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BRIEF REPORT

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

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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 *M pneumoniae*-positive respiratory specimens for 25 additional viral and bacterial respiratory pathogens. It is interesting to note that 1 or more codetections, predominantly bacterial, were identified in approximately 60% of specimens, with codetections being more common in children.

Keywords. community-acquired pneumonia; multipathogen detection; *Mycoplasma pneumoniae*.

Mycoplasma pneumoniae was the most commonly detected bacterial pathogen among children and the second most commonly detected bacteria in adults hospitalized with community-acquired pneumonia (CAP) in the recent US Centers for Disease Control and Prevention (CDC) Etiology of Pneumonia in the Community (EPIC) study [1, 2]. Although we had previously characterized *M pneumoniae* from 216 polymerase chain reaction (PCR)-positive specimens collected during the EPIC study, including P1 subtyping, multilocus variable-number tandem-repeat analysis, and macrolide susceptibility genotyping [3], the goal of the current study was to determine whether other pathogens were codetected with *M pneumoniae* in our 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 χ^2 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

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Table 1. Codetected Respiratory Pathogens in *Mycoplasma pneumoniae*-Positive Specimens Using TaqMan Array Card

Codetection[a]	Total (n = 209) n (%)	Adults (n = 38) n (%)	Children (n = 171) n (%)	*P* Value[b]
Any codetection	125 (59.8)	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
Influenza virus[c]	2 (1.0)	0 (0)	2 (1.2)	1.0
Human coronavirus[d]	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[e]	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

[a] Multiple codetections were identified in a single patient specimen in some cases.

[b] χ^2 or Fisher's exact test as appropriate comparing children with adults.

[c] Includes influenza A, B, and C viruses.

[d] Includes human coronaviruses 229E, NL63, OC43, and HKU1.

[e] 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 codetections per specimen), whereas the highest number of codetections in any specimen from an adult patient was only 2 (Figure 1).

One or more bacterial codetections 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 codetections 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 codetections 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 codetections were found in 34 (19.9%) pediatric specimens but no adult specimens ($P < .01$). The various combinations of codetections are listed in Supplementary Table 1. There were no significant differences in the proportions of specimens in which codetections 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).

Codetections 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

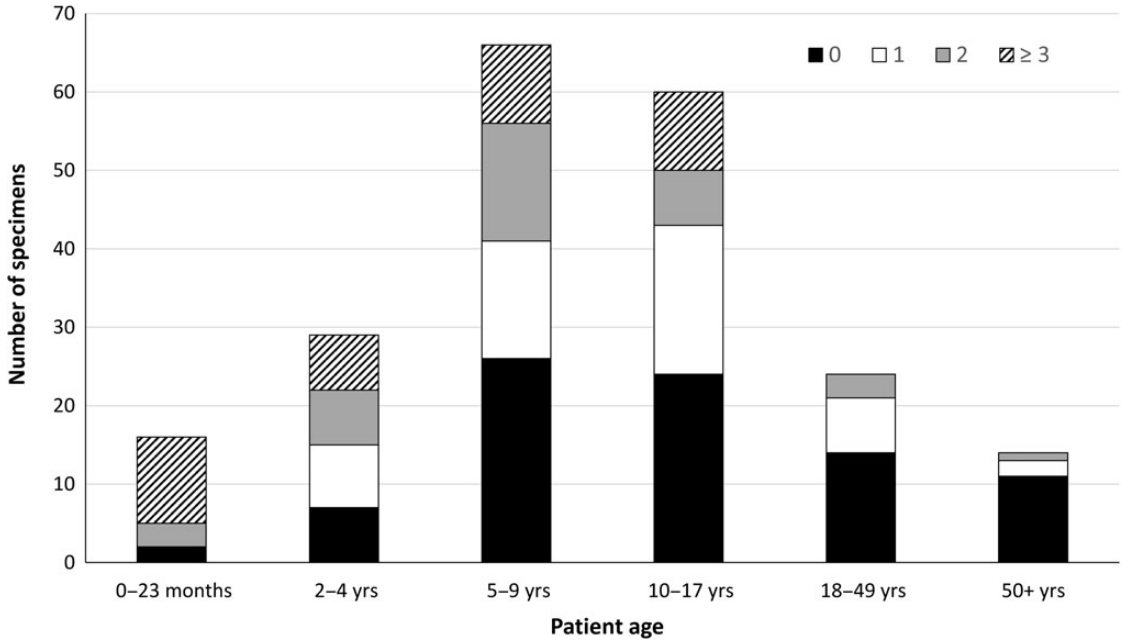

Figure 1. Number of specimens with 0, 1, 2, or ≥3 codetections in addition to *Mycoplasma pneumoniae* in each age category. Specimens having 3 or more codetections (range, 3–7) were combined.

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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References

1. Jain S, Self WH, Wunderink RG, et al. Community-acquired pneumonia requiring hospitalization among U.S. adults. N Engl J Med **2015**; 373:415–27.
2. Jain S, Williams DJ, Arnold SR, et al. Community-acquired pneumonia requiring hospitalization among U.S. children. N Engl J Med **2015**; 372:835–45.
3. Diaz MH, Benitez AJ, Cross KE, et al. Molecular detection and characterization of *Mycoplasma pneumoniae* among patients hospitalized with community-acquired pneumonia in the United States. Open Forum Infect Dis **2015**; 2:ofv106.
4. Chiu CY, Chen CJ, Wong KS, et al. Impact of bacterial and viral coinfection on *Mycoplasmal pneumonia* in childhood community-acquired pneumonia. J Microbiol Immunol Infect **2015**; 48:51–6.
5. Michelow IC, Olsen K, Lozano J, et al. Epidemiology and clinical characteristics of community-acquired pneumonia in hospitalized children. Pediatrics **2004**; 113:701–7.
6. Peng D, Zhao D, Liu J, et al. Multipathogen infections in hospitalized children with acute respiratory infections. Virol J **2009**; 6:155.
7. Waller JL, Diaz MH, Petrone BL, et al. Detection and characterization of *Mycoplasma pneumoniae* during an outbreak of respiratory illness at a university. J Clin Microbiol **2014**; 52:849–53.
8. Thurman KA, Warner AK, Cowart KC, et al. Detection of *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, and *Legionella* spp. in clinical specimens using a single-tube multiplex real-time PCR assay. Diagn Microbiol Infect Dis **2011**; 70:1–9.
9. Hammitt LL, Bruden DL, Butler JC, et al. Indirect effect of conjugate vaccine on adult carriage of *Streptococcus pneumoniae*: an explanation of trends in invasive pneumococcal disease. J Infect Dis **2006**; 193:1487–94.
10. Roberts AL, Connolly KL, Kirse DJ, et al. Detection of group A *Streptococcus* in tonsils from pediatric patients reveals high rate of asymptomatic streptococcal carriage. BMC Pediatr **2012**; 12:3.
11. Tenenbaum T, Franz A, Neuhausen N, et al. Clinical characteristics of children with lower respiratory tract infections are dependent on the carriage of specific pathogens in the nasopharynx. Eur J Clin Microbiol Infect Dis **2012**; 31:3173–82.
12. Skevaki CL, Tsialta P, Trochoutsou AI, et al. Associations between viral and bacterial potential pathogens in the nasopharynx of children with and without respiratory symptoms. Pediatr Infect Dis J **2015**; 34:1296–301.
13. Self WH, Williams DJ, Zhu Y, et al. Respiratory viral detection in children and adults: comparing asymptomatic controls and patients with community-acquired pneumonia. J Infect Dis **2015**; 213:584–91.
14. Chalker VJ, Stocki T, Mentasti M, et al. *Mycoplasma pneumoniae* infection in primary care investigated by real-time PCR in England and Wales. Eur J Clin Microbiol Infect Dis **2011**; 30:915–21.
15. Chen LL, Cheng YG, Chen ZM, et al. [Mixed infections in children with *Mycoplasma pneumoniae* pneumonia]. Zhonghua Er Ke Za Zhi **2012**; 50:211–5.
16. Cimolai N, Wensley D, Seear M, Thomas ET. *Mycoplasma pneumoniae* as a cofactor in severe respiratory infections. Clin Infect Dis **1995**; 21:1182–5.
17. Waites KB, Atkinson TP. The role of *Mycoplasma* in upper respiratory infections. Curr Infect Dis Rep **2009**; 11:198–206.
18. Waites KB, Balish MF, Atkinson TP. New insights into the pathogenesis and detection of *Mycoplasma pneumoniae* infections. Future Microbiol **2008**; 3:635–48.
19. Waites KB, Talkington DF. *Mycoplasma pneumoniae* and its role as a human pathogen. Clin Microbiol Rev **2004**; 17:697–728.
20. Kodani M, Yang G, Conklin LM, et al. Application of TaqMan low-density arrays for simultaneous detection of multiple respiratory pathogens. J Clin Microbiol **2011**; 49:2175–82.