Identification of Bacterial and Viral Codetections With *Mycoplasma pneumoniae* Using the TaqMan Array Card in Patients Hospitalized With Community-Acquired Pneumonia

Maureen H. Diaz,1 Kristen E. Cross,2 Alvaro J. Benitez,1 Laurie A. Hicks,1 Preeta Kutty,1 Anna M. Bramley,1 James D. Chappell,2 Weston Hymas,4 Anami Patel,5,6 Chao Qi,7 Derek J. Williams,6,28 Sandra R. Arnold,6,28 Krow Ampofo,4 Wesley H. Self,2 Carlos G. Grijalva,6 Evan J. Anderson,9 Jonathan A. McCullers,5,6,10 Andrew T. Pavia,4 Richard G. Wunderink,2 Richard M. Edwards,3,8 Seema Jain,2 and Jonas M. Winchell1

1Division of Bacterial Diseases, and 2Influenza Division, Centers for Disease Control, and Prevention, Atlanta, Georgia; 3University of Utah Health Sciences Center, Salt Lake City; 4Le Bonheur Children’s Hospital, Memphis, Tennessee; 5University of Tennessee Health Science Center, Memphis, Tennessee; 6University of Tennessee Health Science Center, Memphis, Tennessee; 7Northwestern University Feinberg School of Medicine, Chicago, Illinois; 8Vanderbilt Vaccine Research Program, Nashville, Tennessee; 9Emory University School of Medicine, Atlanta, Georgia; 10St. Jude Children’s Research Hospital, Memphis, Tennessee.

*Mycoplasma pneumoniae* was detected in a number of patients with community-acquired pneumonia in a recent prospective study. To assess whether other pathogens were also detected in these patients, TaqMan Array Cards were used to test 216 study samples. To achieve this goal, we tested 216 *M pneumoniae*-positive specimens from EPIC for 25 additional respiratory viruses and bacteria using the TaqMan Array Card ([TAC] Thermo Fisher Scientific). Few earlier reports have described multipathogen detection including *M pneumoniae* in the testing algorithm of patients with CAP [4–7], and none included both children and adults.

Children (<18 years old) and adults were enrolled in the EPIC study from January 2010 to June 2012 at 8 hospitals in Chicago, Illinois; Memphis, Tennessee; Nashville, Tennessee; and Salt Lake City, Utah [1, 2]. Informed consent was obtained before enrollment. The study protocol was approved by the institutional review boards at each institution and the CDC. Patients admitted to a study hospital with evidence of acute respiratory infection and radiographic confirmation of pneumonia were included; patients who were recently hospitalized or severely immunocompromised were excluded [1, 2]. For each patient, nasopharyngeal and oropharyngeal (NP/OP) swabs were collected and combined in universal transport media to be tested as a single specimen for respiratory viruses and atypical bacteria, including *M pneumoniae*, using standardized real-time PCR assays at each study site [1, 2].

Nasopharyngeal and oropharyngeal specimens were stored at −70°C and shipped to the CDC for long-term storage. At the CDC, total nucleic acid was extracted using the MagNA Pure Compact System with Total Nucleic Acid Isolation Kit I (Roche Applied Science) according to the manufacturer’s instructions. Of 225 specimens collected within 72 hours of admission from enrolled patients meeting the final CAP case definition [1, 2] and identified as *M pneumoniae*-positive at the study site, 216 (96%) were confirmed upon repeat testing at the CDC using a validated real-time PCR assay [8] and were included in the current study.

Nucleic acid from each *M pneumoniae*-positive specimen was tested for the presence of 25 additional bacterial and viral respiratory pathogens (listed in Table 1) using TAC on the ViiA7 Real-Time PCR System (Thermo Fisher Scientific) as previously described [7]. The proportions of codetections of respiratory pathogens determined using TAC were compared between children and adults using χ² or Fisher’s exact test as appropriate. All analyses were conducted using SAS version 9.3 (SAS Institute, Cary, NC); *P* < .05 was considered significant.

Using TAC, *M pneumoniae* was detected in 209 (96.8%) of 216 specimens. All 7 specimens that were negative for *M pneumoniae* by TAC had a Crossing threshold value ≥33 by the original real-time PCR assay, suggesting that the negative result on TAC was most likely due to low quantity of pathogen-specific nucleic acid in the primary specimen. At least 1 other bacterial or viral...
Table 1. Codetected Respiratory Pathogens in *Mycoplasma pneumoniae*-Positive Specimens Using TaqMan Array Card

| Codetection* | Total (n = 209) n (%) | Adults (n = 38) n (%) | Children (n = 171) n (%) | *P* Valueb |
|--------------|-----------------------|-----------------------|--------------------------|------------|
| Any codetection | 125 (59.6) | 13 (34.2) | 112 (65.5) | <.01 |
| Bacterial only codetections (≥1) | 74 (35.4) | 9 (23.7) | 65 (38.0) | .09 |
| Viral only codetections (≥1) | 17 (8.1) | 4 (10.5) | 13 (7.6) | .5 |
| Bacterial and viral codetections | 34 (16.3) | 0 (0) | 34 (19.9) | <.01 |
| Bordetella pertussis | 0 (0) | 0 (0) | 0 (0) | — |
| Chlamydia pneumoniae | 0 (0) | 0 (0) | 0 (0) | — |
| Haemophilus influenzae | 61 (29.2) | 0 (0) | 61 (35.6) | <.01 |
| Legionella spp | 0 (0) | 0 (0) | 0 (0) | — |
| Moraxella catarrhalis | 30 (14.4) | 3 (7.9) | 27 (15.8) | .3 |
| Staphylococcus aureus | 45 (21.5) | 6 (15.8) | 39 (22.8) | .3 |
| Streptococcus pneumoniae | 50 (23.9) | 3 (7.9) | 47 (27.5) | .01 |
| Streptococcus pyogenes | 12 (5.7) | 0 (0) | 12 (7.0) | .07 |
| Adenoviruses | 3 (1.4) | 0 (0) | 3 (1.8) | 1.0 |
| Human enteroviruses | 12 (5.7) | 0 (0) | 12 (7.0) | 1.0 |
| Influenza virusc | 2 (1.0) | 0 (0) | 2 (1.2) | 1.0 |
| Human coronavirusd | 16 (7.7) | 2 (5.3) | 14 (8.2) | .7 |
| Human metapneumoviruses | 4 (1.9) | 0 (0) | 4 (2.3) | 1.0 |
| Human parechoviruses | 1 (0.5) | 0 (0) | 1 (0.6) | 1.0 |
| Human parainfluenza virus* | 3 (1.4) | 0 (0) | 3 (1.8) | 1.0 |
| Respiratory syncytial virus | 5 (2.4) | 1 (2.6) | 4 (2.4) | 1.0 |
| Human rhinoviruses | 29 (13.9) | 2 (5.3) | 27 (15.8) | .08 |

* Multiple codetections were identified in a single patient specimen in some cases.
* *χ*² or Fisher’s exact test as appropriate comparing children with adults.
* Includes influenza A, B, and C viruses.
* Includes human coronaviruses 229E, NL63, OC43, and HKU1.
* Includes human parainfluenza viruses 1–4.

codetection was identified in 125 of 209 (59.8%) specimens, including 13 of 38 (34.2%) and 112 of 171 (65.5%) specimens from adults and children, respectively (Table 1). The proportion of specimens in which at least 1 codetection was identified was significantly higher among children compared with adults (P < .01). The highest number of codetected organisms was identified in specimens from patients in the 0–23 months and 2–4 years age groups (range, 0–7 codetctions per specimen), whereas the highest number of codetctions in any specimen from an adult patient was only 2 (Figure 1).

One or more bacterial codetctions in addition to *M pneumoniae* was identified in 74 (35.4%) specimens, including 9 (23.7%) adult and 65 (38.0%) pediatric specimens (Table 1). The most frequent bacterial codetctions with *M pneumoniae* were *Haemophilus influenzae* (n = 61), *Streptococcus pneumoniae* (n = 50), *Staphylococcus aureus* (n = 45), and *Moraxella catarrhalis* (n = 30). The predominant bacterial organisms detected using TAC were not included in the primary study site testing algorithm for PCR of NP/OP specimens, although different methods were used to test other specimen types for some of these bacteria [1, 2]. Viral codetctions were less common; 1 or more viruses were found in 4 (10.5%) adults and 13 (7.6%) children; human rhinovirus was the most frequently detected virus (n = 29) and was more common in children (15.8%) compared with adults (5.3%) (Table 1). Mixed bacterial and viral codetctions were found in 34 (19.9%) pediatric specimens but no adult specimens (P < .01). The various combinations of codetctions are listed in Supplementary Table 1. There were no significant differences in the proportions of specimens in which codetctions were identified between sites (data not shown). There were no statistically significant differences in length of stay, intensive care unit admission, invasive mechanical ventilation, or death based on codetection status, although the frequency of these events was low (Supplementary Table 2).

Codetctions with *M pneumoniae* were common, particularly in children. Although most of the codetected organisms have a known pathogenic potential, their contribution to the episodes of CAP in these patients is unclear, given that NP/OP specimens are an indirect measure of what is causing infection in the lung. The proportion of specimens with at least 1 bacterial or viral codetection identified along with *M pneumoniae* in children hospitalized with CAP is consistent with previous reports, ranging from 50 to >90%, depending on the extent of pathogen testing performed [4–6]. A high prevalence of nasopharyngeal colonization with the most commonly codetected bacteria in the current study, including *S pneumoniae* and *H influenzae*, has been reported in children with and without respiratory illness [9–12]. Likewise, human rhinoviruses, commonly codetected with *M pneumoniae* in pediatric specimens in the current study, were found in similar proportions in children with CAP.
enrolled in the EPIC study compared with asymptomatic controls [13]. Codetection of influenza virus, human metapneumovirus, human parechovirus, and respiratory syncytial virus was relatively uncommon (<3.0%) in *M pneumoniae*-positive specimens, similar to previous reports [4–7, 14, 15]. The presence of both viral and bacterial organisms in a single specimen, which was only observed in children and has previously been associated with disease severity [5, 16], warrants further investigation. Additional studies are necessary to understand the mechanisms underlying interactions of codetected organisms with *M pneumoniae* and the potential impact on the severity of CAP, particularly in children.

This analysis has several shortcomings, including previously identified limitations related to the EPIC study design [1–3]. In particular, the presence of codetected pathogens in upper respiratory specimens may not be clinically relevant. Analysis of lower respiratory specimens may be a preferable specimen type for assessment of the potential contribution of these organisms to CAP; however, collection of lower respiratory specimens is more invasive and difficult to obtain. Furthermore, because *M pneumoniae* adheres to and replicates in the nasopharynx or oropharynx [17–19], detection in NP/OP swabs likely represents infectious shedding rather than carriage. Tests performed in the TAC format may be less sensitive compared with individual real-time PCR assays, potentially due to the substantially lower reaction volume [20]. Other variables, including the longer duration of storage, additional thawing of frozen specimen, and different extraction method may also impact the ability to detect respiratory pathogens in these specimens. Finally, the TAC design used in the current study was not customized specifically for this patient population, and thus it may not represent the ideal repertoire of assays for testing of upper respiratory tract specimens from both children and adults. Although TAC is a powerful diagnostic tool that could improve patient management and streamline testing decisions for clinicians during CAP, it is currently used for research only, and further validation is needed to support widespread implementation of this technology in clinical laboratories.

**CONCLUSIONS**

*Mycoplasma pneumoniae* was rarely detected in NP/OP swab specimens collected from asymptomatic controls in the EPIC study primary analysis, [1, 2], suggesting that *M pneumoniae* is not a common colonizer of the upper respiratory tract and, when present, indicates a contribution to ongoing disease. However, further investigation is needed to examine potential interactions of codetected organisms with *M pneumoniae*, including bacterial and viral respiratory pathogens that may be present in a carriage or prolonged shedding state in the upper respiratory tract. Understanding the interplay between *M pneumoniae* and the respiratory microbiome may lend insight into the transmission and clinical spectrum of *M pneumoniae* infections.

**Supplementary Data**

Supplementary material is available online at Open Forum Infectious Diseases online (http://OpenForumInfectiousDiseases.oxfordjournals.org/).
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