**Supplementary Material**

***Laboratory methods***

Briefly, Gram staining and bacterial culture were performed on blood and lower respiratory (including pleural fluid) samples at each study site with the use of standard techniques. Real-time polymerase chain reaction targeting *lytA* (*Streptococcus pneumoniae*) and *spy* (*Streptococcus pyogenes*) genes was performed on whole blood and pleural fluid at CDC. Using PCR, pleural fluid was also tested at the University of Utah for bacterial pathogens (*H. influenzae* and other Gram-negative bacteria, *Staphylococcus aureus*, *Streptococcus anginosus/mitis*, *S. pneumoniae*, and *S. pyogenes*). At each study site nasopharyngeal/oropharyngeal (NP/OP) swabs were tested using CDC-developed real-time PCR methods for detection of viruses such as adenovirus (AdV); coronaviruses; human metapneumovirus (HMV); human rhinovirus; influenza A/B viruses; parainfluenza viruses (PIV 1,2,3); and respiratory syncytial virus (RSV) and bacteria such as *Chlamydia pneumoniae*; in addition to *M. pneumoniae*. Quality assurance and monitoring protocols maintained standardization among sites. Serology for certain viruses such as AdV, HMPV, influenza A/B, PIV, and RSV were performed at CDC on available paired acute and convalescent sera.

*Control Enrollment*

We enrolled a convenience sample of asymptomatic children undergoing elective surgery at two study sites (Nashville and Salt Lake City) from February 1, 2011, to June 30, 2012.[15] Exclusion criteria for the children with pneumonia were also applicable to control children. In addition, controls were excluded if they had fever or respiratory symptoms within 14 days before or after enrollment (per telephone interview); receipt of live attenuated influenza vaccine ≤7 days before enrollment; or were undergoing otolaryngologic surgery.[15] Nasopharyngeal/oropharyngeal specimens from asymptomatic controls were tested using a CDC-developed PCR assay for *M. pneumoniae* detection (Mp-PCR). The pediatric controls were not included in these analysis.

***Macrolide susceptibilities***:

High-resolution melt (HRM) analysis with PCR allows detection of an A to G transition at position 2063 or 2064 within the 23S rRNA gene, the two mutations most commonly associated with macrolide resistance in *M. pneumoniae*. Melt profiles are classified as sensitive or resistant by comparison, to reference strains included in each run. Sequencing analysis was performed on all isolates classified as resistant based on the melt profile in order to identify the specific single-base mutation (A2063G or A2064G) in the 23S rRNA gene. The details of this methodology have been described elsewhere.[17, 18]

***Results***:

*Clinical features by age categories*

When stratified by age, Mp-PCR-positive children <2 years old were less likely to have cough (81% vs. 94%; P<0.03), and Mp-PCR-positive children 2-4 years old were less likely to have rhinorrhea (40% vs. 78%; P<0.01) when compared with Mp-PCR-negative children inthe respective age groups. Mp-PCR-positive children 5-9 years old were more likely to have cough (100% vs. 94%; P=0.03), headache (57% vs. 42%, P=0.02), abdominal pain (57% vs. 43%), P=0.04), diarrhea (31% vs. 18%, P=0.01), ear pain (19% vs. 10%, P=0.04), and rash (15% vs. 7%; P=0.04) but less likely to have wheezing (45% vs. 64%, P<0.01) and chest pain (24% vs. 43%, P<0.01) than Mp-PCR-negative children. Mp-PCR-positive children 10-17 years old were more likely to have chills (77% vs. 57%; P<0.01), and less likely to have wheezing (36% vs. 51%, P=0.04), rhinorrhea (34% vs. 50%, P=0.03), and chest retraction (13% vs. 30%, P<0.01) than Mp-PCR-negative children.

No significant clinical features were distinguishable between children <2 years old with and without *M. pneumoniae*. Mp-PCR-positive children 2-4 years old were more likely to have rales (63% vs. 42%; P=0.02) and less likely to have documented fever (23% vs. 43%, P=0.04) or have asthma (23% vs. 42%; P=0.04) when compared to Mp-PCR-negative children*.* Mp-PCR-positive children 5-9 years old were more likely to have rales (63% vs. 41%; P<0.01) and less likely to have chest indrawing (30% vs. 49%, P<0.01) and wheezing (28% vs. 48%, P<0.01) or have asthma (31% vs. 53%; P<0.01) when compared to Mp-PCR-negative children. Mp-PCR-positive children 10-17 years old were more likely to have rales (59% vs. 36%, P<0.01), hypoxia (42% vs. 28%, P=0.03) and egophony (6% vs. 0.5%; P<0.01) and less likely to have chest indrawing (9% vs. 28%, P<0.01) or history of seizure (5% vs. 17%, P=0.01) when compared to Mp*-*PCR-negative children.

*Asymptomatic controls*

Among 521 asymptomatic pediatric controls, *M. pneumoniae* was detected in only three children (0.6%). The children were all male and from Salt Lake City, Utah; 2(67%) were non-Hispanic white and 1(33%) was Hispanic.

**Supplementary Table 1: *Demographics of children hospitalized for M. pneumoniae community-acquired pneumonia\*, by site* (n=182)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| ***Characteristics*** | ***Memphis***  n=60  n (%) | ***Nashville***  n=41  n (%) | ***Salt lake City***  n=81  n (%) | ***Overall***  (n=182)  n (%) |
| **Age (*years*)** |  |  |  |  |
| Median (IQR) | 5.5 (2.5 –10) | 7 (5 –10) | 9 (6 – 12) | 7 (4 –11) |
| **Age groups** |  |  |  |  |
| 0-23 months | 13 (22) | 4 (10) | 4 (5) | 21 (12) |
| 2-4 years | 11 (18) | 6 (15) | 13 (16) | 30 (17) |
| 5-9 years | 19 (32) | 18 (44) | 30 (37) | 67 (37) |
| 10-17 years | 17 (28) | 13 (32) | 34(42) | 64 (35) |
| **Sex** |  |  |  |  |
| Male | 34 (57) | 22 (54) | 53 (65) | 109 (60) |
| Female | 26 (43) | 19 (46) | 28 (35) | 73 (40) |
| **Race/Ethnicity** |  |  |  |  |
| Non-Hispanic white | 29 (48) | 24 (58) | 60 (74) | 113 (62) |
| Non-Hispanic black | 25 (42) | 7 (17) | 0 | 32 (18) |
| Hispanic | 3 (5) | 8 (20) | 18 (22) | 29 (16) |
| Other† | 3 (5) | 2 (5) | 3 (4) | 8 (4) |
| **Insurance** |  |  |  |  |
| Public | 33 (55) | 22 (54) | 23 (28) | 78 (43) |
| Private | 23 (38) | 19 (46) | 52 (64) | 94 (52) |
| Both | 0 | 0 | 2 (3) | 2 (1) |
| None | 4 (7) | 0 | 4 (5) | 8 (4) |

\* Note: CAP with *M. pneumoniae*: A radiographically-confirmed CAP patient enrolled in EPIC with a positive *M. pneumoniae* PCR.

†Non-Hispanic-Asian; Non-Hispanic-Hawaiian/Pacific Islander; Non-Hispanic-American Indian or Alaska Native; Multiracial; Other

Note: Percentages may not total 100 because of rounding

**Supplementary Table 2. *Pathogens detected* *among children hospitalized with and without M. pneumoniae community-acquired pneumonia*\* *and documented* *co-detections*†**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Co-detection** | | ***M. pneumoniae* PCR-positive**  **n (%)** | ***M. pneumoniae* PCR-negative**  **n (%)** | **OR (95% CI)** |
| Children hospitalized with CAP who had the opportunity to undergo testing for both bacterial and viral testing | | 178 (98) | 2001 (97) |  |
|  | |  |  |  |
| *Co-detection identified* **†**a | | 50 (28) | 523 (26) |  |
| *Any viral co-detection*b | | 49 (98) | 521 (99) | 0.2 (0.02 – 2.1) |
|  | Human rhinovirus | 28 (57) | 230 (44) | 1.7 (0.9 – 3.1) |
|  | Respiratory syncytial  virus | 9 (18) | 254 (49) | 0.2 (0.1 – 0.5) |
|  | Influenza A and B viruses | 7 (14) | 87 (17) | 0.8 (0.4 – 1.9) |
|  | Human metapneumovirus | 6 (12) | 113 (22) | 0.5 (0.2 – 1.2) |
|  | Human coronavirus | 5 (10) | 93 (18) | 0.5 (0.1 – 1.3) |
|  | Adenovirus | 5 (10) | 192 (37) | 0.2 (0.08 – 0.5) |
|  | Parainfluenza virus c | 3 (6) | 81 (16) | 0.3 (0.1 – 1.1) |
| *Any bacterial co-detection*b | | 2 (4) | 105 (20) | 0.2 (0.04 – 0.8) |
|  | *Streptococcus pneumoniae* | 2 (100) | 51 (49) | NAd |

\* Note: *M. pneumoniae* PCR-positive: A radiographically-confirmed CAP patient enrolled in EPIC with a positive *M. pneumoniae* PCR.

*M. pneumoniae* PCR-negative: A patient enrolled in EPIC with radiographically-confirmed pneumonia with a negative *M. pneumoniae* PCR.

**†**Co-detection was defined as detection of more than one bacterial or viral pathogens in any combination, including *M. pneumoniae*.

aCAP with *M. pneumoniae* who did not have co-detections (n=128) and CAP without *M. pneumoniae* who did not have co-detections (n= 1478)

**b**The numbers and percentages are not mutually exclusive

c Parainfluenza virus: PIV 1=2; PIV 3=1

d Fisher’s Exact P=0.2 as 50% of the cells have expected counts less than 5

**Supplementary Table 3. *Select clinical features with significant differences among children hospitalized for community-acquired pneumonia*\* *with M. pneumoniae*† *compared with non-Mycoplasma bacterial*‡ *and viral pneumonia*§**

|  |  |  |  |
| --- | --- | --- | --- |
| **Characteristic** | ***M. pneumoniae* PCR-positive pneumonia†**  **(n=128)**  **n (%)** | ***M. pneumoniae* PCR-negative bacterial pneumonia‡ (n=36) n(%)** | ***M. pneumoniae* PCR-negative viral pneumonia§**  **(n=1559)**  **n (%)** |
| Duration of symptoms prior to hospitalization  Median (interquartile range) in days | 7.5 (5.5 – 9.8) | 4.6 (2.5 – 10.0)a | 3.5 (2.0 – 5.6)a |
| **Study Site** |  |  |  |
| Salt Lake City, Utah (*Reference*) | 60 (47) | 15 (42) | 525 (34) |
| Memphis, Tennessee | 35 (27) | 6 (16) | 634 (41)a |
| Nashville, Tennessee | 33 (26) | 15 (42) | 400 (25) |
| **Age in years** |  |  |  |
| <5 (*Reference*) | 26 (20) | 21 (58) | 1240 (79) |
| 5-9 | 52 (41) | 11 (31)a | 212 (14)a |
| 10-17 | 50 (39) | 4 (11)a | 107 (7)a |
| **Race/Ethnicity** |  |  |  |
| Non-Hispanic white (*Reference*) | 84 (66) | 20 (55) | 529 (34) |
| Non-Hispanic black | 20 (16) | 7 (19) | 577 (37)a |
| Hispanic | 19 (15) | 6 (17) | 317 (20)a |
| **Clinical presentationb** |  |  |  |
| Fatigue | 101 (79) | 30 (83) | 1054 (68)a |
| Dyspnea | 78 (61) | 28 (78) | 1130 (72)a |
| Chills | 77 (60) | 19 (53) | 503 (32)a |
| Headache | 61 (48) | 17 (47) | 271 (17)a |
| Sore throat | 62 (48) | 14 (39) | 408 (26)a |
| Abdominal pain | 58 (45) | 16 (44) | 276 (18)a |
| Wheezing | 52 (41) | 16 (44) | 1033 (66)a |
| Runny nose | 50 (39) | 19 (53) | 1180 (76)a |
| Myalgia | 47 (37) | 18 (50) | 233 (15)a |
| Chest pain | 38 (27) | 18 (50) | 301 (19)a |
| Chest retraction | 29 (23) | 19 (44)a | 730 (47)a |
| Rash | 22 (17) | 1 (3)a | 157 (10)a |
| Conjunctivitis | 4 (3) | 0 | 146 (9)a |
| **Underlying condition** |  |  |  |
| Any condition (≥1 condition) | 55 (43) | 8(22)a | 812 (52)a |
| Asthma/reactive airway disease | 35 (27) | 6 (17) | 555 (36) |
| Chromosomal disorder | 11 (9) | 1 (3) | 68 (4)a |
| Pre-term birthc | 3 (2) | 0 | 161 (10)a |
| **Exam findings at presentation** |  |  |  |
| Decreased breath sounds | 83 (65) | 27 (75) | 599 (38)a |
| Rales | 81 (63) | 14 (39)a | 614 (39)a |
| Tachypnead | 60 (47) | 17 (47) | 556 (36)a |
| Documented fever | 49 (38) | 15 (42) | 803 (52)a |
| Chest indrawing | 36 (28) | 17 (47)a | 936 (60)a |
| Rhonchi | 34 (26) | 11 (31) | 674 (43)a |
| Wheezing | 32 (25) | 4 (11) | 723 (46)a |
| Dullness to percussion | 3 (2) | 2 (6) | 7 (0.45)a,e |
| **Radiographic findingsf** |  |  |  |
| Consolidation | 72 (56) | 29 (81)a | 863 (55) |
| Single lobar infiltrate | 42 (33) | 13 (36) | 348 (22)a |
| Pleural effusion | 33 (26) | 20 (56)a | 139 (9)a |
| Multiple lobar infiltrate | 27 (21) | 15 (42)a | 418 (27) |
| Multiple lobar infiltrate  (Unilateral) | 13 (10) | 10 (28)a | 94 (6) |
| Multiple lobar infiltrate  (Bilateral) | 14 (11) | 5 (14) | 326 (21)a |
| Hilar lymphadenopathy | 13 (10) | 1 (3) | 96 (6) |
| Complicated bronchiolitis | 16 (13) | 3 (8) | 512 (33)a |
| **Laboratory findings** |  |  |  |
| Lymphocytosisg | 2 (2) | 2 (6) | 90 (7)a |
| **Severity of illness** |  |  |  |
| Length of stay, median (interquartile range) in days | 2 (2 – 4) | 6 (3 – 10)a | 3 (2 – 4) |
| ICU admission | 13 (11) | 13 (36)a | 328 (21)a |
| Invasive mechanical ventilation | 1 (8) | 7 (54)a,e | 107 (32) |
| **Antibiotics** |  |  |  |
| Receipt of an outpatient antibiotic | 68 (53) | 12 (33)a | 311 (20)a |
| Receipt of antibiotics prior to admission within 5 days | 52 (41) | 11 (31) | 240 (15)a |

\*Sub-analysis: Only children who had combined nasopharyngeal/oropharyngeal (NP/OP) swabs specimens tested real-time polymerase chain reaction (PCR) *M. pneumoniae* and were tested for both bacteria and viruses were included

†Does not include viral or bacterial co-detections

‡Non-Mycoplasma bacterial pneumonia (*M. pneumoniae* PCR-negative bacterial pneumonia): Detection of *H. influenzae* or other gram-negative bacteria, *S. aureus*, *S. anginosus*, *S. mitis*, *S. pneumoniae*,or *S. pyogenes*. *C. pneumoniae* was not included. This also includes bacterial-bacterial co-detections

§Non-Mycoplasma viral pneumonia (*M. pneumoniae* PCR-negative viral pneumonia): Detection of viruses such as such as adenovirus (AdV); coronaviruses; human metapneumovirus (HMV); human rhinovirus; influenza A/B viruses; parainfluenza viruses 1, 2, 3 (PIV); or respiratory syncytial virus (RSV). This also includes viral-viral co-detections

a P<0.05

bClinical presentation is based on patient history

cOnlyfor those children less than 2 years of age

dTachypnea: For children <2 months: >60 breaths/min; 2 months to <12 months: >50 breaths/min; 12 months to 5 years: >40 breaths/min; >5 years:

e Fisher’s exact test

fThe radiographic findings are not mutually exclusive and could overlap.

gFor children <5 years old, WBC >15,000/mm3 or <5500/mm3 and for children ≥5 years old, WBC >11,000/mm3 or <3000/mm3 were considered abnormal