

Case Reports and Series

Who gets a laboratory positive diagnosis of *Mycoplasma pneumoniae*: A 10-year retrospective analysis

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ABSTRACT

Objectives: *Mycoplasma pneumoniae* (*M. pneumoniae*) is thought to cause up to a third of community acquired pneumonias (CAP), but may be undiagnosed due to limitations with current diagnostics, and untreated given the frequent use of B-lactams to which *M. pneumoniae* is not susceptible. We performed a ten-year retrospective analysis to identify the typical characteristics of a patient with a laboratory positive diagnosis of *M. pneumoniae*.

Methods: Laboratory diagnosis of *M. pneumoniae* was performed using Polymerase Chain Reaction (PCR) and Serology (passive particle agglutination (PPA) and Enzyme-linked immunosorbent (EIA) assays). Data were collected on all patients tested for *M. pneumoniae* between 2009 and 2019.

Results: 19,090 PCR and 4530 serology samples were tested for *M. pneumoniae* with 278 positive results. The positive group had a median age of 40 years (interquartile range 30–41 years); Median C-reactive Protein (CRP) was 71 mg/L, White blood Cell Count (WBC) 7×10^9 . 80% had an abnormal Chest X-ray. Intensive Care Unit (ICU) admission occurred in 4.5%; 1.3% patients died. 29% of patients were positive on both serology and PCR testing platforms.

Conclusions: The characteristics reported here could be used as guidance on who is treated for *M. pneumoniae*. We propose that testing for *M. pneumoniae* needs to be performed systematically in patients with CAP; and that targeted atypical pathogen cover should be considered in preference to B-lactam monotherapy for all patients with these characteristics.

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Background

Mycoplasma pneumoniae (*M. pneumoniae*) is reported to be responsible for up to one third of Community Acquired Pneumonias (CAP), large outbreaks in enclosed environments, and is associated with extra-pulmonary manifestations that may have long-term sequelae (Kashyap and Sarkar, 2010). However, diagnosis remains a challenge as serology lacks sensitivity and specificity, whilst molecular methods are less widely available (CDC, 2018). The difficulty in diagnosis means that there are limited real-life data describing a typical patient with *M. pneumoniae*.

Using a ten-year metropolitan centre dataset of patients with a laboratory diagnosis of *M. pneumoniae*, we develop a representative picture of a patient with *M. pneumoniae* pneumonia as a tool to enable clinicians to identify the illness promptly by ensuring

suitable diagnostic tests are requested, then target treatment, reduce inappropriate B-lactam use, and isolate patients with a suspected positive diagnosis to limit onward transmission.

Methods

Inclusion criteria

Data were extracted from the electronic laboratory software Winpath (v5, CliniSys, UK). All patients with serology and/or Polymerase Chain Reaction (PCR) positive results between May 2009 and March 2019 (serology) and March 2009 and March 2019 (PCR) that did not meet the exclusion criteria (see below) were included. The discrepancy in testing dates in spring 2009 between PCR and serology is due to unavailability of serology prior to May 2009.

Exclusion criteria

Duplicates, samples from hospitals other than the Royal Free London NHS Foundation Trust, and samples that could not be pro-

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cessed were excluded. Reasons for samples not being processed included leaking specimens, incorrectly labelled specimens and laboratory errors. Samples that were incorrectly reported by the laboratory were also excluded. Samples without relevant clinical information are automatically rejected by the laboratory, and these samples were also excluded. The number of samples excluded for each assay type are shown in Fig. 1.

Diagnostic assays

Laboratory testing for *M. pneumoniae* was carried out by on both serological and molecular platforms. Serology testing was performed by passive particle agglutination (PPA) (SERODIA-MYCO-II) between May 2009 and May 2018. The assay was subsequently changed to an immunoglobulin LIASON indirect Enzyme-linked immunosorbent (EIA) capture assay (IgG/M) (DiaSorin, Saluggia, Italy) due to laboratory centralisation. The PCR (Fast Track Diagnostics, Sliema, Malta) assay was performed on sputum and bronchoalveolar lavage (BAL) samples using TaqMan® technology, but not on nasopharyngeal secretions. Testing on nasopharyngeal secretions was not performed at the Trust as sputum testing is felt to provide adequate results for atypical infection diagnosis and was the specimen type on which PCR testing was originally validated.

Definitions

The definitions adopted in this study are:

***M. pneumoniae* Positive serology** is defined as a single serology titre of >1:1000 or 2 paired samples with >2-fold rise in titre by passive particle agglutination. ***M. pneumoniae* equivocal serology** is defined as a serology titre result not meeting the positive criteria but having a value of equal or greater than a single serology titre of 1: 40

***M. pneumoniae* positive IgG/M serology** by the LIASON assay is defined as patients who are IgM positive, IgG negative or IgM positive IgG positive. ***M. pneumoniae* negative by IgG/M serology** is defined as IgM negative, IgG positive or IgM negative and IgG negative. There were no equivocal IgG/M results in our data set.

***M. pneumoniae* Positive PCR** results were reported as positive, negative or equivocal as defined by the manufacturer's protocol.

Data collection and reporting

Data for positive *M. pneumoniae* cases were extracted from electronic patient discharge summaries, laboratory and radiology reports as well as the sample request information and from Win-Path. Missing data were listed as 'unknown'. Where the denominator changes in the results reporting, this reflects unknown data (for

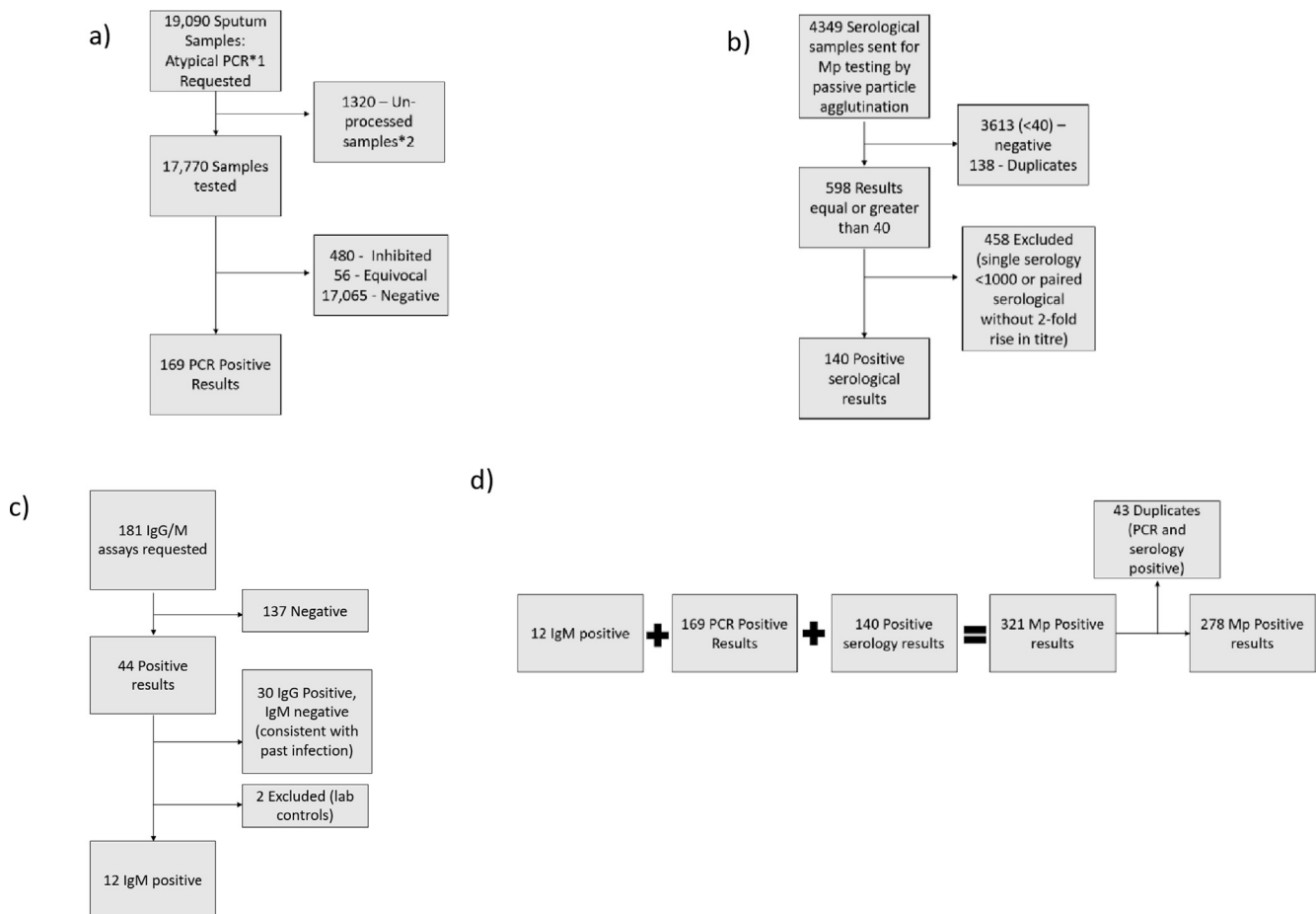


Fig. 1. *M. pneumoniae* Polymerase Chain Reaction (PCR) and serological assays performed between March 2009 and April 2019. 19,090 were tested by PCR of which 169/19,090 (0.89%) were positive. 4349 patients had serology tested, 140/4349 (3.2%) were positive by passive particle agglutination and 12/181 (6.63%) on the LIASON (IgG/M). Excluding duplicates ($n = 43$), there were a total of 278 individual patients with a *M. pneumoniae* laboratory confirmed diagnosis. (a) Total number of *M. pneumoniae* PCR samples tested. (b) Total number of *M. pneumoniae* serological samples tested by passive particle agglutination (Mp = *Mycoplasma pneumoniae*). (c) Total number of *M. pneumoniae* serological samples tested on LIASON (IgG/M). (d) Total number of positive *M. pneumoniae* samples (Mp = *Mycoplasma pneumoniae*). *1 - Atypical PCR panel at this Hospital Trust includes *Mycoplasma pneumoniae* PCR, *Chlamydia pneumoniae* PCR and *Legionella pneumophila* PCR. *2 - Duplicates, samples from hospitals other than the parent trust, and samples that could not be processed were excluded. Reasons for samples not being processed included leaking specimens, incorrectly labelled specimens and laboratory errors. Samples that were incorrectly reported by the laboratory were also excluded.

example, there were 278 positive Mp patients in our data set, 136/267 patients were inpatients, 11 had an unknown setting of diagnosis, so the denominator used here is 267).

Results

23,620 patients were tested for *M. pneumoniae* between March 2009 and April 2019. 1.18% (278) tests were positive on PCR or serology. A Fig. 1 breaks these down in detail.

242 patients in our data set had serology and PCR both tested (Fig. 2). There was poor correlation between PCR and serology positive patients, with only 70/242 (29%) being positive on both platforms.

Table 1 summarises the epidemiologic and clinical features of the 278 positive patients. Median age was 40 years (Q1 30, Q3 41, IQR 11). The youngest patient was 2 years ($n = 2$). 15% (41/278) were ≥ 65 years.

The patients' presenting symptoms are shown in Fig. 3. These were most commonly productive cough 69% (97/140) and fever 76% (101/133). Extra-pulmonary symptoms were less frequently noted, the most common being muco-cutaneous disease at 19% (24/129).

84% patients (87/104) had a symptom duration ≤ 14 days prior to testing positive. Information on symptom duration is shown in Table 1. These data were further analysed in patients with discrepant PCR and serology results. 18 patients had positive serology and negative PCR. Symptom duration data were available for 3. All had a symptom duration of >7 days. In the 30 patients with a pos-

itive PCR and negative serology result, 18 had symptom duration data available. 55% (10/18) reported symptoms ≤ 7 days.

A summary of the key investigation results for our patient population is shown in Table 1. Patients typically had a temperature of 38 °C (Q1 37.3, Q3 38, IQR 0.8) on presentation. Median WBC $\times 10^9/L$ was 7 and CRP 71 mg/L. 149/166 (90%) of our patient group had Chest-X-rays (CXRs), of which 119/149 (80%) were abnormal.

132/139 (95%) patients in our group were given antimicrobials. These are summarised in Fig. 4. Overall, 113/125 (90%) of patients were given antimicrobials with anti-mycoplasma activity. 47/132 (36%) patients had their antibiotics changed based on the *M. pneumoniae* positive result.

The seasonal variation of *M. pneumoniae* in our 10-year data-set is shown in Fig. 5. Peaks in incidence occurred every 4 years; the most cases were seen in 2016.

Discussion

A clear diagnosis of *M. pneumoniae* is important as treatment is different to that for CAP from other causes where first-line therapy is usually B-lactam based, to which *M. pneumoniae* is not susceptible. The British Thoracic Society (BTS) and National Institute of Clinical Excellence (NICE) guidelines currently only recommend treating with antimicrobials to which *M. pneumoniae* is susceptible, such as macrolides and tetracyclines, in certain patient groups (NICE, 2019; BTS, 2009). In addition to this, in secondary care settings, respiratory isolation may be considered in a patient with

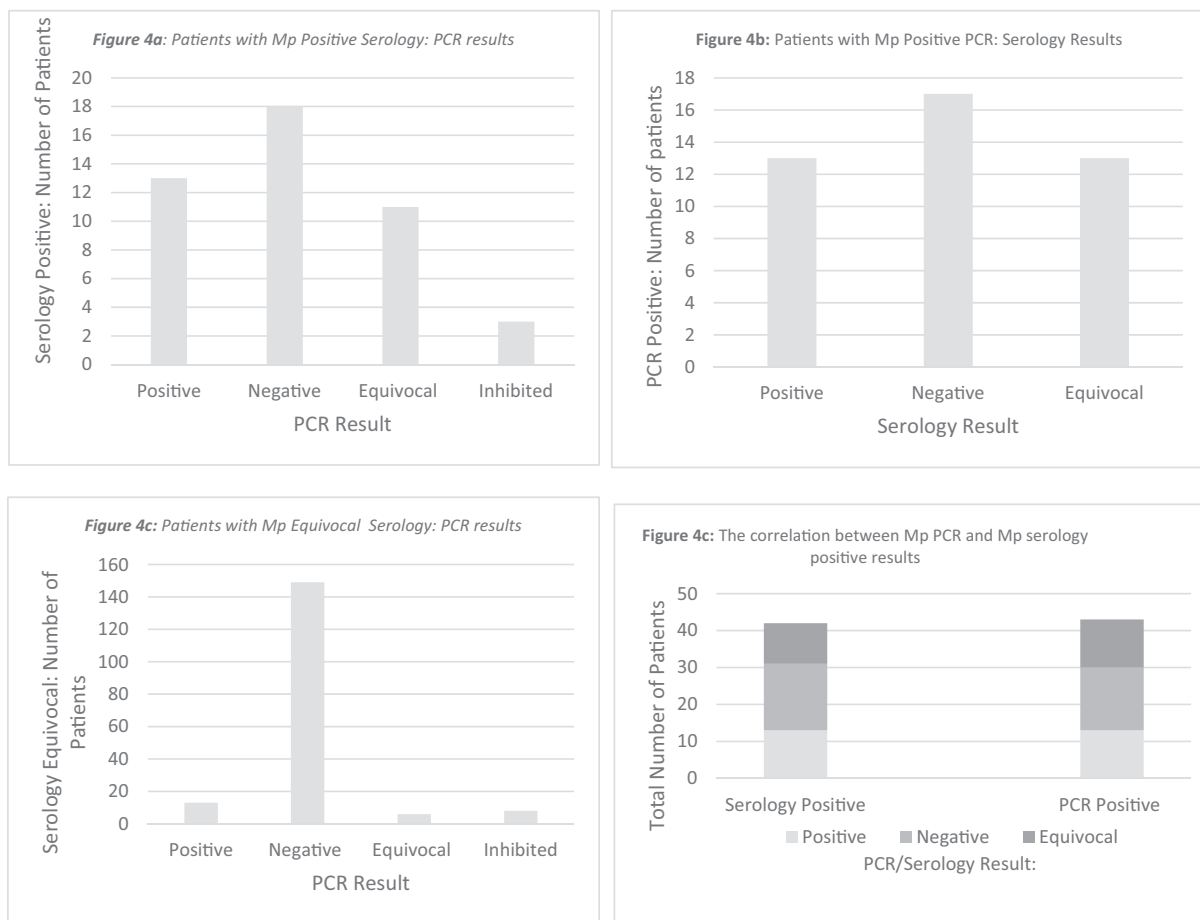


Fig. 2. Patients who were tested for *M. pneumoniae* on both Serology (combined LIASON IgG/M and Passive Particle Agglutination data) and Polymerase Chain Reaction $n = 242$.

Table 1
The Characteristics of *M. Pneumoniae* Positive Patients.

	N (Total), Units, Range	%
Patient Demographics		
Gender (Female)	137 (266)	51.3
Age (Median)	40 (278), Q1 30, Q3 41, IQR 11	N/A
Setting of Diagnosis		
Inpatient	136 (267)	50.9
General Practice	70 (267)	26.2
Emergency Department	24 (267)	9.0
Outpatient Clinic	37 (267)	13.9
Co-morbidities		
Smoker (Yes)	9 (93)	9.7
Ex-Smoker (Yes)	4 (93)	4.3
COPD/Asthma	13 (144)	9.0
Diabetes	5 (144)	3.5
HIV	13 (154)	8
Pregnancy	4 (144)	2.8
Immunocompromised (Yes)	21 (145)	14.5
Details of admission		
Duration of Admission (Median, Days)	4 (Range 0->28)	N/A
Duration of symptoms prior to admission	(104)	
0-3 days	11	10.6
4-7 days	37	35.6
7-14 days	39	38
15-21 days	7	6.7
>22 days	10	9.6
ICU Admission	6 (134)	4.5
Oxygen therapy required	52 (137)	38.0
Mortality	2 (154)	1.3
Temperature at presentation	38, 36.4-39.4 (39), °C, Q1 37.3, Q3 38, IQR 0.8	N/A
Investigation Results		
White Blood cell (WBC)	7, 1.6-24 (153) (10 ⁹ /L, 3-8)	N/A
Neutrophils	6, 6-20 (151) (10 ⁹ /L, 2-7)	N/A
C-reactive Protein (CRP)	71, 1-373 (153) (mg/L, <5)	N/A
Creatinine	71, 19-873 (153, 40-90)	N/A
Alanine Transaminase (ALT)	31.5, 4-2000 (144, 34-50)	N/A
Known HIV positive	13 (154)	8
HIV Test (yes, and result negative)	88 (154)	57
HIV test (yes, and result new positive)	0 (154)	0
Nasopharyngeal swab (yes)	73 (152)	48
Nasopharyngeal swab Negative for viral respiratory pathogens ^{†1}	66 (73)	90
Chest X-Ray (yes)	149 (166)	90
Normal Chest-X-ray	30 (149)	20
Right sided consolidation	40 (114)	28
Left Sided consolidation	54 (114)	38
Bilateral consolidation	20 (114)	18
Computed Topography (CT) performed (yes)	17 (165)	10
Abnormal CT	17 (17)	100

^{†1} PCR panel includes influenza, seasonal coronavirus, rhinovirus/enterovirus, parainfluenza, metapneumovirus).

suspected *M. pneumoniae* to reduce secondary cases and outbreaks (Gdalevich et al., 2018; Hyde et al., 2001).

The data presented here from 278 laboratory positive *M. pneumoniae* diagnoses over a 10-year period at a metropolitan UK hospital, suggest that the typical *M. pneumoniae* patient has demographic characteristics distinct from the typical CAP patient. The median patient age was 40 years with lower incidence of co-morbidity such as diabetes (3.5%) and COPD/Asthma (9%) than that reported for typical patients with CAP. When compared to large national UK audits, such as the BTS CAP audit 2018-2019 (Lim and Lawrence, 2019), the *M. pneumoniae* patient is markedly different. The most recent BTS data showed the mean CAP patient age to be 75 years, with a prevalence of diabetes at 17.5% and COPD 25.4%.

Streptococcus pneumoniae is the commonest cause of bacterial CAP, typically associated with older patients and co-morbidities

(BTS, 2009; Granados et al., 1989). *M. pneumoniae* is described as a cause of atypical pneumonia, along with *Legionella pneumophila* and *Chlamydia pneumoniae*. Differentiating features of *Legionella* are reported to be increased multi-system involvement in younger, male patients, with minimal co-morbidities (Fernández-Sabé et al., 2003), whereas *Chlamydia pneumoniae* is typically associated with a longer duration of symptoms prior to hospital admission and an older patient group (Troy et al., 1997). Our data support the profile of *M. pneumoniae* described in the literature of younger patients, with less co-morbidities and less multisystem involvement.

The rate of ICU admission (4.4% vs 5.2%), length of stay (4 vs 5 days) and oxygen administration rate (38% vs 53%) was similar to nationally-reported CAP outcomes in our data set. However, of note the mortality was significantly lower (1.3% vs 10%). This could be explained by the baseline demographic of our patients as described above, and by less multi-system involvement as is typically seen with other causes of bacterial CAP as described above.

8% patients were HIV positive; significantly higher than the prevalence in North-West London where this study was carried out (0.5-1%) (PHE, 2017). Although this could be significant, the hospital cares for a large number of people living with HIV and routinely sees HIV patients with CAP in an outpatient walk-in setting. Therefore, the higher HIV prevalence seen here may represent our local set-up, rather than a genuine increased population prevalence.

Extra-pulmonary manifestations are an important differentiating feature of *M. pneumoniae*, reported in up to 25% patients (Kashyap and Sarkar, 2010). Mucocutaneous involvement is one of the most widely reported of these, present in up to 22-38% of patients (Kashyap and Sarkar, 2010; Derm NZ, 2019; Schallock and Dinulos, 2009). Our data-set showed an incidence of 19%. Reported features of mucocutaneous involvement is variable, with some patients showing mild self-limiting features such as exanthems and urticaria, and others having more severe, progressive disease such as Stevens-Johnson syndrome and Toxic Epidermal Necrolysis.

Neurological manifestations are one of the most serious complications of *M. pneumoniae* infection, and occurred in 3% (4/131) of our patient group. In the literature, this is described in 5-10% patients (D'Alonzo et al., 2018), and can include both central and peripheral nervous system manifestations, such as encephalitis, aseptic meningitis, transverse myelitis, and neuropathies. Despite the potential long-term impact of neurological disease, the pathophysiology is poorly understood (D'Alonzo et al., 2018). Three main mechanisms of damage to the nervous system have been proposed: direct structural and functional damage; immune-modulated changes; and vascular microthrombotic disease (Tsiodras et al., 2005; D'Alonzo et al. 2018). Treatment options are limited and include immunoglobins and antimicrobials. None of our patients received immunoglobulin treatment, or suffered any long-term neurological manifestations.

Investigation results in our patient group showed a high CRP, median 71 mg/L (normal < 5 mg/L), with a normal WBC 10⁹/L. This is markedly different from typical bacterial CAP, in particular Streptococcal pneumonia, where a WBC > 15 × 10⁹/L is typically indicative of bacterial aetiology (BTS, 2009; Holmberg et al., 1990). CXR abnormalities were seen in 80% of our patients. 94/119 (79%) patients had unilateral consolidation on their CXR. This is in keeping with the literature on *M. pneumoniae* infection, typically describing a lobar pneumonia (Garg et al., 2019).

M. pneumoniae testing is frequently requested as part of routine management for patients with CAP. 23,620 Patients had *M. pneumoniae* serology or PCR tested during our study period with approximately 1.18% of these tests being positive. The low positivity rate may suggest over-zealous testing for atypical pathogens. Serological methods are the most widely used assay to diagnose

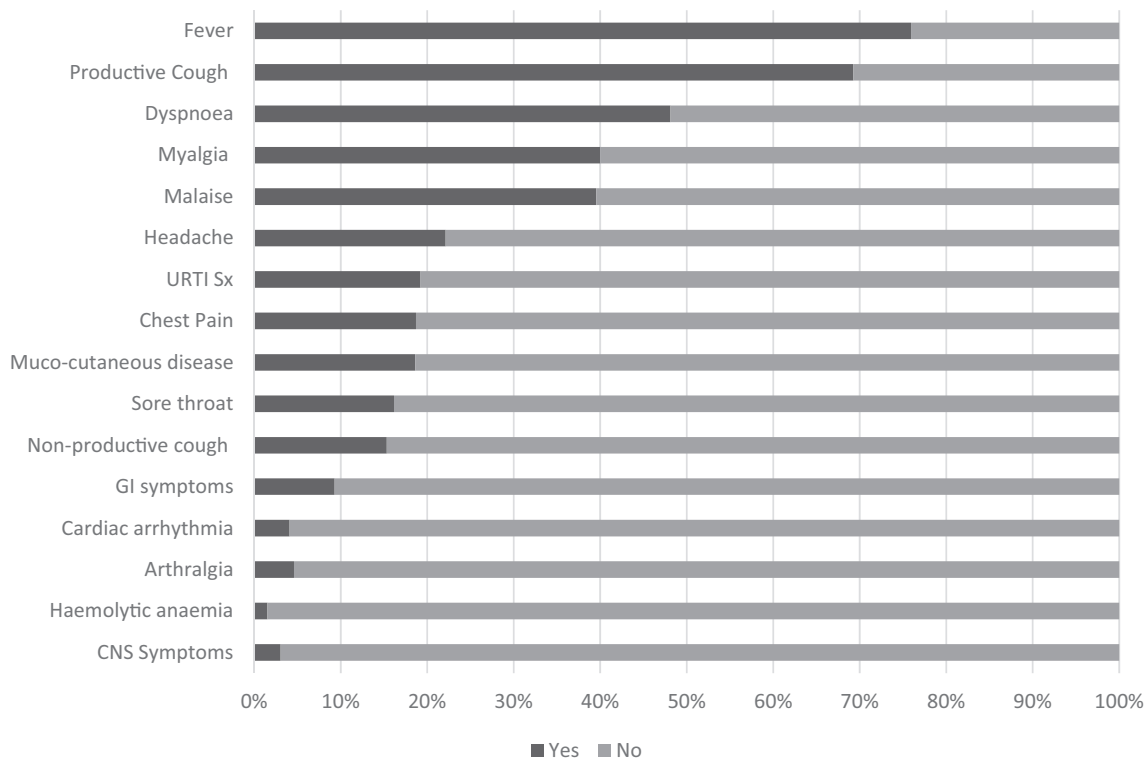


Fig. 3. Presenting symptoms of a *M. pneumoniae* Positive Patient. Key to Abbreviations: URTI: Upper Respiratory Tract Infection, GI: Gastrointestinal, CNS: Central Nervous System.

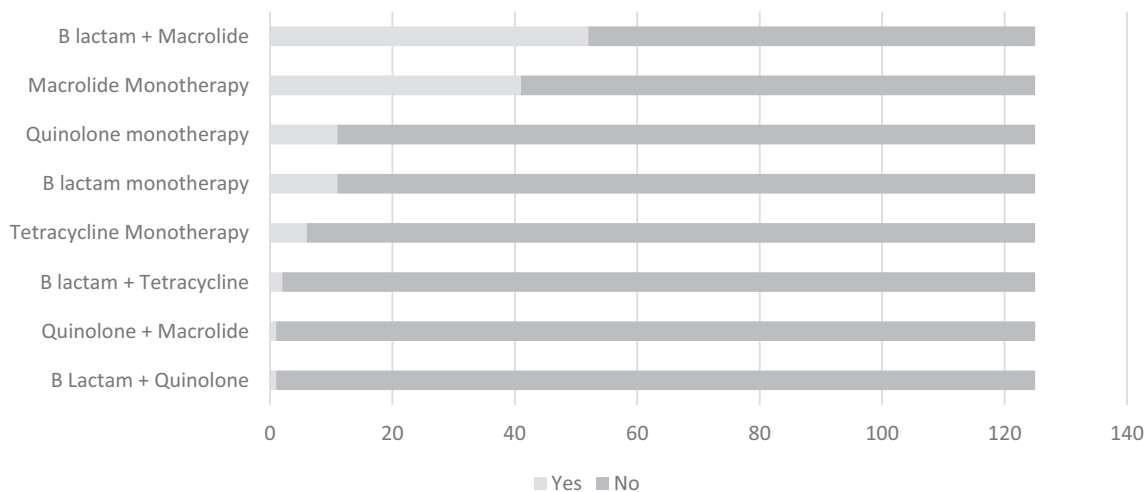


Fig. 4. Antimicrobial Treatment used to treat *Mycoplasma pneumoniae* positive patients (n = 125 patients).

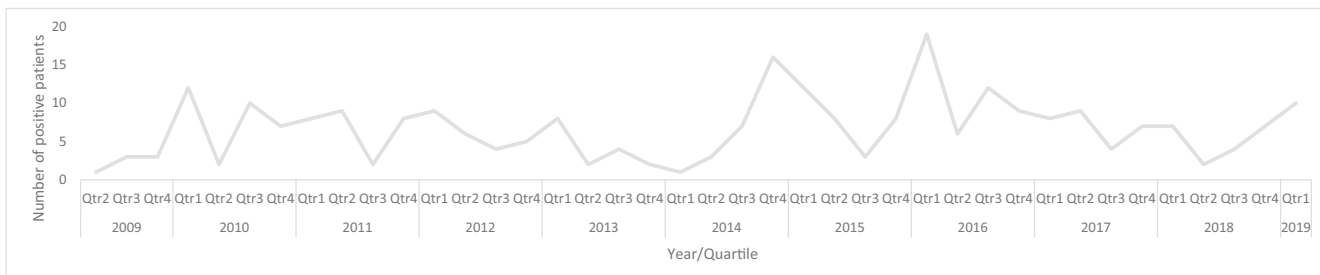


Fig. 5. Incidence of *Mycoplasma pneumoniae* positive cases by quarter during study period (March 2009–March 2019), n = 278.

M. pneumoniae worldwide (CDC, 2018), due to readily available commercial kits. However, serology lacks specificity, multiple samples are required to collect acute and convalescent serology, and time to results is not optimal for treatment decisions. The higher positivity rate in serology in our data set might result from the lack of specificity of this assay, and further work is needed in this area.

We found there to be a poor correlation was seen between PCR and serology, with only 29% patients tested being positive on both assays. This is supported by other studies. Chang et al, studying children, showed only 12.6% of their patient group were positive by both methods (Chang et al., 2014). This brings into question the purpose of doing both PCR and serology.

M. pneumoniae infection can be treated with macrolides, such as clarithromycin, quinolones such as levofloxacin and tetracyclines, such as doxycycline (BTS, 2009). *M. pneumoniae* is not susceptible to B-lactam antibiotics. The BTS and NICE currently recommend the B-lactam amoxicillin as first line treatment for patients admitted from the CAP, with Macrolides reserved for those with severe CAP or where atypical infection such as *M. pneumoniae* is suspected (NICE, 2019; BTS, 2009). Some patients in our group were treated with only B-lactams (9%). Faster *M. pneumoniae* diagnostics would almost certainly improve antimicrobial stewardship here – if the *M. pneumoniae* result were available sooner, this could have been B-lactam-sparing.

Macrolide resistance has been reported in up to 90% patients in some parts of the world, such as south-east Asia (Waites et al., 2019). However, Eurosurveillance data for England in 2015 suggests this to be much lower, at around 9.3% (Brown et al., 2015). 36% patients had their antibiotics changed based on the *M. pneumoniae* positive result, which shows the value of testing for *M. pneumoniae* from a stewardship perspective. This figure could be increased by faster diagnostics. The mortality in our data set was low (1.4%), and we felt there were insufficient data to determine whether a change in antimicrobials had an impact on mortality.

Study limitations

Our work reflects the retrospective experience of a single urban hospital. We made the decision to have a high threshold for defining samples as positive by PPA (see Methods, definitions), therefore the number of true positive cases could have been higher than we describe.

Conclusions

We describe 278 laboratory *M. pneumoniae* positive cases over a 10-year period at a London teaching hospital. The *M. pneumoniae* patient has a different demographic to the typical patient presenting with CAP. The serology and PCR results have poor correlation. Rapid, sensitive and specific diagnostics are needed to ensure patients are isolated early into their admission, and results are back fast enough to guide management. A large proportion of our patients received B-lactam antibiotics. B lactam use could be reduced if *M. pneumoniae* positive results were available sooner, and further work is required to further investigate the wider implications of this.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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