JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 2010, p. 3380–3382 Vol. 48, No. 9
0095-1137/10/$12.00 doi:10.1128/JCM.00321-10

Limited Utility of Culture for *Mycoplasma pneumoniae* and *Chlamydophila pneumoniae* for Diagnosis of Respiratory Tract Infections[▽]

Rosemary C. She,[1,2]* Andy Thurber,[1] Weston C. Hymas,[1] Jeffery Stevenson,[1] Janine Langer,[1] Christine M. Litwin,[1,2] and Cathy A. Petti[1,2,3]

ARUP Laboratories, Salt Lake City, Utah,[1] and Department of Pathology[2] and Department of Medicine,[3] University of Utah School of Medicine, Salt Lake City, Utah

Received 17 February 2010/Returned for modification 20 April 2010/Accepted 28 June 2010

We assessed the utility of culture for *Mycoplasma pneumoniae* and *Chlamydophila pneumoniae* to diagnose respiratory tract infections. Compared to PCR and IgM serology, culture was less sensitive and had extremely low yield. Culture is not recommended for these pathogens, and this method should be eliminated from routine practice.

The etiology of respiratory tract infections can be difficult to diagnose by health care practitioners because clinical history and symptoms are usually nonspecific for most community-acquired pathogens. *Mycoplasma pneumoniae* and *Chlamydophila pneumoniae* cause up to 22% of community-acquired pneumonias and 5 to 10% of cases of tracheobronchitis, pharyngitis, laryngitis, and sinusitis (3–4, 7, 10, 12). Historically, culture has been the gold standard for diagnosis. However, cultivation of these microorganisms can prove challenging because they are fastidious and may require weeks for growth. Serology is more convenient and sensitive than culture, but results are often delayed, and false-negative test results often occur early in the course of illness. Although not standardized, nucleic acid-based tests, such as PCR, provide fast and sensitive results. While such tests are not always available on site in many medical centers, the 24- to 48-h delay in transit time may still be acceptable given the higher diagnostic yield of PCR.

Despite the obvious limitations of culture, physicians continue to order this test frequently. In recent years, ARUP Laboratories has received large numbers of requests, including more than 3,000/year for *M. pneumoniae* and more than 1,500/year for *C. pneumoniae* culture. Studies focusing on the value of culture either have been small in scale or have used type strains or patient isolates rather than direct patient specimens (8, 13). An accurate and reliable diagnosis of *M. pneumoniae* and *C. pneumoniae* is important to differentiate them from other common respiratory pathogens because their treatments differ (1). Being aware of the poor sensitivity of culture for these pathogens, we found the high numbers of test requests for *M. pneumoniae* and *C. pneumoniae* culture from respiratory tract specimens to be concerning and to require further investigation. To examine more closely the utility of culture for diagnosing respiratory syndromes, we compared its performance to those of nucleic acid testing and serology for detection of *M. pneumoniae* and *C. pneumoniae*.

From 2003 to 2008, microbiology results of culture, PCR, and serology performed at ARUP Laboratories for *M. pneumoniae* and *C. pneumoniae* were retrospectively reviewed with a specific focus on respiratory specimens (e.g., nasal wash, nasopharyngeal swab, bronchoalveolar lavage, tracheal aspirate, sputum, and pleural fluid) for PCR and culture. Respiratory specimens were transported either refrigerated or frozen, except for *C. pneumoniae* culture, for which only refrigerated specimens were transported. Additional data were collected for *M. pneumoniae* culture (1995 to 2003). *C. pneumoniae* enzyme-linked immunosorbent assay (ELISA) was performed only in 2005 to 2008, while microimmunofluorescence (MIF) was performed from 2003 to 2008. For serologic tests, data from IgM testing were collected since paired serology for IgG was rarely ordered. Subset analyses were performed for those specimens that were tested by both culture and another method. In 2008, culture-negative specimens for *M. pneumoniae* and *C. pneumoniae* were prospectively collected for PCR testing. The study protocol was approved by the University of Utah Institutional Review Board.

For *M. pneumoniae* culture, respiratory specimens were diluted if viscous, vortexed, supplemented with amphotericin B and penicillin, and inoculated into SP-4 medium. The medium was observed daily for 21 days for a decrease in pH (a red to yellow color change). Positive cultures were confirmed by fluorescent antibody testing (Chemicon MA88285, Temecula, CA) or PCR. For *C. pneumoniae*, specimens were vortexed and inoculated into HEp-2 cells in McCoy medium with cycloheximide. Culture cells were passaged at 72 h into new HEp-2 vials. At day 6, cells were blind stained (Bartels Chlamydia culture confirmation reagent; Trinity Biotech, Bray, Ireland). Positive results were confirmed by a *Chlamydia pneumoniae*-specific reagent (Argene Inc., North Massapequa, NY) or by PCR.

M. pneumoniae and *C. pneumoniae* PCRs were carried out using a laboratory-developed real-time assay which used manual nucleic acid extraction (Qiagen, Valencia, CA), primers

* Corresponding author. Mailing address: ARUP Laboratories, 500 Chipeta Way, Salt Lake City, UT 84108. Phone: (801) 583-2787. Fax: (801) 584-5207. E-mail: rosemary.she-bender@hsc.utah.edu.

▽ Published ahead of print on 7 July 2010.

TABLE 1. Total numbers of samples tested and percentages positive by the various methods used for *Mycoplasma pneumoniae* and *Chlamydophila pneumoniae* diagnosis

Bacterium and method	No. of samples tested	No. positive	% positive	*P* value[a]
M. pneumoniae				
Culture	24,677	10	0.04	NA
PCR	8,509	167	1.9	<0.001
Serology (IgM by ELISA)	92,507	6,049	6.6	<0.001
C. pneumoniae				
Culture	6,981	0	0	NA
PCR	994	6	0.6	<0.001
Serology (IgM by MIF)	58,211	960	1.6	<0.001
Serology (IgM by ELISA)	3,689	143	3.9	<0.001

[a] Compared to culture; determined using Fisher's exact test (one tail). NA, not applicable.

and minor groove-binding hybridization probes from Epoch Biosciences (Bothell, WA), LightCycler Fast Start hybridization probe master mix (Roche, Indianapolis, IN), and the ABI HT7900 sequence detection system (Applied Biosystems, Foster City, CA). The *M. pneumoniae* assay targets a region of the P1 surface protein gene and has a limit of detection of <200 copies/ml. The *C. pneumoniae* assay targets a region of the major outer membrane protein gene and has a limit of detection of <320 copies/ml.

The IgM serologic testing for *M. pneumoniae* was performed by ELISA (values of ≥0.96 U/liter were interpreted as positive results), and for *C. pneumoniae*, it was performed by either MIF or ELISA (titers of ≥1:20 and index values of ≥1.51 were interpreted as positive results, respectively).

For *M. pneumoniae*, culture was less frequently positive than PCR or serology ($P < 0.001$), yielding only 10 positive results out of 24,677 specimens (Table 1). Of 122 paired PCR and culture results, 3 were positive by PCR and none by culture. Of 285 patients for whom both IgM serology and culture performed, 19 were positive by serology and none by culture. Of the 280 prospectively collected, culture-negative specimens, none were positive when tested by PCR.

For *C. pneumoniae*, culture was less frequently positive than PCR or serology ($P < 0.001$), with no positive results in the 6,981 specimens submitted during the study period (Table 1). For 60 cases, both culture and IgM serology by ELISA were performed, 2 of which were positive by ELISA and none by culture. Of 154 cases for which both IgM serology by MIF and culture were performed, 4 were positive by serology and none by culture. There were an insufficient number of cases with both culture and PCR results for retrospective analysis. Of the 225 prospectively collected, culture-negative specimens, 2 were positive when tested by real-time PCR with crossing thresholds of 26.6 cycles (5.74×10^5 copies/ml) and 25.4 cycles (1.36×10^6 copies/ml), respectively.

For diagnosis of acute *M. pneumoniae* and *C. pneumoniae* infections, few studies have focused on the utility of culture compared to other methods. The strengths of this study lie in its extremely comprehensive data set, reviewing large numbers of patient results over a decade from specimens collected across the United States. Our findings confirm that culture is unacceptably insensitive and hence should not be ordered by physicians. Incorrect or delayed diagnosis of the causative organism in community-acquired pneumonia can lead to administration of ineffective antimicrobial agents, adversely impacting patient care (5). *M. pneumoniae* is cell wall deficient and thus cannot be treated with beta-lactam agents. *C. pneumoniae* is an obligate intracellular organism best treated with a macrolide or tetracycline and is not susceptible to beta-lactam drugs. Additionally, physicians' reliance on culture results may cause a delay in exploring alternative etiologies for respiratory tract infections. The prolonged turnaround time of culture makes its result clinically irrelevant, particularly when PCR and serology are widely available options. Although PCR or serology testing may be performed at off-site centralized laboratories, these methods offer more accurate and reliable results and should be considered for cases in which a diagnosis will affect patient management. For *M. pneumoniae* diagnosis, PCR tends to be more sensitive earlier in the disease course, whereas serology becomes more sensitive as the disease persists (6). The diagnosis of *C. pneumoniae* remains difficult, since the positive predictive value of PCR may be low in areas with high asymptomatic carriage, and serology is known to be relatively insensitive (11).

In this study, the positivity rates for culture and PCR were lower than the reported incidence of *M. pneumoniae* and *C. pneumoniae* in respiratory tract infection. The culture-negative specimens that underwent PCR testing also had an unexpectedly low yield. We cannot definitively explain these findings and only hypothesize that a number of variables may be involved. First, we do not fully understand the optimal specimen type (e.g., throat, nasal swab, or sputum) for diagnosing respiratory-associated syndromes and how best to assess the quality of specimen collection. Also, we did not have access to patient records to define the clinical syndrome (e.g., laryngitis, pneumonia, and bronchitis) or timing of specimen collection in relation to symptom onset. Inappropriate testing by culture, serology, or PCR for patients with a low likelihood of disease, e.g., screening of transplant recipients, is also a possibility.

It is acknowledged that this study was limited by the low number of positive cases for a comparison of culture to serology and PCR. However, this is in part due to the extremely low positivity rate of culture. Because this was a retrospective study conducted at a reference laboratory, patient histories were not available for review. Paired IgG serology results would have offered a more reliable diagnosis of recent infection, but the prevailing clinical practice is to request single-point serology due to convenience and the need for a timely diagnosis. When elevated, IgM antibodies for *C. pneumoniae* and *M. pneumoniae* are considered indicative of acute infection, although they may not be elevated early in the disease course or with reinfection. We cannot verify that the two specimens that were culture negative and PCR positive for *C. pneumoniae* represented true disease or colonization given that *C. pneumoniae* is known to asymptomatically colonize the respiratory tract (2, 9). Considering the high quantity of DNA target present, it is likely to represent true disease, but data correlating organism load and disease are lacking.

Given the extremely low yield of culture and the wide availability of molecular testing and serology, we recommend that

culture for *M. pneumoniae* and *C. pneumoniae* no longer be ordered by physicians and be discontinued by clinical microbiology laboratories. The rational use of antibacterial therapy is important, and proper test utilization should be encouraged to improve physicians' ability to accurately define the etiology of infections. When respiratory infections from *M. pneumoniae* and *C. pneumoniae* are being considered in the differential diagnosis, PCR and serology offer better performance and a more rapid means of diagnosis.

This work was supported by the ARUP Institute for Clinical and Experimental Pathology.

REFERENCES

1. **El Sayed Zaki, M., D. Raafat, and A. A. El Metaal.** 2009. Relevance of serology for Mycoplasma pneumoniae diagnosis compared with PCR and culture in acute exacerbation of bronchial asthma. Am. J. Clin. Pathol. **131:**74–80.
2. **Hyman, C. L., M. H. Augenbraun, P. M. Roblin, J. Schachter, and M. R. Hammerschlag.** 1991. Asymptomatic respiratory tract infection with Chlamydia pneumoniae TWAR. J. Clin. Microbiol. **29:**2082–2083.
3. **Kumar, S., and M. R. Hammerschlag.** 2007. Acute respiratory infection due to Chlamydia pneumoniae: current status of diagnostic methods. Clin. Infect. Dis. **44:**568–576.
4. **Martinez, M. A., M. Ruiz, E. Zunino, V. Luchsinger, and L. F. Avendano.** 2008. Detection of Mycoplasma pneumoniae in adult community-acquired pneumonia by PCR and serology. J. Med. Microbiol. **57:**1491–1495.
5. **Nakayama, E., K. Hasegawa, M. Morozumi, R. Kobayashi, N. Chiba, T. Iitsuka, T. Tajima, K. Sunakawa, and K. Ubukata.** 2007. Rapid optimization of antimicrobial chemotherapy given to pediatric patients with community-acquired pneumonia using PCR techniques with serology and standard culture. J. Infect. Chemother. **13:**305–313.
6. **Nilsson, A. C., P. Bjorkman, and K. Persson.** 2008. Polymerase chain reaction is superior to serology for the diagnosis of acute Mycoplasma pneumoniae infection and reveals a high rate of persistent infection. BMC Microbiol. **8:**93.
7. **Normann, E., J. Gnarpe, H. Gnarpe, and B. Wettergren.** 1998. Chlamydia pneumoniae in children with acute respiratory tract infections. Acta Paediatr. **87:**23–27.
8. **Roblin, P. M., W. Dumornay, and M. R. Hammerschlag.** 1992. Use of HEp-2 cells for improved isolation and passage of Chlamydia pneumoniae. J. Clin. Microbiol. **30:**1968–1971.
9. **Schmidt, S. M., C. E. Muller, B. Mahner, and S. K. Wiersbitzky.** 2002. Prevalence, rate of persistence and respiratory tract symptoms of Chlamydia pneumoniae infection in 1211 kindergarten and school age children. Pediatr. Infect. Dis. J. **21:**758–762.
10. **Thom, D. H., J. T. Grayston, S. P. Wang, C. C. Kuo, and J. Altman.** 1990. Chlamydia pneumoniae strain TWAR, Mycoplasma pneumoniae, and viral infections in acute respiratory disease in a university student health clinic population. Am. J. Epidemiol. **132:**248–256.
11. **Verkooyen, R. P., D. Willemse, S. C. Hiep-van Casteren, S. A. Joulandan, R. J. Snijder, J. M. van den Bosch, H. P. van Helden, M. F. Peeters, and H. A. Verbrugh.** 1998. Evaluation of PCR, culture, and serology for diagnosis of Chlamydia pneumoniae respiratory infections. J. Clin. Microbiol. **36:**2301–2307.
12. **Waris, M. E., P. Toikka, T. Saarinen, S. Nikkari, O. Meurman, R. Vainionpaa, J. Mertsola, and O. Ruuskanen.** 1998. Diagnosis of Mycoplasma pneumoniae pneumonia in children. J. Clin. Microbiol. **36:**3155–3159.
13. **Wong, K. H., S. K. Skelton, and Y. K. Chan.** 1992. Efficient culture of Chlamydia pneumoniae with cell lines derived from the human respiratory tract. J. Clin. Microbiol. **30:**1625–1630.