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Pediatric critical respiratory illness associated with *Mycoplasma pneumoniae*: a single-centre, retrospective, cohort study

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Abstract

Objectives. To measure the prevalence of *Mycoplasma pneumoniae* infection in critically ill children with respiratory infections and to determine if children in whom *M. pneumoniae* is detected differ systematically from those that are not.

Study design. A retrospective, single-centre, cohort study. All children aged 2 months – 18 years with presumed respiratory infection who were admitted to the McMaster Children's Hospital pediatric intensive care unit between September 2015-October 2016 were eligible. Participants were grouped by clinical syndrome (viral respiratory infection, asthma exacerbation, undifferentiated/uncomplicated pneumonia, pneumonia complicated by effusion/empyema, and 'other'). Testing for *M. pneumoniae* from nasopharyngeal specimens was done using a lab-developed PCR assay.

Results. There were 227 participants; the median age was 3.1 y, 43% were female, and 79% had medical comorbidities. Those with any pneumonia were significantly less likely to have a respiratory virus identified in their NPS and had significantly higher C-reactive protein values than those in the viral infection and asthma groups. There were 10 participants in whom *M. pneumoniae* was detected (4.4%, 95%CI 2.1-8.0%). *Mycoplasma*-positive children were older (difference 3.5 y, 95%CI 0.54 – 6.4 y) and had fewer viral co-infections (30% compared to 68%, p=0.02). The prevalence of *Mycoplasma* infection in children aged > 5 y with any pneumonia was 12.5% (95%CI 4-27%).

Conclusions. *M. pneumoniae* infection was not rare in a cohort of children admitted to the PICU with critical respiratory infection. Rapid diagnostic testing and targeted treatment should be considered in an effort to avert morbidity and mortality from respiratory infection.

What is known about this topic?

Mycoplasma pneumoniae is commonly detected in children with non-severe pneumonia.

Guidelines for the management of community-acquired pneumonia in children do not advocate empiric treatment with antibiotics active against *Mycoplasma* nor routine testing for this pathogen.

What this study adds:

Mycoplasma pneumoniae was detected in 12.5% of school-aged children with critically severe community-acquired pneumonia.

Systematic screening of school-aged children with pneumonia in the intensive care unit should be considered.

BACKGROUND

Community-acquired pneumonia (CAP) is a leading cause of pediatric hospitalization in North America (1). Children with respiratory disease severe enough to warrant admission to a paediatric intensive care unit (PICU) represent a minority of pneumonia-related hospitalizations (2) but infection-related morbidity and mortality is higher in this subgroup (3).

Streptococcus pneumoniae has long been considered the most important bacterial pathogen causing severe CAP (4,5). *Mycoplasma pneumoniae,* in contrast, is thought of as a less virulent pathogen, possibly due to how often *M. pneumoniae* infection self-resolves (6). Neither the American, Canadian, nor British guidelines recommend antimicrobials with activity against *M. pneumoniae* as first-line empiric treatment for pediatric CAP (7-9). However, this pathogen is a common cause of CAP, especially in school-aged children; *M. pneumoniae* was the most commonly identified bacterial pathogen in American children hospitalized with CAP, being detected in 8% of the overall cohort and in an astonishing 19% of school-aged children (2). A subsequent analysis of this data demonstrated that the children with *M. pneumoniae* infection could not be distinguished reliably on a clinical basis from those without and that, in contrast to dogma (8,9), single lobar infiltrates and pleural effusions were common on chest radiography (32% and 26% of those infected, respectively) (10). Furthermore, 12% of those with *M. pneumoniae* infection required intensive care (10). Clearly, the epidemiology of this common respiratory pathogen – and its effect on the clinical course and prognosis for children with severe CAP – should be evaluated further. The objective of our study was to describe children

admitted to the PICU of McMaster Children's Hospital (MCH) with respiratory infection and explore the epidemiology of *M. pneumoniae* infection in this population.

METHODS

Design

A single center, retrospective cohort study. Eligible children were those aged 2 months to 18 years admitted to the MCH PICU from September 2015 to October 2016 with a presumptive respiratory infection, as defined by a discharge diagnosis of any lower respiratory tract infection; we attempted to capture all those with possible infection, to minimize bias. Children aged less than two months were not included due to the very different epidemiology of respiratory infection in that age group. Furthermore, all eligible participants must have had a nasopharyngeal swab (NPS) taken and a respiratory symptom or sign, including at least one of the following: 1) tachypnoea as per age-specific norms (35); 2) cough; 3) increased work of breathing on exam, or 4) auscultatory findings such as crackles, wheeze, or rhonchi. The study was approved by the Hamilton Integrated Research Ethics Board. Patients or the public were not involved in study design. No formal sample size calculation was done.

Data collection

Information was obtained by retrospective chart review using a standardized data collection form. To group study participants by infection syndrome, the diagnoses of the clinical team were categorized as follows: viral infection without pneumonia (including bronchiolitis and

croup), undifferentiated/uncomplicated pneumonia, pneumonia complicated by effusion/empyema, asthma, and 'other.' If the clinical team recorded multiple diagnoses from the list above, they were classified using the following rules:

1. Participants marked as having both viral infection and pneumonia were classified as having 'pneumonia' if the chest radiograph was read by the radiologist as consistent with pneumonia and as 'viral infection' (without pneumonia) if not.
2. Participants marked as having both asthma and pneumonia were classified as having 'pneumonia' if the chest radiograph was read by the radiologist as consistent with pneumonia and as 'asthma' if not.
3. Participants marked as having both viral infection and asthma were classified as having 'asthma' if they were older than 1 y of age and had a history of atopy; if not, they were classified as 'viral infection.'

Laboratory testing

All children hospitalized with a potentially infectious respiratory ilness at MCH have an NPS performed routinely to identify respiratory viruses. NPSs are assayed using a lab-developed multiplex respiratory virus panel that detects RSV A/B, human metapneumovirus, influenza A/B, parainfluenza I-III, adenovirus, and rhinovirus/enterovirus. NPS specimens from eligible participants were identified and stored. After the surveillance period, NPSs from participants were batch-tested (ie. test results were not available to treating clinicians) using an HRLMP lab-developed multiplex PCR assay to detect *M. pneumoniae* and *Chlamydia pneumoniae*.

Any MP testing ordered prospectively by treating clinicians in the course of routine care was not processed using the lab-developed PCR assay but at Public Health Ontario Laboratories using a commercial multiplex real-time assay (ProPneumo-1 Assay; Gen-Probe Inc., San Diego, CA, USA), which also tests for *Chlamydia pneumoniae*. Samples that tested positive for MP underwent further testing at Public Health Ontario laboratories; nested PCR amplification and DNA sequencing of domain V of the partial 23S rRNA gene were performed to detect mutations at nucleotide positions 2063 and 2064, which are associated with macrolide resistance (11,12).

'Confirmed invasive bacterial infections' were defined as those children with a sterile-site culture (eg. blood, pleural fluid) positive for a pathogen (eg. *S. pneumoniae, S. pyogenes, S. aureus*). Cultures positive for coagulase-negative staphylococci were categorized as contaminants.

Statistical analysis

Descriptive statistics to describe the baseline characteristics were reported as count (percent) for categorical variables, and mean (standard deviation) or median (first quartile-third quartile, labeled as interquartile range [IQR]) for continuous variables depending on the distribution. Linear regression or ANOVA was used to compare normally-distributed continuous variables and Kruskal-Wallis testing was used when the distribution of the variable differed greatly from the normal distribution. Chi-square or Fisher exact testing was used to compare categorical variables between groups. No imputation of missing data was done.

RESULTS

In the study period there were 740 children admitted to the PICU; of these, 227 participants (31%) had a diagnosis of acute respiratory illness, an NPS, and at least one respiratory tract symptom or sign (Table 1). The median age was 3.1 y (IQR 1.4-6.2 y) and 43% were female. The majority of participants (79%) had comorbidities, with 58 (26%) having a neurodevelopmental problem or difficulty handling secretions, 28 (12%) having a cardiac problem, 89 (39%) having atopic disease/asthma, 24 (11%) having another pulmonary issue, 28 (12%) having a genetic disorder, and 21 (9%) being ex-premature infants. There were 13 participants (6%) that had a tracheostomy, 7 (3%) that were receiving home ventilation, 6 (3%) that were receiving home non-invasive ventilation, and 9 (4%) on home oxygen therapy. There were three deaths (1.3%) in the cohort and all had comorbidities. Sixteen participants (7%) were not up-to-date with DPTaP-Hib or PCV13 vaccine and 26 (11%) had not received the influenza vaccine.

In the PICU, the majority of participants (n=143, 63%) received high-flow oxygen support, 52 (29%) received CPAP/BiPAP, 41 (18%) required conventional mechanical ventilation, and 3 (1.3%) were treated with high-frequency oscillatory ventilation. Viral detections were common, with 80 (35%) participants positive for rhinovirus/enterovirus, 38 (17%) positive for RSV, and 24 (11%) positive for parainfluenza; only 76 (33%) tested negative for respiratory viruses. There were 8 participants with confirmed invasive bacterial infections. The median length of stay in the PICU was 3 days (IQR 2-5 d) and the medial length of stay in-hospital was 4 days (IQR 3-10 d).

Of the 227 participants, 51 (22%) were categorized as having had viral infection without pneumonia, 82 (36%) as uncomplicated pneumonia, 13 (5.8%) as pneumonia complicated by effusion/empyaema, 63 (27%) as an asthma exacerbation, and 18 (7.9%) as 'other.' There was considerable overlap in the white blood cell (WBC) distributions between categories. C-reactive protein measurements were clearly different between groups; those with pneumonia (median 45.5 mg/L) had significantly higher median CRP values than those in the viral infection (median 12.6 mg/L)and asthma (median 7.0 mg/L) groups, whereas those with pneumonia complicated by effusion/empyaema (median CRP 222.3 mg/L) had significantly higher CRP values than all other groups (Table 2). There were clear differences in the proportions of participants in each group with respect to viral NPS testing; 90% of the viral infection group and 71% of the asthma group had a respiratory virus detected, while only 60% of the uncomplicated pneumonia group and 23% of the complicated pneumonia group did (p<0.001). All of the participants in the uncomplicated and complicated pneumonia groups were treated with antibiotics, compared to 94% of the 'other' group, 73% of the viral-infection group, and 35% of the asthma group (p<0.0001). The duration of antibacterial treatment was also significantly shorter in the viral infection and asthma groups than all other groups (p<0.0001), as well as significantly longer in the complicated pneumonia group than in the uncomplicated pneumonia group (p=0.02).

There were 3 participants who tested positive, of 10 who had specimens tested, for *M. pneumoniae* through testing that was ordered prospectively by clinicians in the course of routine care (one sputum, one NPS, and one bronchoalveolar lavage [BAL]). There were an additional 7 participants that were found to have an NPS positive for *M. pneumoniae* via

retrospective study testing. The overall prevalence of *M. pneumoniae*-associated respiratory illness in the study cohort was therefore 10/227 (4.4%, 95%CI 2.1-8.0%). *Mycoplasma*-positive participants were significantly older than *Mycoplasma*-negative children (difference 3.5 y, 95% CI 0.54-6.4 y, *p*=0.02)(Table 3). The overall prevalence of *Mycoplasma* infection in participants aged > 5 years with any type of pneumonia was 12.5% (4 of 34 in the uncomplicated pneumonia group and 1 of 6 in the complicated pneumonia group, 95% CI 4-27%). In this older subset, there were zero *Mycoplasma*-positive participants in the viral infection or asthma groups.

None of the *Mycoplasma*-positive group had invasive bacterial infections. Three (30%) of the *Mycoplasma*-positive group had a respiratory viral pathogen detected as compared to 148 (68%) of the *Mycoplasma*-negative group (*p*=0.02). Antimicrobials were prescribed for significantly longer from the time of admission in the *Mycoplasma*-positives (median 11 days, IQR 7-17 d) as compared to the *Mycoplasma*- negatives (median 5 d, IQR 0-8 d, p=0.02); this difference remained significant when the analysis was restricted to only those participants with uncomplicated pneumonia (median 12 d as compared to median 7 d, p=0.004).

Of the 10 *Mycoplasma* isolates, 5 were macrolide-sensitive and 1 harboured the G2063 mutation in the 23S rRNA gene (overall prevalence 17%, 95%CI 0.4-64%); 3 isolates were low-level positives, and so could not be sequenced. One isolate was not retained. Only half of the participants with *Mycoplasma* infection were prescribed macrolide or fluoroquinolone antibacterials.

No study participants had *Chlamydia pneumoniae* detected in their NPS.

DISCUSSION

In this retrospective single-centre study, we found that 4% of all children with acute respiratory illness admitted to the PICU, the majority of whom had comorbidities, had *M. pneumoniae* detected in respiratory specimens. More importantly, 12.5% (95%CI 4-27%) of children diagnosed with pneumonia who were at least 5 years of age were positive for *M. pneumoniae.* Children that were *Mycoplasma*-positive were older, had fewer respiratory virus co-infections, were more often treated with antibacterials before admission, and received a longer course of antibacterials in-hospital than *Mycoplasma*-negative children. Half of the *Mycoplasma*-positive children did not receive antibacterials active against *Mycoplasma*. One of the six *Mycoplasma* isolates that could be sequenced harboured a macrolide resistance gene.

The fact that *Mycoplasma* was commonly detected in critically ill children would argue that routine surveillance for this pathogen should be considered, as others have suggested (13). Our results are consistent with the findings of the EPIC study, which also demonstrated that *M. pneumoniae* is found commonly in school-aged children with CAP (2), including children admitted to the intensive care unit (10). A recent retrospective cohort study of all children admitted to two PICUs in Australia over a 6-year period revealed 30 cases identified by testing done as part of routine clinical care among 3005 “nonelective infection-related admissions”, for a prevalence of ~1% (14). Those authors stated “*M. pneumoniae* infections in critically ill

children are uncommon" and noted that outcomes were comparable to those children without *Mycoplasma* detected. However, there is considerable variability in what constitutes an "infection-related admission" (14); furthermore, given the range of presenting symptoms/signs associated with this pathogen (10,15,16), one questions whether these clinicians would have been able to reliably identify which children merited testing, and so the true prevalence of *Mycoplasma* infection in this cohort could be much higher. The incidence of *M. pneumoniae* infection does vary widely by location and season (10,17) and so we cannot exclude the possibility that the prevalence observed in our study was higher than in years before or after.

An older iteration of the Canadian Paediatric Society guidelines for the management of CAP (circa 2011) recommended routine use of azithromycin for all children with 'severe' pneumonia because of the possibility of 'atypical infection', though diagnostic testing to identify atypical pathogens was not suggested or even mentioned (18). At that time, we thought that this practice would not represent appropriate antimicrobial stewardship, given that the majority of severe pediatric CAP is likely to be caused by *S. pneumoniae.* The CPS guidelines were later revised in 2015 and no longer recommend routine treatment with macrolides (8). They state that atypical pneumonia should be suspected in children with 'subacute, nonsevere pneumonia, presenting with features such as prominent cough, minimal leukocytosis, and a nonlobar infiltrate' and that azithromycin is recommended 'for suspected or proven *Mycoplasma* or *Chlamydia pneumoniae*' (8). Unfortunately, it has been repeatedly demonstrated that these symptoms and signs cannot reliably identify atypical pneumonia (10,15,16); it seems likely that many clinicians may not consider the possibility that *M.*

pneumoniae may play a significant role in the pathogenesis of critically ill children with respiratory compromise. Based on our data, we would suggest that clinicians be aware that a reasonable proportion of school-aged children with CAP admitted to the PICU may have an active *M. pneumoniae* infection and recommend empiric treatment with anti-*Mycoplasma* agents (eg. macrolides, doxycycline, fluoroquinolones) until diagnostic (molecular) testing results are available.

One obvious issue is that we cannot be certain of the therapeutic benefit of antibacterials (such as macrolides or doxycycline) for pediatric CAP presumed to be caused at least in part by *M. pneumoniae.* (We suspect that almost all clinicians would treat suspected or presumed *Legionella* CAP with antibacterials, but this pathogen is rare in pediatrics (19).) Furthermore, the detection of *Mycoplasma* in the respiratory tract does not prove causation, as coinfections have been shown to be common (10) and some investigators have documented high rates of PCR-positivity in control persons (20) (although others have not (10,21)). We would agree with other authors who have suggested that specific anti-*Mycoplasma* treatment might yield significant benefit, especially for those with severe disease, and have called for the execution of a randomized treatment trial (10,13). However, until results of a definitive treatment trial are available, we feel that the potential benefit of treating critically ill children with *Mycoplasma* detected in respiratory symptoms outweighs the potential antimicrobial stewardship harms of this strategy.

In conclusion, we found that *Mycoplasma pneumoniae* was detected in 12.5% of children aged 5 years and older admitted with CAP to the PICU of a children's hospital over a 13-month period. Consideration should be given to empiric anti-*Mycoplasma* antimicrobial therapy pending the result of rapid molecular diagnostic testing in this subset of critically ill children.

Contributorship Statement

HA designed the study, wrote the protocol, did the chart reviews, wrote the abstract, and revised the manuscript critically. JMP conceived the study question, provided input on study design, performed statistical analyses, and wrote the manuscript. KL and MS provided input on microbiologic methods, revised the protocol, did testing to identify *M. pneumoniae,* and revised the manuscript critically. AE and JBG provided input on microbiologic methods, did testing to identify resistance genes, and revised the manuscript critically.

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Table 1. Whole-cohort characteristics.

Median age (IQR)	3.14 y (1.39 – 6.15 y)
# Female (%)	98 (43%)
Fever recorded (%)	124 (56%)
Median duration of fever (IQR)	3 d (2 – 6.5 d)
Symptoms	
Cough	195 (30%)
Increased work of breathing	206 (91%)
Stridor	13 (5.8%)
Wheeze	112 (50%)
Chest pain	4 (1.8%)
Antibiotics given before presentation?	
Yes	45 (20%)
Amoxicillin	18
Amoxicillin/clavulanate	2
Cephalosporins	11
Macrolides	10
Other	11
Comorbid medical conditions	179 (79%)
neurologic/neurodevelopmental	58
tracheostomy	13
prematurity	44
chronic lung disease/bronchopulmonary dysplasia	20
asthma	89
cystic fibrosis	1
other pulmonary disease	24
genetic disease	28
cardiac disease	28
chronic liver disease	1
chronic kidney disease	7
endocrine disorders	14
malignancy	4
immunodeficiency	3
haemoglobinopathies	5
other	26
Home ventilation/oxygenation	16 (7%)
Mechanical ventilation	7
Noninvasive ventilation	6
Oxygen therapy without ventilation	3
Highest level of respiratory support given in PICU	
High frequency oscillatory ventilation	3 (1.3%)
Conventional mechanical ventilation	38 (17%)
BiPAP/CPAP	34 (15%)

High flow oxygen by nasal cannula	98 (43%)
Low flow oxygen (FiO2 > 0.4)	10 (4.4%)
Antibiotics given in PICU	171 (75%)
ampicillin	14
ceftriaxone	154
vancomycin	33
clarithromycin	3
azithromycin	37
ciprofloxacin	1
levofloxacin	11
tetracyclines	0

Table 2. Differences between diagnostic categories.

	Viral infection	Asthma	Pneumonia (uncomplicated/ undifferentiated)	Pneumonia (complicated by effusion)	Other
Count	51 (22%)	63 (27%)	82 (36%)	13 (5.8%)	18 (7.9%)
mean WBC (SD)	12.2 (4.3)	13.6 (5.0)	12.9 (8.1)	17.5 (11.8)	11.8 (7.3)
median CRP, mg/L (IQR)	12.6 (3.5-28.6)	7.0 (3.6-16.4)	45.5[1] (15.2-103)	222.3[2](177.6-259.1)	25.7 (15.0-82.6)
No respiratory virus detected	5 (9.8%)	18 (29%)	33 (40%)	10 (77%)	10 (56%)
median duration of antibiotics, days (IQR)	2[3] (0-4)	0[3] (0-1)	7 (7-10)	23[4] (14-27)	10 (7-14)

[1]median of pneumonia group significantly greater than that of viral infection group (p=0.007) and asthma group (p=0.0009) but significantly lower than that of complicated pneumonia group (p=0.009)
[2]median of complicated pneumonia group significantly greater than viral infection and asthma groups (p<0.0001), other group (p=0.01), and pneumonia group (p=0.009)
[3]median of viral infection and asthma groups significantly smaller than all other groups (p<0.0001)
[4]median of complicated pneumonia group also significantly higher than pneumonia group (p=0.02)

Table 3. Comparison of *Mycoplasma*-positive and *Mycoplasma*-negative participants.

	Mycoplasma-positive	*Mycoplasma*-negative	p
Count	10	217	n/a
mean age, y(SD)	8.1 (6.1)	4.7 (4.5)	0.02
# with viral infection or asthma diagnosis (%)	3 (30%)	111 (51%)	0.2

(restricted to participants > 5 y)	0	30 (41%)	0.08
# with no detectable respiratory virus in NPS	7 (70%)	69 (32%)	0.02
(restricted to participants > 5 y)	5 (83%)	36 (49%)	0.2
median duration of antibiotic treatment, d (IQR)	11 (7-17)	5 (0-8)	0.02
(restricted to participants with uncomplicated pneumonia)	12 (10-13)	7 (7-10)	0.004
Median length of stay in PICU, d (IQR)	4.5 (2-8)	3 (2-5)	0.1
(restricted to participants with uncomplicated pneumonia)	7 (2-8)	4 (2-7)	0.7
Median length of stay in hospital, d (IQR)	10 (5-13)	4 (3-9)	0.04
(restricted to participants with uncomplicated pneumonia)	13 (7-14)	7 (3-14)	0.3

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Pediatric critical respiratory illness: a single-centre, retrospective, cohort study

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Abstract

Objectives. To describe children admitted with respiratory infections to a paediatric intensive care unit (PICU), classify them by infection syndrome type, and determine the prevalence of *Mycoplasma pneumoniae* infection.

Study design. A retrospective, single-centre, cohort study. All children aged 2 months – 18 years with presumed respiratory infection who were admitted to the McMaster Children's Hospital PICU between September 2015-October 2016 were eligible. Subjects were grouped by clinical syndrome (viral respiratory infection, asthma exacerbation, undifferentiated/uncomplicated pneumonia, pneumonia complicated by effusion/empyema, and 'other'). Testing for *M. pneumoniae* from nasopharyngeal specimens was done using a lab-developed PCR assay.

Results. There were 221 subjects; the median age was 3.1 y, 44% were female, and 78% had medical comorbidities. Those with any pneumonia were significantly less likely to have a respiratory virus identified in their nasopharynges and had significantly higher C-reactive protein (CRP) values than those in the viral infection and asthma groups. There were 10 subjects in whom *M. pneumoniae* was detected (4.5%, 95%CI 2.2-8.2%). *Mycoplasma*-positive children were older (difference 3.5 y, 95%CI 0.66 – 6.4 y) and had fewer viral co-infections (30% compared to 69%, p=0.02). The prevalence of *Mycoplasma* infection in children aged > 5 y with any pneumonia was 13.2% (95%CI 4.4-28%).

Conclusions. There were differences in CRP and viral prevalence observed between children with different infection syndrome types. *M. pneumoniae* infection was not rare in school-aged children with pneumonia admitted to the PICU. Rapid diagnostic testing for *Mycoplasma* and targeted treatment in older, critically ill children should be considered in an effort to avert morbidity and mortality from respiratory infection.

What is known about this topic?

Mycoplasma pneumoniae is commonly detected in children with non-severe pneumonia.

Guidelines for the management of community-acquired pneumonia in children do not advocate first-line empiric treatment with antibiotics active against *Mycoplasma* nor routine testing for this pathogen.

What this study adds:

There are clear biochemical (eg. CRP) and microbiologic (eg. respiratory virus prevalence) differences between critically ill children with different respiratory infection syndromes.

Mycoplasma pneumoniae was detected in 13.2% of school-aged critically ill children with severe community-acquired pneumonia.

BACKGROUND

Community-acquired pneumonia (CAP) is a leading cause of pediatric hospitalization in North America (1). Children with respiratory disease severe enough to warrant admission to a paediatric intensive care unit (PICU) represent a minority (~20%) of pneumonia-related hospitalizations (2) but infection-related morbidity and mortality is higher in this subgroup (3).

Streptococcus pneumoniae has long been considered the most important bacterial pathogen causing severe CAP (4,5). *Mycoplasma pneumoniae,* in contrast, is thought of as a less virulent pathogen, possibly due to the fact that *M. pneumoniae* infection often self-resolves (6). Neither the American, Canadian, nor British guidelines recommend antimicrobials with activity against *M. pneumoniae* as first-line empiric treatment for pediatric CAP (7-9). However, this pathogen is a common cause of CAP, especially in school-aged children; *M. pneumoniae* was the most commonly identified bacterial pathogen in American children hospitalized with CAP, being detected in 8% of the overall cohort and in 19% of school-aged children (2). A subsequent analysis of this data demonstrated that the children with *M. pneumoniae* infection could not be distinguished reliably on a clinical basis from those without and that, in contrast to dogma (8,9), single lobar infiltrates and pleural effusions were common on chest radiography (32% and 26% of those infected, respectively) (10). Furthermore, 12% of those with *M. pneumoniae* infection required intensive care (10). Clearly, the epidemiology of this common respiratory pathogen – and its effect on the clinical course and prognosis for children with severe CAP – should be evaluated further. The objective of our study was to describe children admitted to the PICU of

McMaster Children's Hospital (MCH) with respiratory infection and determine the prevalence of *M. pneumoniae* infection in this population.

METHODS

Setting

MCH is a tertiary care centre serving a population of approximately 2.3 million residents. At the time of the study, the centre had 159 beds (12 PICU beds) and, on a yearly basis, admitted approximately 6500 children, with over 40 000 emergency department visits.

Design

A single center, retrospective cohort study. Eligible children were those aged 2 months to 18 years admitted to the MCH PICU from September 2015 to October 2016 with a presumptive respiratory infection, as defined by a discharge diagnosis of any lower respiratory tract infection. Discharge diagnoses for all patients leaving the PICU were reviewed on a biweekly basis by the principal investigator (HA); we attempted to capture all those with possible respiratory infection, to minimize bias. Children aged less than two months were not included due to the very different epidemiology of respiratory infection in that age group. Furthermore, all eligible subjects must have had a nasopharyngeal swab (NPS) taken less than a week after admission to hospital and a respiratory symptom or sign, including at least one of the following: 1) tachypnoea as per age-specific norms (35); 2) cough; 3) increased work of breathing on exam, or 4) auscultatory findings such as crackles, wheeze, or rhonchi. The study was approved

by the Hamilton Integrated Research Ethics Board, who waived the requirement for consent in this retrospective study. Patients or the public were not involved in study design. No formal sample size calculation was done.

Data collection

Information was obtained by retrospective chart review using a standardized data collection form. To group study subjects by infection syndrome, the discharge diagnoses of the clinical team were categorized as follows: viral infection without pneumonia (including bronchiolitis and croup), undifferentiated/uncomplicated pneumonia, pneumonia complicated by effusion/empyema, asthma, and 'other.' If the clinical team recorded multiple diagnoses from the list above, they were classified using the following rules:

1. Subjects marked as having both viral infection and pneumonia were classified as having 'pneumonia' if the chest radiograph was read by the radiologist as consistent with pneumonia and as 'viral infection' (without pneumonia) if not.
2. Subjects marked as having both asthma and pneumonia were classified as having 'pneumonia' if the chest radiograph was read by the radiologist as consistent with pneumonia and as 'asthma' if not.
3. Subjects marked as having both viral infection and asthma were classified as having 'asthma' if they were older than 1 y of age and had a history of atopy; if not, they were classified as 'viral infection.'

Laboratory testing

All children hospitalized with a potentially infectious respiratory illness at MCH have an NPS performed routinely to identify respiratory viruses, as per the institutional Acute Respiratory Infection Surveillance Protocol. NPSs are assayed using a lab-developed multiplex respiratory virus panel (11) that detects RSV A/B, human metapneumovirus, influenza A/B, parainfluenza I-III, adenovirus, and rhinovirus/enterovirus. NPS specimens from eligible subjects were identified and stored. After the surveillance period, NPSs from subjects were batch-tested (ie. test results were not available to treating clinicians) using an Hamilton Regional Laboratory Medicine Program lab-developed multiplex PCR assay to detect *M. pneumoniae* and *Chlamydia pneumoniae* that was validated against sequencing and external quality control materials.

Any MP testing ordered prospectively by treating clinicians in the course of routine care was not processed using the lab-developed PCR assay but at Public Health Ontario Laboratories using a commercial multiplex real-time assay (ProPneumo-1 Assay; Gen-Probe Inc., San Diego, CA, USA), which also tests for *Chlamydia pneumoniae*. Samples that tested positive for MP underwent further testing at Public Health Ontario laboratories; nested PCR amplification and DNA sequencing of domain V of the partial 23S rRNA gene were performed to detect mutations at nucleotide positions 2063 and 2064, which are associated with macrolide resistance (12,13).

'Confirmed invasive bacterial infections' were defined as those children with a sterile-site culture (ie. blood, pleural fluid) positive for a recognized pathogen. Cultures positive for coagulase-negative staphylococci were categorized as contaminants.

Statistical analysis

Descriptive statistics to describe the baseline characteristics were reported as count (percent) for categorical variables, and mean (standard deviation) or median (first quartile-third quartile, labeled as interquartile range [IQR]) for continuous variables depending on the distribution. Normality was assessed visually. T-tests or linear regression were used to compare normally-distributed continuous variables. Kruskal-Wallis testing was used when the distribution of the variable differed greatly from the normal distribution. If Kruskal-Wallis testing identified significant differences, nonparametric pairwise multiple comparisons of the groups using Dunn's test with Bonferroni adjustment were done. Chi-square or Fisher exact testing was used to compare categorical variables between groups. Alpha was set at 0.05, with no adjustments for multiple comparisons in this exploratory study. No imputation of missing data was done. Analyses were conducted using Stata v11.2 (College Station, TX).

RESULTS

In the study period there were 740 children admitted to the PICU; of these, 221 subjects (31%) had a diagnosis of acute respiratory illness, an NPS taken less than a week after admission, and at least one respiratory tract symptom or sign (Table 1). The median age was 3.1 y (IQR 1.4-6.0 y) and 44% were female. The majority of subjects (78%) had comorbidities (see Table 1). There were 13 subjects (6%) that had a tracheostomy, 7 (3%) that were receiving home ventilation, 6 (3%) that were receiving home non-invasive ventilation, and 9 (4%) on home oxygen therapy. There were three deaths (1.3%) in the cohort and all had comorbidities. Fourteen subjects (6%) were not up-to-date with DPTaP-Hib or PCV13 vaccine.

In the PICU, the majority of subjects (n=139, 63%) received high-flow oxygen support, 49 (22%) received CPAP/BiPAP, 38 (17%) required conventional mechanical ventilation, and 1 (0.45%) were treated with high-frequency oscillatory ventilation (see Table 2). Viral detections were common, with 79 (36%) subjects positive for rhinovirus/enterovirus, 37 (17%) positive for RSV, and 24 (11%) positive for parainfluenza; only 72 (33%) tested negative for respiratory viruses (see Table 3). There were 7 subjects with confirmed invasive bacterial infections. The median length of stay in the PICU was 3 days (IQR 2-5 d) and the medial length of stay in-hospital was 4 days (IQR 3-8 d).

Of the 221 subjects, 50 (23%) were categorized as having had viral infection without pneumonia, 81 (37%) as uncomplicated pneumonia, 12 (5.4%) as pneumonia complicated by effusion/empyaema, 63 (29%) as an asthma exacerbation, and 15 (6.8%) as 'other.' There was considerable overlap in the white blood cell (WBC) distributions between categories (see Tabl;e 4). C-reactive protein measurements were clearly different between groups; those with pneumonia (median 45.5 mg/L) had significantly higher median CRP values than those in the viral infection (median 12.6 mg/L)and asthma (median 7.0 mg/L) groups, whereas those with pneumonia complicated by effusion/empyaema (median CRP 203.8 mg/L) had significantly higher CRP values than all other groups. There were clear differences in the proportions of subjects in each group with respect to viral NPS testing; 90% of the viral infection group and 72% of the asthma group had a respiratory virus detected, while only 60% of the uncomplicated pneumonia group and 25% of the complicated pneumonia group did (p<0.0001). All of the subjects in the uncomplicated and complicated pneumonia groups were treated with

antibiotics, compared to 93% of the 'other' group, 74% of the viral-infection group, and 35% of the asthma group (p<0.0001). The duration of antibacterial treatment was also significantly shorter in the viral infection and asthma groups than all other groups (p<0.0001), as well as significantly longer in the complicated pneumonia group than in the uncomplicated pneumonia group (p=0.02).

There were 3 subjects who tested positive, of 10 who had specimens tested, for *M. pneumoniae* through testing that was ordered prospectively by clinicians in the course of routine care (one sputum, one NPS, and one bronchoalveolar lavage [BAL]). There were an additional 7 subjects that were found to have an NPS positive for *M. pneumoniae* via retrospective study testing. The overall prevalence of *M. pneumoniae*-associated respiratory illness in the study cohort was therefore 10/221 (4.5%, 95%CI 2.2-8.2%). *Mycoplasma*-positive subjects were significantly older than *Mycoplasma*-negative children (difference 3.5 y, 95% CI 0.66-6.4 y, p=0.02)(Table 4). The overall prevalence of *Mycoplasma* infection in subjects aged > 5 years with any type of pneumonia was 13.2% (4 of 33 in the uncomplicated pneumonia group and 1 of 5 in the complicated pneumonia group, 95% CI 4.4-28%). In this older subset, there were zero *Mycoplasma*-positive subjects in the viral infection or asthma groups.

None of the *Mycoplasma*-positive group had invasive bacterial infections. Three (30%) of the *Mycoplasma*-positive group had a respiratory viral pathogen detected as compared to 146 (69%) of the *Mycoplasma*-negative group (p=0.02, see Table 5). Antimicrobials were prescribed for significantly longer from the time of admission in the *Mycoplasma*-positives (median 11

days, IQR 7-17 d) as compared to the *Mycoplasma*- negatives (median 5 d, IQR 0-8 d, p=0.02); this difference remained significant when the analysis was restricted to only those subjects with uncomplicated pneumonia (median 12 d as compared to median 7 d, p=0.004).

Of the 10 *Mycoplasma* isolates, 5 were macrolide-sensitive and 1 harboured the G2063 mutation in the 23S rRNA gene (overall prevalence 17%, 95%CI 0.4-64%); 3 isolates were low-level positives, and so could not be sequenced. One isolate was not retained. Only half of the subjects with *Mycoplasma* infection were prescribed macrolide or fluoroquinolone antibacterials.

No study subjects had *Chlamydia pneumoniae* detected in their NPS.

DISCUSSION

In this retrospective single-centre study, we found that 4.5% of all children with acute respiratory illness admitted to the PICU, the majority of whom had comorbidities, had *M. pneumoniae* detected in respiratory specimens. More importantly, 13.2% (95%CI 4.4-28%) of children diagnosed with pneumonia who were at least 5 years of age were positive for *M. pneumoniae.* Children that were *Mycoplasma*-positive were older, had fewer respiratory virus co-infections, were more often treated with antibacterials before admission, and received a longer course of antibacterials in-hospital than *Mycoplasma*-negative children. Half of the *Mycoplasma*-positive children did not receive antibacterials active against *Mycoplasma*. One of the six *Mycoplasma* isolates that could be sequenced harboured a macrolide resistance gene.

Our results would argue that routine surveillance for this pathogen should be considered, as others have suggested (14), although infection was more rare in infants or preschool-aged children. Our results are consistent with the findings of the EPIC study, which also demonstrated that *M. pneumoniae* is found commonly in school-aged children with CAP (2), including children admitted to the intensive care unit (10). A recent retrospective cohort study of all children admitted to two PICUs in Australia over a 6-year period revealed 30 cases identified by testing done as part of routine clinical care among 3005 "nonelective infection-related admissions", for a prevalence of ~1% (15). Those authors stated "*M. pneumoniae* infections in critically ill children are uncommon" and noted that outcomes were comparable to those children without *Mycoplasma* detected. However, there is considerable variability in what constitutes an "infection-related admission" (15); furthermore, given the range of presenting symptoms/signs associated with this pathogen (10,16,17), one questions whether these clinicians would have been able to reliably identify which children merited testing, and so the true prevalence of *Mycoplasma* infection in this cohort could be much higher. The incidence of *M. pneumoniae* infection does vary widely by location and season (10,18) and so we cannot exclude the possibility that the prevalence observed in our study was higher than in years before or after.

An older iteration of the Canadian Paediatric Society guidelines for the management of CAP (circa 2011) recommended routine use of azithromycin for all children with 'severe' pneumonia because of the possibility of 'atypical infection', though diagnostic testing to identify atypical

pathogens was not suggested or even mentioned (19). One might question whether this practice would represent appropriate antimicrobial stewardship, given that the majority of severe pediatric CAP is likely to be caused by *S. pneumoniae.* The CPS guidelines were later revised in 2015 and no longer recommend routine treatment with macrolides (8). They state that atypical pneumonia should be suspected in children with 'subacute, nonsevere pneumonia, presenting with features such as prominent cough, minimal leukocytosis, and a nonlobar infiltrate' and that azithromycin is recommended 'for suspected or proven *Mycoplasma* or *Chlamydia pneumoniae*' (8). Unfortunately, it has been repeatedly demonstrated that these symptoms and signs cannot reliably identify atypical pneumonia (10,16,17); it seems likely that many clinicians may not consider the possibility that *M. pneumoniae* may play a significant role in the pathogenesis of critically ill children with respiratory compromise. Based on our data, we would suggest that clinicians be aware that a reasonable proportion of school-aged children with CAP admitted to the PICU may have an active *M. pneumoniae* infection and recommend empiric treatment with anti-*Mycoplasma* agents (eg. macrolides, doxycycline, fluoroquinolones) until diagnostic (molecular) testing results are available.

There were obvious limitations to our study. As noted previously, this was a retrospective design and included only a single centre over a 13-month period; as outbreaks with this pathogen have been frequently described (20), we cannot be certain that the prevalence of infection documented in this study is an accurate estimate of children hospitalized with critical respiratory illness in our region of Canada. It is also quite possible that hospital clinicians may

not have strictly followed hospital infection control policy and failed to sample the nasopharynges of some patients who otherwise would have been eligible. The study cohort only comprised 221 children and there were only 10 found to be positive for *M. pneumoniae;* consequently, 95% confidence intervals around our point estimates are wide. Having said that, the prevalence of *Mycoplasma* infection found in this small study was similar to that found in a much larger study conducted recently in the United States (2).

One obvious issue is that we cannot be certain of the therapeutic benefit of antibacterials (such as macrolides or doxycycline) for pediatric CAP presumed to be caused at least in part by *M. pneumoniae*; one systematic review found no clear difference in outcomes between children treated with *Mycoplasma*-active agents and those without (21). Furthermore, the detection of *Mycoplasma* in the respiratory tract does not prove causation, as coinfections have been shown to be common (10) and some investigators have documented high rates of PCR-positivity in control persons (22) (although others have not (10,23)); some investigators have identified novel serologic tests that can confirm active infection (24). We would agree with other authors who have suggested that specific anti-*Mycoplasma* treatment might yield significant benefit, especially for those with severe disease, and have called for the execution of a randomized treatment trial (10,14). However, until results of a definitive treatment trial are available, we feel that the potential benefit of treating critically ill children with *Mycoplasma* detected in respiratory symptoms outweighs the potential antimicrobial stewardship harms of this strategy.

In conclusion, we found that *Mycoplasma pneumoniae* was detected in 12.5% of children aged 5 years and older admitted with CAP to the PICU of a children's hospital over a 13-month period. Consideration should be given to empiric anti-*Mycoplasma* antimicrobial therapy pending the result of rapid molecular diagnostic testing in this subset of critically ill children.

Contributorship Statement

HA designed the study, wrote the protocol, did the chart reviews, wrote the abstract, and revised the manuscript critically. JMP conceived the study question, provided input on study design, performed statistical analyses, and wrote the manuscript. KL and MS provided input on microbiologic methods, revised the protocol, did testing to identify *M. pneumoniae,* and revised the manuscript critically. AE and JBG provided input on microbiologic methods, did testing to identify resistance genes, and revised the manuscript critically.

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Table 1. Whole-cohort baseline characteristics.

Median age (IQR)	3.11 y (1.39 – 6.02 y)
Age	
< 1 y	36 (16%)
1-2 y	45 (20%)
2-5 y	63 (29%)
5-10 y	45 (20%)
10-15 y	32 (14%)
# Female (%)	96 (44%)
Fever recorded (%)	120 (55%)
Median duration of fever (IQR)	3 d (2 – 6 d)
Symptoms	
Cough	191 (87%)
Increased work of breathing	202 (91%)
Stridor	13 (5.9%)
Wheeze	112 (51%)
Chest pain	4 (1.8%)
Antibiotics given before presentation?	
Yes	44 (20%)
Amoxicillin	18
Amoxicillin/clavulanate	2
Cephalosporins	10
Macrolides	10
Other	11
Comorbid medical conditions	174 (78%)
neurologic/neurodevelopmental	52
tracheostomy	13
other lung disease (including bronchopulmonary dysplasia)	36
asthma	89
cystic fibrosis	1
genetic disease	26
cardiac disease	26
chronic liver disease	1
chronic kidney disease	7
endocrine disorders	13
malignancy	3
immunodeficiency/immunosuppressant drugs	5
haemoglobinopathies	5
other	27
Home ventilation/oxygenation	16 (7%)
Mechanical ventilation	7
Noninvasive ventilation	6
Oxygen therapy without ventilation	3

Highest level of respiratory support given in PICU	
High frequency oscillatory ventilation	1 (0.45%)
Conventional mechanical ventilation	37 (17%)
BiPAP/CPAP	34 (15%)
High flow oxygen by nasal cannula	96 (43%)
Low flow oxygen (FiO2 > 0.4)	10 (4.4%)
Antibiotics given in PICU	166 (75%)
ampicillin	14
ceftriaxone	149
piperacillin-tazobactam	12
carbapenems	3
vancomycin	29
clindamycin	25
clarithromycin	3
azithromycin	35
levofloxacin	11
tetracyclines	0

Table 2. Whole-cohort clinical course in the PICU

Median length of stay in PICU (IQR)	3 d (2-5 d)
Highest level of respiratory support given in PICU	
High frequency oscillatory ventilation	1 (0.45%)
Conventional mechanical ventilation	37 (17%)
BiPAP/CPAP	34 (15%)
High flow oxygen by nasal cannula	96 (43%)
Low flow oxygen (FiO2 > 0.4)	10 (4.4%)
Antibiotics given in PICU	166 (75%)
ampicillin	14
ceftriaxone	149
piperacillin-tazobactam	12
carbapenems	3
vancomycin	29
clindamycin	25
clarithromycin	3
azithromycin	35
levofloxacin	11
tetracyclines	0

Table 3. Whole-cohort microbiology

Mucosal testing	
RSV	37 (17%)
Influenza	6 (3%)
Metapneumovirus	7 (3%)

Adenovirus	5 (2%)
Parainfluenza	24 (11%)
Rhino/enterovirus	79 (36%)
Mycoplasma	10 (5%)
Pleural fluid testing	
group A Streptococcus	2
Streptococcus anginosus	1
Blood culture testing	
Streptococcus pneumoniae	1
Haemophilus influenzae	1
Escherichia coli	1
Enterococcus faecalis	1

Table 4. Differences between diagnostic categories.

	Viral infection	Asthma	Pneumonia (uncomplicated/ undifferentiated)	Pneumonia (complicated by effusion)	Other
Count (%)	50 (22%)	63 (29%)	81 (37%)	12 (5.4%)	15 (6.8%)
mean WBC (SD)	12.1 (4.3)	13.6 (5.0)	13.0 (8.1)	19.0[1] (11.0)	12.8 (7.5)
missing	1	1	1	0	0
median CRP, mg/L (IQR)	12.6 (3.5-28.6)	7.0 (3.6-16.4)	45.5[2] (15.2-103)	203.8[3] (146.8-274.7)	23.6 (14.6-80.2)
missing	28	47	31	4	2
No respiratory virus detected	5 (10%)	18 (29%)	32 (40%)	9 (75%)	8 (53%)
missing	0	0	0	0	0
median duration of antibiotics, days (IQR)	2[4] (0-4)	0[4] (0-1)	7 (7-10)	23[5] (14-27)	10 (7-14)
missing	0	0	0	0	0

[1]mean of complicated pneumonia group significantly greater than the others (p=0.002).
[2]median of pneumonia group significantly greater than that of viral infection group (p=0.007) and asthma group (p=0.0009) but significantly lower than that of complicated pneumonia group (p=0.02)
[3]median of complicated pneumonia group significantly greater than viral infection and asthma groups (p<0.0001) and pneumonia group (p=0.009)
[4]median of viral infection and asthma groups significantly smaller than all other groups (p<0.0001)
[5]median of complicated pneumonia group also significantly higher than pneumonia group (p=0.02)

Table 5. Comparison of *Mycoplasma*-positive and *Mycoplasma*-negative subjects.

	Mycoplasma-positive	*Mycoplasma*-negative	p
Count	10	211	n/a
age, years mean (SD) median (IQR) % greater than 5 y	 8.1 (6.1) 7.2 (2.0 – 16) 60%	 4.6 (4.4) 3.0 (1.3 – 6.0) 34%	0.02
# with viral infection or asthma diagnosis (%)	3 (30%)	110 (51%)	0.2
(restricted to subjects > 5 y)	0	30 (42%)	0.08
# with no detectable respiratory virus in NPS	7 (70%)	65 (31%)	0.02
(restricted to subjects > 5 y)	5 (83%)	33 (46%)	0.1
median duration of antibiotic treatment, d (IQR)	11 (7-17)	5 (0-8)	0.02
(restricted to subjects with uncomplicated pneumonia)	12 (10-13)	7 (7-10)	0.004
Median length of stay in PICU, d (IQR)	4.5 (2-8)	3 (2-5)	0.1
(restricted to subjects with uncomplicated pneumonia)	7 (2-8)	4 (2-7)	0.7
Median length of stay in hospital, d (IQR)	10 (5-13)	4 (3-8)	0.03
(restricted to subjects with uncomplicated pneumonia)	13 (7-14)	7 (3-14)	0.3

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Pediatric critical illness associated with respiratory infection: a single-centre, retrospective, cohort study

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Pediatric critical illness associated with respiratory infection: a single-centre, retrospective, cohort study

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Abstract

Objectives. To describe critically ill children with respiratory infections, classify them by infection syndrome type, and determine the prevalence of *Mycoplasma pneumoniae* detection.

Study design. A retrospective, single-centre, cohort study. All children aged 2 months – 18 years with presumed respiratory infection who were admitted to a tertiary hospital paediatric intensive care unit between September 2015-October 2016 were eligible. Subjects were grouped by clinical syndrome (viral respiratory infection, asthma exacerbation, undifferentiated/uncomplicated pneumonia, pneumonia complicated by effusion/empyema, and 'other'). All subjects had nasopharyngeal swabs tested for respiratory viruses, *Mycoplasma pneumoniae,* and *Chlamydia pneumoniae*.

Results. There were 221 subjects; the median age was 3.1 y, 44% were female, and 78% had medical comorbidities. A majority (75%) was treated with antibiotics, most often ceftriaxone (90% of treated children). Those with any pneumonia were significantly less likely to have a respiratory virus identified in their nasopharynges and had significantly higher C-reactive protein (CRP) values than those in the viral infection and asthma groups. There were 10 subjects in whom *M. pneumoniae* was detected (4.5%, 95%CI 2.2-8.2%). *Mycoplasma*-positive children were older (difference 3.5 y, 95%CI 0.66 – 6.4 y) and had fewer viral co-infections (30% compared to 69%, p=0.02). The prevalence of *Mycoplasma* infection in children aged > 5 y with any pneumonia was 13.2% (95%CI 4.4-28%).

Conclusions. The majority of participants had respiratory viruses detected and were treated with broad-spectrum antibiotics. Differences in CRP and viral prevalence were observed between children with different infection syndrome types. *M. pneumoniae* infection was not rare in school-aged children with pneumonia admitted to the PICU. Attention to antibiotic treatment and rapid diagnostic testing for *Mycoplasma* in older, critically ill children should be considered to optimize management and avert morbidity and mortality from respiratory infection.

What is known about this topic?

Respiratory viruses and *Mycoplasma pneumoniae* are commonly detected in children with non-severe pneumonia.

Guidelines for the management of community-acquired pneumonia in children do not advocate first-line empiric treatment with antibiotics active against *Mycoplasma* nor routine testing for this pathogen.

What this study adds:

There are clear biochemical (eg. CRP) and microbiologic (eg. respiratory virus prevalence) differences between critically ill children with different respiratory infection syndromes.

Respiratory viruses were detected in 67% of the entire study cohort and *Mycoplasma pneumoniae* was detected in 13.2% of school-aged critically ill children with severe community-acquired pneumonia.

BACKGROUND

Community-acquired pneumonia (CAP) is a leading cause of pediatric hospitalization in North America (1). Children with respiratory disease severe enough to warrant admission to a paediatric intensive care unit (PICU) represent a minority (~20%) of pneumonia-related hospitalizations (2) but infection-related morbidity and mortality is higher in this subgroup (3).

Streptococcus pneumoniae has long been considered the most important bacterial pathogen causing severe CAP (4,5). *Mycoplasma pneumoniae,* in contrast, is thought of as a less virulent pathogen, possibly due to the fact that *M. pneumoniae* infection often self-resolves (6). Neither the American, Canadian, nor British guidelines recommend antimicrobials with activity against *M. pneumoniae* as first-line empiric treatment for pediatric CAP (7-9). However, this pathogen is a common cause of CAP, especially in school-aged children; *M. pneumoniae* was the most commonly identified bacterial pathogen in American children hospitalized with CAP, being detected in 8% of the overall cohort and in 19% of school-aged children (2). A subsequent analysis of this data demonstrated that the children with *M. pneumoniae* infection could not be distinguished reliably on a clinical basis from those without and that, in contrast to dogma (8,9), single lobar infiltrates and pleural effusions were common on chest radiography (32% and 26% of those infected, respectively) (10). Furthermore, 12% of those with *M. pneumoniae* infection required intensive care (10). Clearly, the epidemiology of this common respiratory pathogen – and its effect on the clinical course and prognosis for children with severe CAP – should be evaluated further. The objective of our study was to describe children admitted to the PICU of

McMaster Children's Hospital (MCH) with respiratory infection and determine the prevalence of *M. pneumoniae* detection in this population.

METHODS

Setting

MCH is a tertiary care centre serving a population of approximately 2.3 million residents. At the time of the study, the centre had 159 beds (12 PICU beds) and, on a yearly basis, admitted approximately 6500 children, with over 40 000 emergency department visits.

Design

A single center, retrospective cohort study. Eligible children were those aged 2 months to 18 years admitted to the MCH PICU from September 2015 to October 2016 with a presumptive respiratory infection, as defined by a discharge diagnosis of any lower respiratory tract infection. Discharge diagnoses for all patients leaving the PICU were reviewed on a biweekly basis by an investigator (HA); we attempted to capture all those with possible respiratory infection, to minimize bias. Children aged less than two months were not included due to the different epidemiology of respiratory infection in that age group. Furthermore, all eligible subjects had to have had a nasopharyngeal swab (NPS) taken less than a week after admission to hospital and a respiratory symptom or sign, including at least one of the following: 1) tachypnoea as per age-specific norms (35); 2) cough; 3) increased work of breathing on exam, or 4) auscultatory findings such as crackles, wheeze, or rhonchi. The study was approved by the

Hamilton Integrated Research Ethics Board, who waived the requirement for consent in this retrospective study. Patients or the public were not involved in study design. No formal sample size calculation was done.

Data collection

Information was obtained by retrospective chart review using a standardized data collection form. To group study subjects by infection syndrome, the discharge diagnoses of the clinical team were categorized as follows: viral infection without pneumonia (including bronchiolitis and croup), undifferentiated/uncomplicated pneumonia, pneumonia complicated by effusion/empyema, asthma, and 'other.' If the clinical team recorded multiple diagnoses from the list above, they were classified using the following rules:

1. Subjects marked as having both viral infection and pneumonia were classified as having 'pneumonia' if the chest radiograph was read by the radiologist as consistent with pneumonia and as 'viral infection' (without pneumonia) if not.
2. Subjects marked as having both asthma and pneumonia were classified as having 'pneumonia' if the chest radiograph was read by the radiologist as consistent with pneumonia and as 'asthma' if not.
3. Subjects marked as having both viral infection and asthma were classified as having 'asthma' if they were older than 1 y of age and had a history of atopy; if not, they were classified as 'viral infection.'

Laboratory testing

All children hospitalized with a potentially infectious respiratory illness at MCH have an NPS performed routinely to identify respiratory viruses, as per the institutional Acute Respiratory Infection Surveillance Protocol. NPSs are assayed using a lab-developed multiplex respiratory virus panel (11) that detects respiratory syncytial virus (RSV) A/B, human metapneumovirus, influenza A/B, parainfluenza I-III, adenovirus, and rhinovirus/enterovirus. NPS specimens from eligible subjects were identified and stored. After the surveillance period, NPSs from subjects were batch-tested (ie. test results were not available to treating clinicians) using an Hamilton Regional Laboratory Medicine Program lab-developed multiplex PCR assay to detect *M. pneumoniae* and *Chlamydia pneumoniae* that was validated against sequencing and external quality control materials.

Any MP testing ordered prospectively by treating clinicians in the course of routine care was not processed using the lab-developed PCR assay but at Public Health Ontario Laboratories using a commercial multiplex real-time assay (ProPneumo-1 Assay; Gen-Probe Inc., San Diego, CA, USA), which also tests for *Chlamydia pneumoniae*. Samples that tested positive for MP underwent further testing at Public Health Ontario laboratories; nested PCR amplification and DNA sequencing of domain V of the partial 23S rRNA gene were performed to detect mutations at nucleotide positions 2063 and 2064, which are associated with macrolide resistance (12,13).

'Confirmed invasive bacterial infections' were defined as those children with a sterile-site culture (ie. blood, pleural fluid) positive for a recognized pathogen. Cultures positive for coagulase-negative staphylococci were categorized as contaminants.

Statistical analysis

Descriptive statistics to describe the baseline characteristics were reported as count (percent) for categorical variables, and mean (standard deviation) or median (first quartile-third quartile, labeled as interquartile range [IQR]) for continuous variables depending on the distribution. Normality was assessed visually. T-tests or linear regression were used to compare normally-distributed continuous variables. Kruskal-Wallis testing was used when the distribution of the variable differed greatly from the normal distribution. If Kruskal-Wallis testing identified significant differences, nonparametric pairwise multiple comparisons of the groups using Dunn's test with Bonferroni adjustment were done. Chi-square or Fisher exact testing was used to compare categorical variables between groups. Alpha was set at 0.05, with no adjustments for multiple comparisons in this exploratory study. No imputation of missing data was done. Analyses were conducted using Stata v11.2 (College Station, TX).

RESULTS

In the study period there were 740 children admitted to the PICU; of these, 221 subjects (31%) had a diagnosis of acute respiratory illness, an NPS taken less than a week after admission, and at least one respiratory tract symptom or sign (Table 1). The median age was 3.1 y (IQR 1.4-6.0 y) and 44% were female. The majority of subjects (78%) had comorbidities (see Table 1). There were 13 subjects (6%) that had a tracheostomy, 7 (3%) that were receiving home ventilation, 6 (3%) that were receiving home non-invasive ventilation, and 9 (4%) on home oxygen therapy.

There were three deaths (1.3%) in the cohort and all had comorbidities. Fourteen subjects (6%) were not up-to-date with DPTaP-Hib or PCV13 vaccine.

In the PICU, the majority of subjects (n=139, 63%) received high-flow oxygen support, 49 (22%) received CPAP/BiPAP, 38 (17%) required conventional mechanical ventilation, and 1 (0.45%) were treated with high-frequency oscillatory ventilation (see Table 2). Viral detections were common, with 79 (36%) subjects positive for rhinovirus/enterovirus, 37 (17%) positive for RSV, and 24 (11%) positive for parainfluenza; only 72 (33%) tested negative for respiratory viruses (see Table 3). There were 7 subjects with confirmed invasive bacterial infections. The median length of stay in the PICU was 3 days (IQR 2-5 d) and the medial length of stay in-hospital was 4 days (IQR 3-8 d).

Of the 221 subjects, 50 (23%) were categorized as having had viral infection without pneumonia, 81 (37%) as uncomplicated pneumonia, 12 (5.4%) as pneumonia complicated by effusion/empyaema, 63 (29%) as an asthma exacerbation, and 15 (6.8%) as 'other.' There was considerable overlap in the white blood cell (WBC) distributions between categories (see Tabl;e 4). C-reactive protein measurements were clearly different between groups; those with pneumonia (median 45.5 mg/L) had significantly higher median CRP values than those in the viral infection (median 12.6 mg/L)and asthma (median 7.0 mg/L) groups, whereas those with pneumonia complicated by effusion/empyaema (median CRP 203.8 mg/L) had significantly higher CRP values than all other groups. There were clear differences in the proportions of subjects in each group with respect to viral NPS testing; 90% of the viral infection group and

72% of the asthma group had a respiratory virus detected, while only 60% of the uncomplicated pneumonia group and 25% of the complicated pneumonia group did (p<0.0001). All of the subjects in the uncomplicated and complicated pneumonia groups were treated with antibiotics, compared to 93% of the 'other' group, 74% of the viral-infection group, and 35% of the asthma group (p<0.0001). The duration of antibacterial treatment was also significantly shorter in the viral infection and asthma groups than all other groups (p<0.0001), as well as significantly longer in the complicated pneumonia group than in the uncomplicated pneumonia group (p=0.02).

There were 3 subjects who tested positive, of 10 who had specimens tested, for *M. pneumoniae* through testing that was ordered prospectively by clinicians in the course of routine care (one sputum, one NPS, and one bronchoalveolar lavage [BAL]). There were an additional 7 subjects that were found to have an NPS positive for *M. pneumoniae* via retrospective study testing. The overall prevalence of *M. pneumoniae*-associated respiratory illness in the study cohort was therefore 10/221 (4.5%, 95%CI 2.2-8.2%). *Mycoplasma*-positive subjects were significantly older than *Mycoplasma*-negative children (difference 3.5 y, 95% CI 0.66-6.4 y, p=0.02)(Table 4). The overall prevalence of *Mycoplasma* detection in subjects aged > 5 years with any type of pneumonia was 13.2% (4 of 33 in the uncomplicated pneumonia group and 1 of 5 in the complicated pneumonia group, 95% CI 4.4-28%). In this older subset, there were zero *Mycoplasma*-positive subjects in the viral infection or asthma groups.

None of the *Mycoplasma*-positive group had invasive bacterial infections. Three (30%) of the *Mycoplasma*-positive group had a respiratory viral pathogen detected as compared to 146 (69%) of the *Mycoplasma*-negative group (p=0.02, see Table 5). Antimicrobials were prescribed for significantly longer from the time of admission in the *Mycoplasma*-positives (median 11 days, IQR 7-17 d) as compared to the *Mycoplasma*- negatives (median 5 d, IQR 0-8 d, p=0.02); this difference remained significant when the analysis was restricted to only those subjects with uncomplicated pneumonia (median 12 d as compared to median 7 d, p=0.004).

Of the 10 *Mycoplasma* isolates, 5 were macrolide-sensitive and 1 harboured the G2063 mutation in the 23S rRNA gene (overall prevalence 17%, 95%CI 0.4-64%); 3 isolates were low-level positives, and so could not be sequenced. One isolate was not retained. Only half of the subjects with *Mycoplasma* infection were prescribed macrolide or fluoroquinolone antibacterials.

No study subjects had *Chlamydia pneumoniae* detected in their NPS.

DISCUSSION

In this retrospective single-centre study, we found that children with acute respiratory illness admitted to the PICU were predominantly preschool-aged, often had medical comorbidities, and frequently had viral pathogens detected in their nasopharynges. A minority had *M. pneumoniae* detected in respiratory secretions and even fewer had documented invasive bacterial infections. Despite this, 75% of the cohort was treated with antibacterials, most

commonly ceftriaxone (90% of treated children). Children diagnosed with asthma or viral infections were found to differ microbiologically (more viral pathogens detected) and biochemically (lower CRP values) from children diagnosed with pneumonia. Interestingly, 13.2% (95%CI 4.4-28%) of children diagnosed with pneumonia who were at least 5 years of age were positive for *M. pneumoniae.* Children that were *Mycoplasma*-positive were older, had fewer respiratory virus co-infections, were more often treated with antibacterials before admission, and received a longer course of antibacterials in-hospital than *Mycoplasma*-negative children. Half of the *Mycoplasma*-positive children did not receive antibacterials active against *Mycoplasma*.

The fact that respiratory viruses were frequently detected in critically ill paediatric patients with respiratory illness is not surprising, given the epidemiology of respiratory infection in children. Respiratory viruses have been long known to be important causes of paediatric pulmonary disease; for example, it has been estimated that there are at least 50 000 RSV-associated hospitalizations per year in the United States, with more than a quarter requiring intensive care (14). One large recent cohort study enrolling over two thousand children hospitalized for pneumonia (21% of whom required PICU admission) at three American hospitals detected respiratory viral pathogens in 73% (2). Viral coinfections may be even more common in children with critical illness, given that paediatric patients with bacterial pneumonia with confirmed viral coinfection have been found to have worse outcomes than those without (15).

It is unfortunate that almost three-quarters of all patients thought to have a purely viral syndrome received treatment with antibacterials. Needless to say, neither the Canadian, American, nor British guidelines recommend antibiotic treatment for viral lower respiratory tract infections (16-18). Furthermore, the vast majority of treated patients received ceftriaxone, which would be appropriate for some children with pneumonia (eg. immunocompromised patients) but not for others (eg. group A streptococcal empyaema). It is difficult to make definitive statements about appropriateness given that we did not examine the precise sequence of antibiotic administration in each patient in relation to the timing of microbiologic results. However, the fact that the vast majority of CAP in children is caused by pneumococcus or group A streptococcus, coupled with the observation that only 14 children (6%) received ampicillin, is very suggestive that antimicrobial stewardship was sub-optimal in the PICU during the study period.

Our results would argue that routine surveillance for *Mycoplasma* in school-aged children should be considered, as others have suggested (19). Our findings are consistent with other studies that demonstrated that *M. pneumoniae* is found commonly in school-aged children with CAP (2), including children admitted to the intensive care unit (10). The incidence of *M. pneumoniae* infection does vary widely by location and season (10,20) and so we cannot exclude the possibility that the prevalence observed in our study was higher than in years before or after. An older iteration of the Canadian Paediatric Society guidelines for the management of CAP (circa 2011) recommended routine use of azithromycin for all children with 'severe' pneumonia because of the possibility of 'atypical infection', though diagnostic

testing to identify atypical pathogens was not suggested or even mentioned (21). One might question whether this practice would represent appropriate antimicrobial stewardship, given that the majority of severe pediatric CAP is likely to be caused by *S. pneumoniae.* The CPS guidelines were later revised in 2015 and no longer recommend routine treatment with macrolides (8). They state that atypical pneumonia should be suspected in children with 'subacute, nonsevere pneumonia, presenting with features such as prominent cough, minimal leukocytosis, and a nonlobar infiltrate' and that azithromycin is recommended 'for suspected or proven *Mycoplasma* or *Chlamydia pneumoniae*' (8). Unfortunately, it has been repeatedly demonstrated that these symptoms and signs cannot reliably identify atypical pneumonia (10,22,23); it seems likely that many clinicians may not consider the possibility that *M. pneumoniae* may play a significant role in the pathogenesis of critically ill children with respiratory compromise. Based on our data, we would suggest that clinicians be aware that a reasonable proportion of school-aged children with CAP admitted to the PICU may have an active *M. pneumoniae* infection and recommend empiric treatment with anti-*Mycoplasma* agents (eg. macrolides, doxycycline, fluoroquinolones) until diagnostic (molecular) testing results are available. Of course, we cannot be certain of the therapeutic benefit of antibacterials targeting *M. pneumoniae*; one systematic review found no clear difference in outcomes between children treated with *Mycoplasma*-active agents and those without (24). Furthermore, the detection of *Mycoplasma* in the respiratory tract does not prove causation, as coinfections have been shown to be common (10) and some investigators have documented high rates of PCR-positivity in control persons (25) (although others have not (10,26)); some investigators have identified novel serologic tests that can confirm active infection (27). We

would agree with other authors who have suggested that specific anti-*Mycoplasma* treatment might yield significant benefit, especially for those with severe disease, and have called for the execution of a randomized treatment trial (10,19). However, until results of a definitive treatment trial are available, we feel that the potential benefit of treating critically ill children with *Mycoplasma* detected in respiratory symptoms outweighs the potential antimicrobial stewardship harms of this strategy.

There were obvious limitations to our study. As noted previously, this was a retrospective design and included only a single centre over a 13-month period; as outbreaks with this pathogen have been frequently described (28), we cannot be certain that the prevalence of infection documented in this study is an accurate estimate of children hospitalized with critical respiratory illness in our region of Canada. It is also quite possible that hospital clinicians may not have strictly followed hospital infection control policy and failed to sample the nasopharynges of some patients who otherwise would have been eligible. The study cohort only comprised 221 children and there were only 10 found to be positive for *M. pneumoniae;* consequently, 95% confidence intervals around our point estimates are wide. Having said that, the prevalence of viral and *Mycoplasma* detection found in this small study was similar to other estimates (2).

In conclusion, we found that the majority of children admitted to the PICU with respiratory illness over a 13-month period were positive for respiratory viruses and potentially inappropriate antibiotic treatment was common. *Mycoplasma pneumoniae* was detected in

13.2% of children aged 5 years and older diagnosed with CAP. Effort should be made to distinguish those with plausible bacterial infections from those without and consideration should be given to empiric anti-*Mycoplasma* antimicrobial therapy pending the result of rapid molecular diagnostic testing in a subset of critically ill children.

Contributorship Statement

HA designed the study, wrote the protocol, did the chart reviews, wrote the abstract, and revised the manuscript critically. JMP conceived the study question, provided input on study design, performed statistical analyses, and wrote the manuscript. KL and MS provided input on microbiologic methods, revised the protocol, did testing to identify *M. pneumoniae,* and revised the manuscript critically. AE and JBG provided input on microbiologic methods, did testing to identify resistance genes, and revised the manuscript critically.

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Table 1. Whole-cohort baseline characteristics.

Median age (IQR)	3.11 y (1.39 – 6.02 y)
Age	
< 1 y	36 (16%)
1-2 y	45 (20%)
2-5 y	63 (29%)
5-10 y	45 (20%)
10-15 y	32 (14%)
# Female (%)	96 (44%)
Fever recorded (%)	120 (55%)
Median duration of fever (IQR)	3 d (2 – 6 d)
Symptoms	
Increased work of breathing	202 (91%)
Cough	191 (87%)
Wheeze	112 (51%)
Stridor	13 (5.9%)
Chest pain	4 (1.8%)
Antibiotics given before presentation?	
Yes	44 (20%)
Amoxicillin	18
Amoxicillin/clavulanate	2
Cephalosporins	10
Macrolides	10
Other	11
Comorbid medical conditions	174 (78%)
asthma	89
other lung disease (including bronchopulmonary dysplasia)	36
neurologic/neurodevelopmental	52
genetic disease	26
cardiac disease	26
endocrine disorders	13
tracheostomy	13
chronic kidney disease	7
immunodeficiency/immunosuppressant drugs	5
haemoglobinopathies	5
malignancy	3
chronic liver disease	1
other	27
Home ventilation/oxygenation	16 (7%)
Mechanical ventilation	7
Noninvasive ventilation	6
Oxygen therapy without ventilation	3
Antibiotics given in PICU	166 (75%)

ampicillin	14
ceftriaxone	149
piperacillin-tazobactam	12
carbapenems	3
vancomycin	29
clindamycin	25
clarithromycin	3
azithromycin	35
levofloxacin	11
tetracyclines	0

Table 2. Whole-cohort clinical course in the PICU

Median length of stay in PICU (IQR)	3 d (2-5 d)
Highest level of respiratory support given in PICU	
High frequency oscillatory ventilation	1 (0.45%)
Conventional mechanical ventilation	37 (17%)
BiPAP/CPAP	34 (15%)
High flow oxygen by nasal cannula	96 (43%)
Low flow oxygen (FiO2 > 0.4)	10 (4.4%)
Antibiotics given in PICU	166 (75%)
ceftriaxone	149
azithromycin	35
vancomycin	29
clindamycin	25
ampicillin	14
piperacillin-tazobactam	12
levofloxacin	11
carbapenems	3
clarithromycin	3

Table 3. Whole-cohort microbiology

Mucosal testing	positive in 156 (71%)
Rhino/enterovirus	79 (36%)
RSV	37 (17%)
Parainfluenza	24 (11%)
Mycoplasma	10 (5%)
Metapneumovirus	7 (3%)
Influenza	6 (3%)
Adenovirus	5 (2%)
Pleural fluid testing	
group A Streptococcus	2
Streptococcus anginosus	1
Blood culture testing	

Streptococcus pneumoniae	1
Haemophilus influenzae	1
Escherichia coli	1
Enterococcus faecalis	1

Table 4. Differences between diagnostic categories.

	Viral infection	Asthma	Pneumonia (uncomplicated/ undifferentiated)	Pneumonia (complicated by effusion)	Other
Count (%)	50 (22%)	63 (29%)	81 (37%)	12 (5.4%)	15 (6.8%)
mean WBC (SD)	12.1 (4.3)	13.6 (5.0)	13.0 (8.1)	19.0[1] (11.0)	12.8 (7.5)
missing	1	1	1	0	0
median CRP, mg/L (IQR)	12.6 (3.5-28.6)	7.0 (3.6-16.4)	45.5[2] (15.2-103)	203.8[3] (146.8-274.7)	23.6 (14.6-80.2)
missing	28	47	31	4	2
No respiratory virus detected	5 (10%)	18 (29%)	32 (40%)	9 (75%)	8 (53%)
missing	0	0	0	0	0
median duration of antibiotics, days (IQR)	2[4] (0-4)	0[4] (0-1)	7 (7-10)	23[5] (14-27)	10 (7-14)
missing	0	0	0	0	0

[1]mean of complicated pneumonia group significantly greater than the others (p=0.002).
[2]median of pneumonia group significantly greater than that of viral infection group (p=0.007) and asthma group (p=0.0009) but significantly lower than that of complicated pneumonia group (p=0.02)
[3]median of complicated pneumonia group significantly greater than viral infection and asthma groups (p<0.0001) and pneumonia group (p=0.009)
[4]median of viral infection and asthma groups significantly smaller than all other groups (p<0.0001)
[5]median of complicated pneumonia group also significantly higher than pneumonia group (p=0.02)

Table 5. Comparison of *Mycoplasma*-positive and *Mycoplasma*-negative subjects.

	Mycoplasma-positive	*Mycoplasma*-negative	p
Count	10	211	n/a
age, years			0.02
mean (SD)	8.1 (6.1)	4.6 (4.4)	
median (IQR)	7.2 (2.0 – 16)	3.0 (1.3 – 6.0)	
% greater than 5 y	60%	34%	

# with viral infection or asthma diagnosis (%)	3 (30%)	110 (51%)	0.2
(restricted to subjects > 5 y)	0	30 (42%)	0.08
# with no detectable respiratory virus in NPS	7 (70%)	65 (31%)	0.02
(restricted to subjects > 5 y)	5 (83%)	33 (46%)	0.1
median duration of antibiotic treatment, d (IQR)	11 (7-17)	5 (0-8)	0.02
(restricted to subjects with uncomplicated pneumonia)	12 (10-13)	7 (7-10)	0.004
Median length of stay in PICU, d (IQR)	4.5 (2-8)	3 (2-5)	0.1
(restricted to subjects with uncomplicated pneumonia)	7 (2-8)	4 (2-7)	0.7
Median length of stay in hospital, d (IQR)	10 (5-13)	4 (3-8)	0.03
(restricted to subjects with uncomplicated pneumonia)	13 (7-14)	7 (3-14)	0.3