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Mycoplasma pneumoniae detection in children with respiratory tract infections and influence on management–a retrospective cohort study in Switzerland

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Short title: Prevalence of *Mycoplasma pneumoniae* in children

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Abstract

Aim

To evaluate the frequency of *Mycoplasma pneumoniae* in nasopharyngeal specimens from children with respiratory tract infections (RTI) and to detail clinical characteristics and management.

Methods

The study was designed as a retrospective cohort study. All children with RTI and nucleic acid amplification testing from nasopharyngeal specimens were analysed. Clinical data was extracted from electronic health records for all *M.pneumoniae* positive cases. Stored samples of cases and a random selection of matched controls were re-tested using a *M.pneumoniae-specific* nucleic acid amplification test.

Results

Of 4460 children, 70 (1.6%) were positive for *M.pneumoniae* with a median age of 6.4 (IQR:2.7–9.7). *M.pneumoniae* was the only organism identified in 50/64 (78%) cases. Macrolide treatment was prescribed in 52/65 (80%); prescription was empirical in 29/52 (56%) and targeted in 23/52 (44%) with no difference regarding patient age, oxygen requirement or duration of hospitalisation.

Conclusion

Prevalence of *M.pneumoniae* in nasopharyngeal specimens of children with RTI was low. Detection of *M.pneumoniae* influenced antibiotic prescriptions, but the benefit of early empirical versus targeted treatment remains unclear.

Keywords: Pneumonia, LRTI, x-ray, bronchitis, macrolides

Key notes

- This Swiss study investigated the prevalence of *M. pneumoniae* in 4460 children with respiratory tract infection
- In children with respiratory tract infection, in whom *M. pneumoniae* was detected by nucleic acid amplification testing, the majority had no other organism detected suggesting causal relationship
- Detection of *M. pneumoniae* resulted in prescription of a macrolide antibiotic in the majority of cases and empirical versus delayed macrolide treatment did not result in improved short-term outcome

Introduction

Mycoplasma pneumoniae respiratory tract infections (RTI) are common particularly in children and young adults between 5 and 20 years of age (1). *M. pneumoniae* causes upper and lower RTI and has been shown to be the most common bacterial pathogen for community-acquired pneumonia together with *Streptococcus pneumoniae* (2,3).
M. pneumoniae-associated RTI are most often mild and self-limiting in nature, and severe courses of disease are not common (4,5). In the past, detection of pathogen specific IgM

and IgG antibodies in serum has been the standard diagnostic test for *M. pneumoniae* RTI. However, elevated *M. pneumoniae*-specific serum antibodies have been shown to persist for several months after infection and are also common in asymptomatic children, thus limiting their use for detection of an acute infection (6). Molecular methods for the diagnosis of *M. pneumoniae* have therefore emerged and are now being used in clinical practice. However, the relationship between the detection of *M. pneumoniae* by nucleic acid amplification testing from a nasopharyngeal specimen and disease has been questioned in light of evidence that it is also commonly detected among asymptomatic children (6). The aim of this study was to investigate the frequency of *M. pneumoniae* in routine nasopharyngeal specimens from children with a RTI and to detail clinical characteristics and management of *M. pneumoniae*-positive patients.

Methods

Study setting and participants

Eligible for inclusion were children with an acute RTI and undergoing routine multiplex nucleic acid amplification testing from nasopharyngeal specimens, who presented to the emergency department of the University of Basel Children's Hospital, Basel, Switzerland, between June 2010 and December 2014. They were identified using laboratory records of the Division of Infection Diagnostics, Department of Biomedicine (Haus Petersplatz) at the University of Basel.

There were no further in- or exclusion criteria. Electronic health records were used to extract the following data: age at presentation, gender, date of presentation, admission status, clinical examination findings, results of multiplex nucleic acid amplification test from nasopharyngeal specimens, laboratory inflammatory markers, results of chest radiography as reported by senior radiologist, oxygen requirement, antibiotic treatment and duration of admission. Data from patients without a complete electronic health record was included in

the analysis and specified were applicable. The study was approved by the ethics committee of the University of Basel (EKNZ 2015-277).

Analysis of nasopharyngeal specimens

All nasopharyngeal specimens were routinely taken by experienced clinical staff and analysed using a commercially available multiplex nucleic acid amplification test (Respifinder®, Pathofinder, Maastricht, Netherlands) (7,8). The multiplex nucleic acid amplification test used detects *M. pneumoniae*, *Bordetella pertussis, Chlamydophila (synonym: Chlamydia) pneumoniae*, adenovirus, bocavirus, coronavirus (229E,HKU1, OC43, and NL63), human metapneumovirus (hMPV), influenzavirus (A, B and A(H1NA)pdm09), rhinovirus/enterovirus, parainfluenza virus (1-4), and respiratory syncytial virus (RSV) A and B. Routinely stored nasopharyngeal specimens were retested with an independent *M. pneumoniae-specific* nucleic acid amplification test based on Nilsson et al. using the primers GGCAGTCAACAAACCACGTATG, GGTGGTTGATGCGGTCAAA, and the Taqman probe CCCACCCGAACCGAAGCGG (9). This was done in all children where *M. pneumoniae* was detected in the multiplex nucleic acid amplification test and in 1:2-matched children with negative initial *M. pneumoniae* detection. Matching criteria were age (+/- 24 months), gender and season of presentation. Season was defined as autumn/winter (22 Sep – 19 March) and spring/summer (20 March – 21 Sept). The analysis of all samples were done at the Division of Infection Diagnostics in the Department of Biomedicine (Haus Petersplatz) at the University of Basel.

Statistical analysis

Statistical analysis for numerical data was performed using Mann-Whitney-U or Kruskal-Wallis tests; for categorical data, Pearsons-Chi-Square test was used. A p-value <0.05 was considered statistically significant.

Results

Characteristics of all children with nasopharyngeal specimen results

Over the 4.5-year study period 4460 nasopharyngeal specimens were obtained from children (median age 1.3 years) with acute RTI undergoing routine multiplex nucleic acid amplification testing from nasopharyngeal specimens. Of those, 70 (1.6%, 95%CI 1.2% to 1.9%) were positive for *M. pneumoniae* with a median age of 6.4 (IQR 2.7–9.7) years. The annual proportion for *M. pneumoniae*-positive samples ranged between 0.7 and 2.5%. Figure 1 shows age-stratified numbers and proportions of *M. pneumoniae* positive versus negative cases in the study period.

For the re-analysis of nasopharyngeal specimens 135 matched controls were identified. Complete medical records including all variables of interest were available in 63 (90%) of 70 *M. pneumoniae*-positive cases and in 124 (92%) of 135 controls. The most frequent discharge diagnoses of *M. pneumoni*ae-positive cases were; pneumonia 46/65 (71%), bronchitis/bronchiolitis 9/65 (14%) and upper respiratory tract infection 4/65 (6%).

In controls the discharge diagnoses were more diverse: upper respiratory tract infection 42/125 (33%), pneumonia 32/125 (26%) and bronchitis/bronchiolitis 30/125 (24%) were the most frequently recorded. Further baseline characteristics including clinical characteristics of both groups are summarised in Table 1.

Organisms identified

In 50 (78%) of 64 cases, *M. pneumoniae* was the only organism detected in nasopharyngeal specimens. Concomitant organisms were identified in 22%; 13 (20%) had two and one (1.5%) had three organisms detected. In *M. pneumoniae*-negative patients 80/124 (64%) had one organism detected, with rhinovirus/enterovirus being the most frequent organism,

and 7 (6%) had two organisms detected. Of the stored nasopharyngeal specimen samples 64/70 (91%) of the *M. pneumoniae*-positive cases and 126/135 (93%) of the *M. pneumoniae*-negative cases were available for retesting using a *M. pneumoniae*-specific nucleic acid amplification test. Of the samples with sufficient RNA/DNA, 55 (92%) were confirmed positive and none of those initially *M. pneumoniae* negative tested positive (Table 2). Therefore, the *M. pneumoniae*-specific nucleic acid amplification test of stored samples showed a sensitivity of 91,7% and a specificity of 100%.

Antibiotic treatment of cases and controls

At total of 52/65 (80%) cases were treated with a macrolide, which was Clarithromycin in 50 (96%) of them (Table 1). While 34 (38%) cases received a macrolide only, 18 (15%) were also prescribed concomitant treatment with a beta-lactam antibiotic. In 5 (10%) cases macrolide treatment was initiated before presentation to the hospital, in 24 (46%) it was started empirically at presentation and in 23 (44%) targeted; i.e. it was initiated after the result of *M. pneumoniae* detection had become available. Children receiving empiric compared to targeted macrolide treatment were statistically not different for age (p=0.21), oxygen requirement (p=0.78) or duration of hospitalisation (p=0.56). Thirteen cases did not receive an antibiotic treatment. In 11/13 (85%) cases clinical improvement and/or discharge from the hospital prior to the result being available were documented. In 2/13 (14%) the reason for withholding macrolide treatment is unknown. In controls, 19/125 (15%) were treated with a macrolide (Table 1). Of those, 13 (10%) received a macrolide only and 6 (5%) a combination treatment with a beta-lactam antibiotic. In addition, 33/125 (26%) of controls received a beta-lactam antibiotic only.

Chest radiography

A chest x-ray (CXR) was performed in 53 (81%) of 65 cases. Of those, 31 (58%) were classified as pneumonia/infiltrate, 18 (34%) as bronchitis/bronchial wall thickening, 3 (6%) as normal and for one the report was missing. In 126 children with negative *M. pneumoniae* detection, 64 (51%) had a CXR performed. Of those, 32 (50%) were classified as pneumonia/infiltrate, 17 (27%) as bronchitis/bronchial wall thickening, 14 (22%) as normal and one (2%) as an effusion. A CXR was more commonly performed in cases compared to controls ($p<0.001$), and they were more commonly abnormal in cases ($p=0.013$).

Discussion

We investigated the prevalence of *M. pneumoniae* in nasopharyngeal specimens from children with RTI. Of 4464 samples, only 70 (1.6%) were positive for *M. pneumoniae*. This is in line with findings from two previous studies in England/Wales and Germany in which *M. pneumoniae* was detected by PCR in 0.5 to 4% of patients with RTI (10,11). These studies were performed before the occurrence of the most recent *M. pneumoniae* epidemics in Europe in 2010 - 2012 and other recent studies describing much higher rates of *M. pneumoniae* detection in children with RTI (6,12-17). For example, a study in the Netherlands including children aged 3 months to 16 years from July 2008 to November 2011, showed that 16% of children with RTI (mean age 2.7 years) and 21% of asymptomatic children (mean age 5.6 years) were positive for *M. pneumoniae* by PCR (6). Importantly the prevalence of *M. pneumoniae* in children with RTI was lower in the years 2008/2009 compared to 2010/2011, with 4-7% and 21-24%, respectively, suggesting a considerable year to year variation likely influenced by the presence of local or national *M. pneumoniae* epidemics. The lower prevalence of *M. pneumonia*e in our study might be explained by an absence of an *M. pneumoniae* epidemic between 2010 and 2014 in our region. Such a diverse regional prevalence of *M. pneumoniae* seems possible as other European regions or

countries in the same time interval also did not report an increasing *M. pneumoniae* prevalence (18). Unfortunately, Switzerland has no national surveillance of *M. pneumoniae* related RTI and it remains therefore difficult to confer our data to the whole country.

Further to this, younger age has been shown in several studies to be associated with lower detection rates of *M. pneumoniae* (11,19,20). In the Dutch study mentioned above, the prevalence of *M. pneumoniae* in nasopharyngeal specimens in children under five years of age was 15% compared to 23% in older children. A study from Italy including children admitted to hospital with RTI with a mean age of 5.2 years showed a *M. pneumoniae* prevalence of 23% (12). Similarly, in a study in Chile including children with community acquired pneumonia at a mean age of 4.9 years, 26.4% were positive for *M. pneumoniae* by PCR (13). A recent study in the United States showed only 3% *M. pneumoniae* prevalence in children below 5 years of age with community acquired pneumonia, compared to 17% and 24% in children aged 5-9 years and 10-17 years, respectively (21). In our study, the median age of children included was 1.3 years and therefore lower than in all previous published studies. This has likely contributed to the low prevalence of *M. pneumoniae* in our setting as younger age is associated with lower *M. pneumoniae* detection rates.

The use of macrolides prior to testing might have also influenced the results, as effective antibiotic treatment may shorten the period of *M. pneumoniae* DNA persistence. In our study, 15% of the negative controls were already treated with a macrolide before a nasopharyngeal specimen was obtained. However, it is not known how long DNA might persist in children on effective treatment and as it is also impossible to reconstruct the duration of antimicrobial treatment these children received prior to testing, the use of macrolides might have had an influence, but this is uncertain.

As asymptomatic carriage rates as high as 56% have been reported, we had expected higher *M. pneumoniae* rates in our study, including in cases with a likely other cause of RTI such as influenza or RSV (6,22). However, the proportion of other pathogens identified was low in the group of *M. pneumoniae*-positive children. This strongly suggests that the detection of *M. pneumoniae* in the nasopharyngeal specimen as a single organism increases the likelihood of causality for the RTI. This notion is supported by another study reporting that over 40% of symptomatic children with *M. pneumoniae* detected had no other pathogen identified in their nasopharyngeal specimen (6). There is limited data comparing sensitivity of upper versus lower respiratory tract samples for *M. pneumoniae.* However, the available evidence indicates that in most cases both specimens are positive. Thus, detection of *M. pneumoniae* in the nasopharyngeal specimen of a symptomatic child with lower RTI strongly implicates this pathogen as the cause of the pneumonia (23,24). This interpretation is also in line with evidence showing the narrowing of the microbiota present in nasopharyngeal specimen from children with RTI (25). Further to this, CXR were more frequently performed and abnormal in the group of children with *M. pneumoniae* detected in nasopharyngeal specimens compared to those with negative results. This suggests an association of pneumonia in those with positive results.

Detection of *M. pneumoniae* led to a change of the empiric antibiotic treatment in almost half of the cases in the present study. However, data on the efficacy of macrolide treatment are controversial. Two recent systematic reviews concluded that there is insufficient evidence to draw conclusions about the efficacy of macrolides in the treatment of *M. pneumonia*e lower respiratory tract infections in children, and despite the fact that there is considerable evidence on anti-inflammatory properties of these agents (26-28). Interestingly, in our study no short-term treatment benefit such as reduced oxygen requirement or shorter hospital stay was found when comparing patients with empiric administration of a macrolide and those with prescription 48 hours later after *M. pneumoniae* infection had been confirmed by nucleic

acid amplification testing. This is consistent with another recently published study, which failed to show a significant difference in duration of admission in children with *M. pneumoniae* positive community-acquired pneumonia treated with a macrolide compared to no treatment (21).

One potential limitation of our study is that in our emergency department multiplex nucleic acid amplification testing from NPS is not a standard diagnostic tool for children not requiring admission. This may lead to a bias of testing children with clinically more severe RTI presentations, thereby rendering our findings not applicable to children with mild RTI. A further limitation is the potential lower sensitivity of multiplex PCR assays. However, re-testing of stored samples with an independent *M. pneumoniae*-specific nucleic acid amplification testing did not suggest lower sensitivity in line with recent results (29). However, sensitivity may also be influenced by the storage itself, which cannot be excluded in the current study setting. The study was done in a limited region and time frame and this may limit generalisability of the study results. Further to this, results and comparisons of results from the group with negative *M. pneumoniae* testing require cautious interpretation as they represent only a small and potentially non-representative group of *M. pneumoniae* negative patients.

Conclusions

Multiplex nucleic acid amplification testing is a sensitive screening tool and detection of *M. pneumoniae* influenced antibiotic prescription. Empiric treatment did not result in shorter admission duration. In view of the uncertainties about efficacy of antibiotic treatment and increasing antibiotic resistance worldwide, the significance of *M. pneumoniae* detection in children with RTI needs to be further investigated.

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Conflict of Interest Statement

JAB declares that her husband is employed by Novartis. All other authors have nothing to declare.

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List of abbreviations

CXR: chest x-ray

M. pneumoniae: Mycoplasma pneumoniae

PCR: polymerase chain reaction

RSV: respiratory syncytial virus

RTI: respiratory tract infection

CAP: community-acquired pneumonia

Table 1: Characteristics of cases with nasopharyngeal specimen positive for *M. pneumoniae* and matched *M. pneumoniae*-negative controls.

	Cases		Matched controls	
	n *	**Result**	**n ***	**Result**
Median (IQR) age in years	70	6.4 (2.7-9.7)	133	6.1 (4.5-9.2)
Number (%) male gender	70	42(60)	133	81 (61)
Number (%) sampled in winter season	70	46(66)	133	87 (65)
Number (%) other pathogen identified[†]	64	14 (22)	124	80 (64)
Number (%) admission to hospital	65	48 (74)	125	85 (68)
Median (IQR) CRP (mg/l)	52	17.5 (8.3 – 42.5)	85	42.5 (4.1- 32)
Number (%) X-ray performed	65	53(81)	126	64 (51)
Number (%) oxygen requirement	64	18 (28)	125	44 (35)
Pneumonia as diagnosis (%)	65	46 (71)	125	32 (26)
Number (%) any antibiotic treatment	65	55 (85)	125	52 (42)
Number (%) macrolide prescribed	65	52 (80)	125	19 (15)
Number (%) non-macrolide antibiotic prescribed	65	4 (5)	125	33 (26)
Number (%) >1 antibiotic prescribed	65	17 (26)	125	6 (5)

* information available

[†]matching criteria

CRP = C-reactive protein, IQR = interquartile range

Table 2: Results for retesting of *M. pneumoniae-specific nucleic acid amplification test*

	Cases (n=70)	**Controls (n=135)**
Number (%) available for retesting	64 (91)	128 (95)
Number PCR/DNA empty	4	2
Number (%) positive	55 (92)	0 (0)
Number (%) negative	5 (8)	126 (100)

Figure legends

Figure 1: Age-stratified analysis of *Mycoplasma pneumoniae* cases between 2010 – 2014

Years	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21 - 25
Mycoplasma positive	4	6	9	4	9	1	3	6	4	8	1	2	3	3	5	1	1	0	0	0	0	0
Mycoplasma negative	189	704	382	252	200	165	124	86	79	86	54	43	12	50	67	42	32	30	16	12	10	18
proportion	0,2	0,8	2,3	1,6	4,5	0,6	2,4	7	5,1	9,3	1,8	4,6	25	6	7,5	2,4	3,1	0	0	0	0	0