Detection of macrolide resistant *Mycoplasma pneumoniae* in England, September 2014 to September 2015

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Mycoplasma pneumoniae **infection can cause pneumonia, particularly in children. Global increase in macrolide-resistant *M. pneumoniae* is of concern due to limited therapeutic options. We describe the detection of macrolide resistance-conferring mutations in 9.3% of 43 clinical specimens where *M. pneumoniae* was detected in England and Wales from September 2014-September 2015. This study aims to impact by highlighting the presence of macrolide resistance in *M. pneumoniae* positive patients, promoting increased clinical vigilance.**

Here we report the detection of mutations associated with macrolide resistance in *M. pneumoniae*-positive specimens from four patients with pneumonia in Englandin the periodSeptember 2014 to September 2015. Prior to 2014, in the United Kingdom seven cases of macrolide-resistant *M. pneumoniae* infections were reported between 2008 and 2011, mainly from Scotland [1,2].

Macrolide resistance determination

The Bacteriology Reference Department, Public Health England (PHE), London, receives specimens from England and Wales for *M. pneumoniae* testing and confirmatory testing. Here we detected *M. pneumoniae* by qPCR in 60 clinical specimens from 60 patients (Cambridge, Leeds, London, Manchester, Nottingham and Oxford) that were submitted to PHE between 1 September 2014 and 1 September 2015. DNA extractions from specimens, where *M. pneumoniae* was detected, were screened for point mutations known to confer macrolide resistance. Mutations in domain V of the 23S rRNA were detected by a modified version of the method described by Li et al., 2009 [3], wherein the entire region of interest is amplified and sequenced as one product. Primers used were as follows: forward primer 5'-ATCTCTTGACTGTCTCGGC-3' and reverse primer 5'-TACAACTGGAGCATAAGAGGTG-3'.

Of the 60 specimens, 17 (28.3%; 95% confidence interval (CI): 18.4--40.8) contained insufficient DNA to determine macrolide resistance-conferring mutations. Of the remaining 43 specimens mutations in the 23S rRNA known to confer macrolide resistance were found in four (9.3%; 95% CI: 3.1–22.2). Of these 43 specimens, 32 were from a single city in England, Leeds, and a single specimen among these was positive for the mutation, 3.1% (95% CI: 0.01–17.1). The cases identified with point mutations known to confer macrolide-resistant *M. pneumoniae* were in two women and two men, respectively, aged › 15 to ‹65 years old. Three were hospitalised with pneumonia (Table) with no known connection between patients.

Interestingly, two of the macrolide-resistant cases were patients that had recently arrived from the United States (exact timeline unknown); of which one had received clarithromycin whilst undergoing treatment in the UK. The origins of the infecting *M. pneumoniae* strains in these two cases may have been external to England and Wales. The other two cases were from separate cities in England. All macrolide resistance-conferring mutations were A2058G (*Escherichia coli* numbering) point mutation in the 23S rRNA.

Background

Mycoplasma pneumoniae can be isolated from patients with lower respiratory tract infection, including pneumonia, and has also been associated with prolonged persistent cough and exacerbation of asthma [4]. *M. pneumoniae* infections may manifest infrequently as extra-pulmonary sequelae after the onset of or even in the absence of respiratory illness [5]; including encephalitis [6], dermatological manifestations such

Table

Details of patients with macrolide-resistant *Mycoplasma pneumoniae*-positive clinical specimens, England and Wales, September 2014–September 2015 (n=4)

Case	Age group (years)	Sample type	Pneumonia	Hospitalised	Macrolide before sampling
1	45–65	TS	Yes	Yes	Unknown
2	15–25	BAL	Yes	Yes	Yes
3	45–65	BAL	Yes	Yes	Unknown; Antibiotics class unknown administered before admission
4	15–25	TS	Yes	Unknown	Unknown

BAL: bronchoalveolar lavage; TS: throat swab.

as Stevens-Johnson syndrome [7], and haemolytic anaemia [8]. Asymptomatic carriage of *M. pneumoniae* has been documented in nasopharyngeal swabs at low levels in England, e.g. at 0.25% based on PCR in a 2001 carriage study [9], however, a study from the Netherlands reported a much higher carriage rate (21.2%) [10]. In England and Wales, *M. pneumoniae* infection can be found in all age groups, with a higher prevalence in children of school age [9]. In England and Wales, seasonal peaks of infection are detected from December to February each year with epidemics at approximately four-yearly intervals, lasting 12 to 15 months [9]. A large increase in reported *M. pneumoniae* cases was documented in several European countries, including England and Wales, in 2011 [11].

Discussion

In the past 15 years, a significant increase in macrolide-resistant *M. pneumoniae* has been reported globally, of increasing concern and importance to the international community [12]. In Asia, resistance rates of over 90% have been reported [13], particularly in China, whereas in Europe and North America resistance rates of up to 25% have been documented [14,15]. Macrolide-resistant strains of *M. pneumoniae* have not been documented to show cross-resistance to other classes of antibiotics i.e. tetracyclines and fluoroquinolones [16].

Prior to 2014, in the United Kingdom, seven cases of macrolide-resistant *M. pneumoniae* infections were reported between 2008 and 2011, one case in England and Wales and six cases in Scotland [1,2]. This is the second report of macrolide-resistant *M. pneumoniae* strains detected in England and Wales, with one case previously documented for a single patient specimen from 2008 [1]. Macrolide resistance in *M. pneumoniae* has been reported in Scotland at 19% (6/32) [2], considerably higher than the 9.3% documented here. This may reflect low sample numbers or sampling differences and it is important to note that the specimens examined for macrolide resistance in Scotland were from patients in whom macrolide resistance was considered most likely based on their clinical presentation or history, being one of the following: repeated specimen positive, remaining symptomatic following antibiotic treatment, admitted to critical care or having an underlying condition.

In this study a high number of samples were from a single city in England and a local epidemic cannot be excluded. There is no requirement for referral of *M. pneumoniae*-positive specimens to the reference laboratory in England and Wales. Systematic testing and referral of positive specimens does not occur. Therefore regional comparison was not possible. Nonetheless, the focus of this article was to highlight macrolide resistance rather than a specific regional cluster analysis.

Macrolides are currently recommended as the first-line treatment for *M. pneumoniae* infection in the UK [17]. The 2011 British Thoracic Society guidelines for the management of community acquired pneumonia in children and adults suggest empirical macrolide treatment at any age if there is no response to first-line beta-lactam antibiotics or in the case of very severe disease [17,18]. Tetracyclines (minocycline and doxycycline) and fluoroquinolones (levofloxacin and moxifloxacin) can be used to treat *M. pneumoniae* infections as an alternative to macrolides when clinically relevant [19], however, their use in children is limited due to effects on bone toxicity and cartilage development, respectively [20,21].

We did not isolate *M. pneumoniae* by culture from those specimens wherein *M. pneumoniae* was detected by PCR and therefore we were not able to confirm phenotypic macrolide resistance. However, point mutations within the 23SrRNA gene in clinical specimens and isolates, including the A2058G mutation, have previously been shown to confer resistance [16]. Acquisition of resistance has been documented in patients receiving macrolides and resistance may develop as a consequence of antibiotic selective pressure [22]. This is supported by the highest macrolide resistance rates being reported in countries with extensive macrolide use [15]. Increased vigilance pertaining to macrolide-resistant *M. pneumoniae* in the UK is recommended.

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Conflict of interest

None declared.

Authors' contributions

RJB wrote the manuscript, LMS undertook PCR and local study conception, SP performed macrolide resistance analysis, VJC designed, oversaw the study and wrote the manuscript.

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