus</td>
<td>6 (7)</td>
</tr>
<tr>
<td>Human parechovirus</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Human metapneumovirus</td>
<td>1 (1)</td>
</tr>
</tbody>
</table>

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given other antibiotics such as β-lactams that did not cover *M. pneumoniae*.

**Macrolide resistance**

Specimens from patients in whom macrolide resistance was considered most likely, based on their clinical picture, were tested for genotypic macrolide resistance. Forty-four *M. pneumoniae* PCR-positive specimens from 32 patients were tested. In six patient samples (19%) within this subset, mutations conferring macrolide resistance were found in the 23S rRNA gene (Table 5). The A2063G mutation was found in four cases and A2064G and A2064C were each found in one case, respectively. Three of the six patients were children with co-morbidities, two of whom were immunocompromised. The six cases of macrolide resistance were detected mainly at the end of the study period; there was one case in May 2010 and one in June 2011, with the remainder in October 2011 (*n*= 1), November 2011 (*n*= 2) and March 2012 (*n*= 1).

In four of the six cases, the macrolide-resistant strain appeared to have arisen *de novo* during or following macrolide treatment. In three of these cases, a susceptible genotype was found on presentation and the patients were treated with oral macrolides. However, a resistant genotype was found in a specimen taken 2–10 weeks later, following representation with on-going symptoms. These three patients recovered fully. In the fourth case, macrolide-resistant *M. pneumoniae* was detected on one of four occasions during a 4-month period in a patient with a significant co-morbidity who later died due to their underlying condition (Table 5). In two of the six cases, macrolide resistance was detected at initial presentation. One patient was immunocompromised and recovered fully, although their hospital stay was several weeks. In the other case, resistance was found on presentation in two respiratory specimens from a patient who was visiting Scotland from northern Europe; this patient required intensive therapy unit admission due to severe pneumonia, but survived.

**DISCUSSION**

The *M. pneumoniae* epidemic of 2011 provided us with the opportunity to study the clinical features of a cohort of patients testing positive by PCR in a hospital setting and to determine the local prevalence of macrolide resistance by genetic methods. With the now widespread use of molecular tools for diagnosis and the recent increase in cases due to the epidemic, it is important to assess clinical outcomes and existence of macrolide resistance in preparation for the next epidemic. With this aim, we carried out a retrospective descriptive study of consecutive *M. pneumoniae* cases detected during the epidemic period in south-east Scotland, UK.

The age distribution of patients with *M. pneumoniae* respiratory infection diagnosed in our centre was similar to that seen across Scotland in the 2011 epidemic, with a significant proportion of cases in children below school age (Gadsby et al., 2012). In the present study, however, adults were more likely than children to have markers of disease severity such as oxygen requirement, hospital admission and intensive care admission. Presenting symptoms were non-specific, with cough and/or fever being the most common. However, three-quarters of *M. pneumoniae*-positive patients tested negative for respiratory viruses in our multiplex real-time PCR screen and viral co-infection was not associated with markers of disease severity. An association between respiratory viral co-infection and increased severity of respiratory tract infection in children has been demonstrated in some studies (Semple et al., 2005; Richard et al., 2008) but not others (Wolf et al., 2006), and the impact of viral/bacterial co-infection with *M. pneumoniae* specifically is not clear (Bezerra et al., 2011; Dhanoo et al., 2011). Around one-quarter of patients in whom *M. pneumoniae* was detected had co-morbidities, and these patients were more likely to be admitted to hospital than those without co-morbid conditions. We also found a substantial burden on hospital services during the *M. pneumoniae* epidemic, with 38% of *M. pneumoniae*-positive patients requiring oxygen and 59% admitted to hospital, frequently staying for 2 or more days.

This study suggests that, although the burden of *M. pneumoniae* detection was numerically concentrated in the younger age groups during the epidemic in Scotland, the burden of clinical disease may have been focused in adults and in those with underlying illness. This finding is interesting in the context of the high levels of *M. pneumoniae* DNA detection in both symptomatic and asymptomatic children in a recent study (Spuesens et al., 2013). The pathogenic potential of *M. pneumoniae* has been well described (Chanock et al., 1961; Waites & Talkington, 2004; Templeton et al., 2005; Atkinson et al., 2008; Sánchez-Vargas & Gómez-Duarte, 2008; Kannan et al., 2012). However, the asymptomatic DNA detection rate in non-paediatric populations has not yet been studied. The most recent *M. pneumoniae* epidemic coincided with the now widespread use of PCR-based detection methods, enabling us to diagnose more infections but presumably to detect more carriage. Therefore, the significance of an *M. pneumoniae*-positive PCR result should be interpreted in the context of clinical findings and other microbiological test results; in our study, a number of paediatric and adult patients were admitted to respiratory wards or critical care with respiratory tract illness from whom no organisms other than *M. pneumoniae* were detected.

In our centre, patients are typically tested for *M. pneumoniae* on presentation to hospital with respiratory symptoms. Therefore, a limitation of the study is that we did not have a control group consisting of asymptomatic individuals who were *M. pneumoniae* PCR positive. Although the majority of patients were negative in our respiratory viral screen, other pathogens such as coronaviruses, which are not part of our routine testing.
<table>
<thead>
<tr>
<th>Case no.</th>
<th>Age group (years)</th>
<th>Presence of co-morbidity</th>
<th>Genotypic macrolide resistance test on <em>M. pneumoniae</em> PCR-positive specimen</th>
<th>Antibiotic treatment</th>
<th>Clinical course</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0–4</td>
<td>No</td>
<td>Initial presentation</td>
<td>Sensitive</td>
<td>Clarithromycin</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Representation 4 weeks later</td>
<td>Resistant*</td>
<td>Clarithromycin</td>
</tr>
<tr>
<td>2</td>
<td>5–9</td>
<td>No</td>
<td>Initial presentation</td>
<td>Sensitive</td>
<td>Clarithromycin</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Representation 2 weeks later</td>
<td>Resistant*</td>
<td>Clarithromycin</td>
</tr>
<tr>
<td>3</td>
<td>10–14</td>
<td>Yes</td>
<td>Initial presentation</td>
<td>Sensitive</td>
<td>Azithromycin</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Representation 10 weeks later</td>
<td>Resistant†</td>
<td>NK</td>
</tr>
<tr>
<td>4</td>
<td>0–4</td>
<td>Yes‡</td>
<td>Initial presentation</td>
<td>ND</td>
<td>NK</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Same episode 3 weeks later</td>
<td>Sensitive</td>
<td>NK</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Same episode 4 weeks later</td>
<td>Sensitive</td>
<td>NK</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Same episode 10 weeks later</td>
<td>Resistant§</td>
<td>NK</td>
</tr>
<tr>
<td>5</td>
<td>0–4</td>
<td>Yes‡</td>
<td>Initial presentation</td>
<td>Resistant*</td>
<td>Clarithromycin</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Same episode 2 days later</td>
<td>Resistant*</td>
<td>NK</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Same episode 11 days later</td>
<td>Resistant*</td>
<td>NK</td>
</tr>
<tr>
<td>6</td>
<td>30–34</td>
<td>No</td>
<td>Initial presentation</td>
<td>Resistant*</td>
<td>NK</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Same episode 1 day later</td>
<td>Resistant*</td>
<td>NK</td>
</tr>
</tbody>
</table>

ND, Not done; NK, not known.

*Macrolide resistance mutation A2063G.
†Macrolide resistance mutation A2064G.
‡Immunocompromised patient.
§Macrolide resistance mutation A2064C.
||Patient was visiting the UK from northern Europe.
repertoire, may account for the respiratory symptoms in a proportion of our *M. pneumoniae*-positive cohort. It is also possible that clinicians may be more inclined to test children for *M. pneumoniae* who present to hospital with respiratory symptoms regardless of severity, but only test for *M. pneumoniae* in those adults who are more severely ill, thus introducing a testing bias.

Around half the patients presenting to hospital in this study were empirically given a macrolide antibiotic to cover *M. pneumoniae*; this choice was likely to have been informed by the patients’ lack of response to other antibiotics prescribed in primary care. Local primary care guidelines, both currently and during the *M. pneumoniae* epidemic, recommend the use of amoxicillin as first-line treatment for community-acquired pneumonia managed in the community, and help to prevent inappropriate use of macrolides. Our data regarding the low level of community macrolide prescribing suggests that general practitioners were following local guidelines for lower respiratory tract infection appropriately. However, patients successfully treated in the community with macrolides would not have presented to hospital and therefore would not have been included in our study. Prescribing of macrolides greatly increased in Norway and Sweden during the recent *M. pneumoniae* epidemic (Blystad et al., 2012; Linde et al., 2012), but it is not yet known if similar trends are apparent in the UK.

Low levels of macrolide resistance have been reported in Europe (Dumke et al., 2010; Chironna et al., 2011; Uldum et al., 2012), but only a single case has been reported previously in the UK from a sample taken in 2008 (Chalker et al., 2012). In the present study, we have described the first series of macrolide-resistant *M. pneumoniae* cases from the UK and found six patients out of 32 tested (19%) had genotypic evidence of macrolide resistance. Although we concentrated our efforts on only a subset of patients in whom macrolide resistance was considered most likely clinically, this is an important finding. The previous UK study, which is only published in summary format, examined specimens taken up until October 2011 (Chalker et al., 2012). However, we found four of the six resistance cases in our study occurred from October 2011 onwards, which may explain the lower prevalence of macrolide resistance in the previous study and indicates that macrolide resistance is only recently emerging in the UK.

We documented the development of *de novo* resistance over time in children with on-going symptoms despite first-line treatment, as well as the presence of resistance in patients at initial presentation, including in one visitor to the UK from northern Europe. A limitation of the study is that although all patients with macrolide resistance recovered from their infection, limited prescribing records meant that we were not able to determine if there had been any switch of antibiotic treatment from a macrolide to an alternative agent due to clinical suspicion of resistance. The development of *M. pneumoniae* macrolide resistance in an immunocompromised child treated with macrolides has been described previously (Hantz et al., 2012). Our finding of two cases of macrolide resistance in immunocompromised children further illustrates the importance of considering this possibility when treating *M. pneumoniae* in this vulnerable patient group.

These results suggest that, even in the context of an overall low prevalence of *M. pneumoniae* macrolide resistance in the UK, prevalence may be higher in some patient groups and may be an emerging problem. Therefore, it is appropriate for clinicians to consider macrolide resistance in patients giving cause for concern, and it is important to bear this in mind for the next epidemic. Prospective surveillance of macrolide-resistant *M. pneumoniae* in the UK is warranted.

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