MAJOR ARTICLE

Impact of Rapid Detection of Viral and Atypical Bacterial Pathogens by Real-Time Polymerase Chain Reaction for Patients with Lower Respiratory Tract Infection

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(See the editorial commentary by Murdoch on pages 1445–7)

Background. Rapid diagnostic tests with a high sensitivity for lower respiratory tract infection (LRTI) could lead to improved patient care and reduce unnecessary antibiotic use and associated costs. Diagnostic yields, feasibility, and costs of real-time polymerase chain reaction (PCR) of nasopharyngeal and oropharyngeal swab specimens in the routine diagnostic work-up for LRTI were determined.

Methods. In a randomized controlled trial, nasopharyngeal and oropharyngeal swab specimens from patients admitted for antibiotic treatment of LRTI were evaluated by means of real-time PCR for respiratory viruses and atypical pathogens, as well as by conventional diagnostic procedures. Real-time PCR results for patients in the intervention group were reported to the treating physician; results for patients in the control group were not made available.

Results. A total of 107 patients (mean age [$\pm$ standard deviation], 63.6 $\pm$ 16.3 years) were included, of whom 55 were allocated to the intervention group. The pathogens detected most frequently were influenza virus (14 patients), *Streptococcus pneumoniae* (8), coronavirus (6), *Staphylococcus aureus* (5), and rhinoviruses (5). Real-time PCR increased the diagnostic yield from 23 cases (21% of patients) to 47 cases (43% of patients), compared with conventional diagnostic tests. The detection of viral pathogens by PCR was associated with the winter season, less infiltrates on chest radiographs, lower C-reactive protein levels, and shorter duration of symptoms. Use of real-time PCR results resulted in partial or total cessation of antibiotic treatment for 6 patients (11%; 95% confidence interval, 2–19), but overall antibiotic use was comparable in the intervention group and the control group (median duration of treatment, 10.0 vs. 9.0 days; $P =$ not significant). Use of real-time PCR increased treatment and diagnostic costs with €318.17 per patient.

Conclusions. Implementation of real-time PCR for the etiological diagnosis of LRTI increased the diagnostic yield considerably, but it did not reduce antibiotic use or costs.

The health and economic burden from community-acquired lower respiratory tract infections (LRTIs), such as those due to community-acquired pneumonia and those associated with exacerbations of chronic obstructive pulmonary disease, is large and continues to increase [1–4]. Empirical antimicrobial therapy of LRTI is based on the expected etiological role of bacteria, such as *Streptococcus pneumoniae, Haemophilus influenzae, Staphylococcus aureus, Legionella pneumophila, Chlamydia pneumoniae,* and *Mycoplasma pneumoniae* [5–7]. Because of the improved sensitivity of laboratory techniques, respiratory viruses recently have been identified as more frequent and important causes of severe LRTI than was previously assumed [8–12]. Rapid identification of a viral etiology of LRTI may improve effective patient management by influencing the decision

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whether to withhold antibiotic treatment, initiate antiviral therapy, or implement infection-control measures to prevent transmission [13].

Rapid assessment of a viral and bacterial etiology is now possible with novel sensitive and highly specific TaqMan-based real-time PCR assays (Applied Biosystems) [12, 14]. However, these diagnostic tests are costly, and the diagnostic yield and feasibility of implementation in routine diagnostic work-up has not been evaluated sufficiently. Therefore, a randomized controlled trial was conducted to evaluate the feasibility and clinical and economic impact of the use of rapid TaqMan PCR for detection of respiratory viruses and atypical pathogens in patients hospitalized on general wards with LRTI.

PATIENTS, MATERIALS, AND METHODS

Setting and study population. A multicenter, randomized clinical trial was conducted in a 1042-bed university hospital (University Medical Center) and a 627-bed teaching hospital (Diakonessenhuis) in Utrecht, The Netherlands. The trial included patients with LRTI who were referred to one of these hospitals by their general practitioner. Before the study began, study procedures and test characteristics of real-time PCR that were based on previous research in our center [12, 14, 15] were explained to physicians at the participating hospitals by means of an introductory presentation and written information.

Between November 2002 and March 2004, all consecutive patients aged $\geqslant$18 years who were admitted to one of the participating hospitals and who needed immediate antimicrobial treatment for LRTI were eligible for inclusion. LRTI was defined as the onset or increase of cough, sputum production, shortness of breath, wheezing, chest pain, or focal or diffuse signs on chest examination, as well as the presence of at least 1 constitutional symptom, including fever, confusion, sweating, headaches, and leukocytosis [16]. Pneumonia was defined as LRTI with a new or progressive infiltrate detected on a chest radiograph. Patients who had severe immunosuppression (defined as the presence of neutropenia [neutrophil count, $<0.5 \times 10^9$ neutrophils/L] and a CD4 cell count of <200 cells/mm^3), concurrent nonrespiratory infection requiring antibiotic treatment, or severe LRTI requiring treatment in an intensive care unit and patients who were expected not to comply with the study procedures were excluded. The study was approved by the medical ethical committees of both hospitals, and all patients provided written informed consent.

Study procedures. Patients were included within 24 h after admission to the hospital. Demographic data, information on previous treatments, duration of symptoms, and high-risk comorbid conditions were recorded, and laboratory tests to determine WBC counts and C-reactive protein levels and chest radiography were performed.

Pathogen detection. Sputum samples (when available) and blood samples were cultured and processed in accordance with standard microbiological procedures. Nasopharyngeal samples were obtained by inserting 1 swab into both nostrils parallel to the palate for a few seconds to absorb secretions. In addition, with a second swab, posterior pharynx and tonsillar areas were sampled. A urinary antigen assay was used for detection of *L. pneumophila* (Binax Now). Within 24 h after admission, nasopharyngeal and oropharyngeal swab specimens were collected and transported immediately in viral transport medium to the laboratory. The specimens were vortexed for 10 s and centrifuged at 2000 *g* for 15 min, and the supernatants were used for virus isolation and real-time PCR.

For virus isolation, conventional as well as shell-vial cultures of tertiary monkey kidney cells and of human diploid fibroblast cells were inoculated with 0.1 mL of clinical specimen and incubated for a maximum of 14 days. Conventional cultures were examined twice weekly for the development of a cytopathological effect. In cultures with positive results, virus was identified by immunofluorescence with monoclonal antibodies to adenoviruses, influenza A and B viruses, respiratory syncytial virus, and parainfluenza viruses 1–3 (Dako Imagen). Rhinoviruses were identified by acid-lability testing. In shell-vial cultures, an immunofluorescence test using the above-mentioned monoclonal antibodies was performed after 2 days of culture, irrespective of the development of a cytopathological effect.

Identification of respiratory pathogens by real-time PCR analysis. Tests for detection of viral and atypical pathogens were performed in parallel by means of real-time PCR specific for influenza A and B viruses, respiratory syncytial viruses A and B, coronaviruses OC43 and 229E, parainfluenza viruses 1–4, rhinoviruses, all adenoviruses, *M. pneumoniae, C. pneumoniae,* and *L. pneumophila.* Real-time PCR procedures were performed as described elsewhere [12, 14, 15]. In brief, after addition of generic internal virus controls to monitor for inhibition of extraction and amplification, 200 μL of nasopharyngeal swab and oropharyngeal swab supernatants were used for total nucleic acid (i.e., RNA plus DNA) extraction with HighPure Nucleic Acid extraction columns (Roche Diagnostics). Subsequently, the purified RNA was used for cDNA synthesis using random hexamers (Applied Biosystems). Thereafter, purified DNA and cDNA were used as input in individual real-time PCR reactions and were amplified using the Sequence Detection System 7700 (Applied Biosystems). Detection of amplified products was performed using pathogen-specific FAM-dye–labeled TaqMan probes. All pathogens were detected in parallel amplification reactions in 1 assay run. In addition, positive controls and negative controls were included in each run for each individual pathogen. An assay result was validated on the basis of preset C_t criteria for both the internal control and the positive controls.

Randomization and diagnostic intervention. In an open

study, all patients were randomly allocated to the intervention group or to the control group by means of a computer-generated table. The study team was available 24 h per day to enroll patients. In the intervention group, results of the real-time TaqMan PCR had to be reported as soon as possible (⩽48 h after samples were obtained) to the appropriate clinicians. To mimic real-life situations, decisions regarding treatment changes after results of PCR analysis were available were left at the discretion of the physician. In the control group, real-time PCR was performed, but results of PCR analysis were not made available to the treating physicians. Physicians complied with the hospital guidelines described in the hospital antibiotic formulary. Changes in antibiotic treatment and reasons for such changes were recorded.

Follow-up and outcome measurements. All relevant microorganisms cultured from blood or sputum samples or detected by urinary antigen tests, in virus cultures, or by real-time PCR were considered to be a cause of LRTI. Patients were followed up for a maximum of 28 days. Inhospital clinical data, such as diagnostic procedures performed and antibiotics used, were recorded. If patients were discharged within 28 days after admission, clinical outcome and health care–related costs after discharge were recorded at the outpatient clinic. A change in antibiotic treatment that was based on the results of PCR was defined as the primary outcome measure. Investigators were not blinded to patient randomization.

Cost calculations. The health care perspective was used to calculate costs associated with the diagnostic intervention. Costs per patient were calculated by multiplying the resources used by their unit costs. Costs of medical drugs were determined using 2003 Dutch prices [17]. Other costs included hospital stay, which was estimated at to be €512.00 per day and included standard diagnostic procedures [18]. Costs for real-time PCR, including labor costs and costs of reagents, depreciation equipment, and overhead costs (20%), were €330.78 per sample. Other diagnostic costs were based on prices indicated by The Netherlands College for Tariffs in Healthcare in 2004.

Sample size calculation and statistical analysis. In the control group, all patients were expected to receive a complete course of antibiotic treatment. On the basis of an expected detection rate of 20% for atypical and viral pathogens in the intervention group [19] and an estimate of the number of possible drop outs, 100 persons would need to be included to demonstrate a reduction in the use of antibiotic treatment from 100% to 80% ($\alpha = 0.05$; $1 - \beta = 0.80$). Statistical analysis was performed according to the intention-to-treat principle. Differences in the values of continuous variables between the comparison groups were evaluated by means of Student's *t* tests for normally distributed variables and by means of the Mann-Whitney *U* test for variables with a skewed distribution. χ^2 tests were used to test for differences in proportions among categorical variables between the 2 groups.

RESULTS

A total of 107 patients (mean age [±SD], 63.6 ± 16.3 years, mean Fine score [20], 88.0 ± 29.9) were included in the study. Of these, 55 were randomized to the intervention group, and 52 were randomized to the control group. Baseline characteristics were comparable in both study groups, but slightly more patients in the intervention group had received previous antibiotic treatment (table 1).

Table 1. Characteristics of patients with lower respiratory tract infection (LRTI) in a study to detect viral and atypical bacterial pathogens by real-time PCR.

Variable	Intervention group (*n* = 55)	Control group (*n* = 52)
Demographic feature		
Age, mean years ± SD	65.3 ± 14.6	61.7 ± 17.9
Male sex	30 (55)	27 (52)
Clinical feature		
Diagnosis		
Pneumonia	28 (51)	27 (52)
Exacerbation of COPD	12 (22)	10 (19)
Other LRTI	15 (27)	15 (29)
Temperature, mean °C ± SD	38.6 ± 0.99	38.5 ± 0.99
Respiratory rate, mean breaths/min ± SD	23.6 ± 7.3	23.6 ± 6.6
Leukocyte level, median leukocytes $\times 10^9$/L (range)	12.0 (1–70)	12.5 (4.3–45.3)
Lymphocyte level, median lymphocytes $\times 10^9$/L (range)	6.5 (0–41)	5.5 (0–36)
C-reactive protein level, median mg/L (range)	82.5 (0–793)	95 (6–527)
Cough	44 (80)	47 (90)
Sputum production	34 (62)	38 (73)
Previous antibiotic treatment	23 (42)	12 (23)
Fine score, mean ± SD	89.8 ± 27.3	86.1 ± 32.5
Duration of LRTI symptoms, median days (range)	4 (0–14)	3 (1–28)
Initial antibiotic treatment		
β-Lactam	33 (60)	38 (73)
β-Lactam plus macrolide	9 (16)	6 (12)
β-Lactam plus aminoglycoside	3 (5)	0 (0)
Fluoroquinolone	4 (7)	3 (6)
Macrolide	3 (5)	2 (4)
Other	3 (5)	3 (6)
Comorbidity		
COPD	18 (33)	23 (44)
Diabetes mellitus	4 (7)	6 (12)
Malignancy	11 (20)	11 (21)

NOTE. Data are no. (%) of patients, unless otherwise indicated. COPD, chronic obstructive pulmonary disease.

Etiology. An etiological diagnosis could be made for 53 patients (50%) in the study. Conventional diagnostic analyses (blood and sputum cultures and urinary antigen tests) showed a potential pathogen for LRTI in 23 patients (21%). Real-time PCR increased the diagnostic yield from 24 cases (22% of patients) to 47 cases (43% of patients). In 6 patients (6%), standard cultures yielded and real-time PCR detected pathogens (table 2). The most frequently detected pathogens were influenza virus (16 patients [15%]), *S. pneumoniae* (8 [7%]), coronavirus (6 [6%]), and *S. aureus* (5 [5%]). No infections with *C. pneumoniae, M. pneumoniae,* or *L. pneumophila* were detected by real-time PCR. There were no significant differences in etiology between both study groups.

Patients with a virus identified by PCR had significantly shorter duration of symptoms, lower C-reactive protein levels, fewer chest radiographs that showed infiltrates, and were admitted more frequently during the winter season, compared with PCR-negative patients (table 3). In multivariate analysis, the best predictive model for prediction of PCR positivity included all of these features (area under the receiver operating characteristic curve, 0.74; 95% CI, 0.63–0.86).

Feasibility of PCR analysis and impact on treatment decisions. In the intervention group, results of real-time PCR were reported as a mean duration ($\pm$SD) of 30 ± 13 h after sampling, and 14 patients (25%) had positive results. On the basis of PCR results, antibiotic treatment was modified in 6 patients (11%) in the intervention group. In 4 of these 6 patients, treatment for possible infection due to *M. pneumoniae, C. pneumoniae,* or *L. pneumophila* was discontinued because of negative PCR results. In each of these 4 patients, β-lactam therapy was continued, and no definitive etiological diagnosis was made. In the 2 other patients, antibiotic treatment was discontinued when PCR was positive for coronavirus and influenza virus. Because no treatment adaptations were made in the control group, the relative reduction in the number of completed antibiotic courses was 4% (95% CI, −1% to 9%), and if treatment adaptations with continued β-lactam treatment are included in the analysis, the relative reduction was 11% (95% CI, 2%–19%). All 6 patients for whom treatment was adapted were clinically cured by day 28. Four patients (7%) underwent barrier isolation after PCR indicated infection with influenza virus, and 2 patients (4%) received treatment with a neuramidase inhibitor.

Clinical and economic evaluation. Three patients in each study group died (5.5% in the intervention group and 5.8% in the control group). The duration of antimicrobial treatment was comparable in both study groups, with median durations of 10 days (range, 1–46 days) in the intervention group and 9 days (range, 1–31 days) in the control group ($P =$ not significant). Mean antibiotic costs were €90.60 (range, €4.62–€882.00) per patient. Total antibiotic costs were comparable for both study groups (table 4). Treatment with neuramidase inhibitors added €18.10 per treated patient to the costs in the intervention group. Importantly, the use of real-time PCR had no effect on the duration of hospital stay, with a median length of stay of 8.0 days (range, 1–24 days) in the intervention group and 8.0 days (range, 1–19 days) in the control group, and did not reduce the number extra diagnostic procedures that were required to exclude or confirm other related diagnoses, with comparable numbers of additional cultures, blood-gas analyses, CT (angio) scans, or bronchoscopies performed in both study

Table 2. Results of etiologic investigations for patients with lower respiratory tract infection in a study to detect viral and atypical bacterial pathogens by real-time PCR.

Pathogen(s), according to etiologic detection method(s)	Intervention group, no. (%) of patients (n = 55)	Control group, no. (%) of patients (n = 52)
Conventional diagnostic techniques	10 (18)	13 (25)
Streptococcus pneumoniae	3 (5)	5 (10)
Staphylococcus aureus	2 (4)	1 (2)
Haemophilus influenzae	1 (2)	2 (4)
Escherichia coli	1 (2)	…
Pseudomonas aeruginosa	…	1 (2)
Klebsiella pneumoniae	2 (4)	1 (2)
Moraxella catarrhalis	2 (4)	…
Legionella pneumophila	1 (2)	1 (2)
Other	1 (2)	3 (6)
Mixed bacterial etiology[a]	3 (5)	1 (2)
TaqMan-based real-time PCR	11 (20)	13 (25)
Influenza virus	7 (13)	7 (13)
Coronavirus	2 (4)	3 (6)
Rhinovirus	2 (4)	1 (2)
Parainfluenza virus	…	1 (2)
Respiratory syncytial virus	1 (2)	…
Adenovirus	…	1 (2)
Mixed viral etiology[a]	1 (2)	…
Virus culture	10 (18)	6 (12)
Influenza virus	3 (5)	5 (10)
Herpes simplex virus	3 (5)	
Enterovirus	4 (7)	1 (2)
Conventional methods or TaqMan-based real-time PCR	3 (5)	3 (6)
Parainfluenza virus and *Haemophilus parainfluenzae*	1 (2)	…
Rhinovirus and *E. coli*	1 (2)	…
Rhinovirus and *S. aureus*	1 (2)	…
Influenza virus, coronavirus, and *H. influenzae*	…	1 (2)
Influenza virus, *S. aureus,* and *M. catarrhalis*	…	1 (2)
Coronavirus and *L. pneumophila*	…	1 (2)
No cause	31 (56)	23 (44)

[a] Pathogens associated with mixed infections are also counted individually.

Table 3. Multivariate logistic regression analysis to detect characteristics associated with positive PCR results in patients with lower respiratory tract infection (LRTI).

Variable	Patients with positive PCR result (n = 29)	Patients with negative PCR results (n = 78)	OR or difference (95% CI)	P
Infiltrate on chest radiograph	10 (34)	45 (58)	OR, 0.39 (0.16–0.94)	.05[a]
Admitted in winter season	19 (66)	25 (32)	OR, 4.03 (1.64–9.92)	<.01[a]
Chronic obstructive pulmonary disease	14 (48)	27 (35)	OR, 1.76 (0.74–4.19)	.25
Sputum production	21 (72)	51 (65)	OR, 1.39 (0.54–3.55)	.64
Cough	27 (93)	64 (82)	OR, 2.95 (0.62–13.89)	.25
Previous antibiotic treatment	11 (38)	24 (31)	OR, 1.43 (0.58–3.51)	.49
Duration of LRTI symptoms, days	3.79	5.52	Difference, 1.73 (0.74–3.39)	.04[a]
C-reactive protein level, mg/L	89.8	158.7	Difference, 68.9 (6.78–131.05)	.03[a]
Leukocyte level, $\times 10^9$ leukocytes/L	12.4	15.9	Difference, 3.50 (−0.96–7.97)	.12
Age, years	62.5	63.9	Difference, 1.39 (−5.65–8.44)	.70
Temperature, °C	38.5	38.6	Difference, 0.11 (−0.31–0.54)	.60

NOTE. Data are no. (%) of patients, unless otherwise indicated. Other tested characteristics included Fine score, respiratory rate, lymphocytes, blood urea level, serum sodium level, serum glucose level, sex, coronary artery disease, presence of renal disease, cerebrovascular disease, presence of diabetes mellitus, presence of malignancy, and sore throat. None were predictive of positive PCR results.

[a] Included in multivariate analysis.

groups. The total cost per patient for hospitalization, diagnostic procedures, and treatment was €5117.05 in the intervention group and €4741.30 in the control group (table 4).

In the intervention group, the actual antibiotic cost savings attributed to the reporting of PCR results was €699.30, compared with continued treatment. In contrast to these savings, the total cost of PCR testing was €18,193 per patient. Thus, if all patients are included in the analysis, use of real-time PCR would increase the average costs with €318.17 per patient, which is 6.3% of the treatment and diagnostic costs per patient in the intervention group.

DISCUSSION

Implementation of real-time PCR in the diagnostic work-up of patients hospitalized with community-acquired LRTI in-

Table 4. Economical outcome associated with hospitalization, diagnostic procedures, and treatment for lower respiratory tract infection.

Variable	Average quantity of resources used per patient[a]		Unit cost in €	Average cost in € per group	
	Intervention group	Control group		Intervention group	Control group
Hospitalization	9.0 days	8.9 days	512	4608	4557
Diagnostic procedure					
Real-time PCR	1	0	331	331	0
CT of thorax and/or pulmonary angiogram	0.07	0.08	164	11.48	13.12
Additional blood-gas analysis	0.11	0.14	4.05	0.44	0.57
Additional blood culture	0.04	0.12	23.15	0.93	2.78
Additional sputum culture	0.06	0.08	8.68	0.52	0.69
Spirometry	0.07	0.04	15	1.05	0.60
Bronchoscopy	0.13	0.04	301	39.13	12.04
Total	…	…	…	384.55	29.80
Duration of antibiotic treatment	12.3 days	10.3 days	15	184.50	154.50
Total hospitalization, diagnostic, and treatment costs per patient	…	…	…	5177.05	4741.30

[a] Data are no. of analyses, unless otherwise indicated.

creased the etiologic diagnosis from 21% to 43%. However, clinical treatment of patients hardly changed. Antibiotics were partially or totally discontinued in only 6 patients (11%), and rapid diagnosis of viral LRTI did not reduce the duration of hospital stay or the number and costs of other diagnostic procedures and antibiotics.

Despite advances in health care, LRTI is still one of the leading causes of hospital admissions and mortality and is associated with considerable antibiotic use and health care–related costs. For example, community-acquired pneumonia is responsible for ∼500,000 hospitalizations in the United States each year, and the annual costs of treating these patients is approximately $9.7 billion [2, 3, 21, 22]. In addition, there is considerable overuse of antibiotics in the treatment of LRTI, especially for viral infections [23]. Unnecessary antibiotic use is regarded as a driving force in the global increase of antibiotic resistance. The clinical value of conventional diagnostic methods, such as microbiological culturing or Gram staining, in guiding treatment of LRTI is limited, because of low sensitivity and considerable delay in obtaining results [24–28]. Because of small fractions of positive samples, Gram staining of sputum specimens probably cannot lead to great decreases in antibiotic use or costs [29]. Therefore, improvements of diagnostic methods and policies to decrease antibiotic use in patients with LRTI are necessary. Recently, a promising method has been evaluated: the measurement of procalcitonin serum levels for patients with clinical symptoms of LRTI resulted in a significant decrease in antibiotic use without an adverse effect on patient outcome, especially among patients with acute bronchitis [30].

In theory, rapid detection of respiratory viruses might also result in clinical and economical benefits. In a before-after study, rapid diagnosis of viral LRTI resulted in a 52% reduction in antibiotic use in children, compared with the preceding year, but no statistically significant financial benefit was achieved for adult patients in another study with a similar design [13, 31]. Drawbacks of these studies were the use of immunofluorescence techniques, which are less sensitive for virus detection in adult patients [32, 33], and the use of historical control subjects for evaluation of costs and outcomes. In addition, comparisons were made only for those patients with viral infections identified. In the present study, the use of sensitive real-time PCRs for all patients admitted with LRTI did not increase the economic benefits, compared with the contemporary control group.

The cost-effectiveness of real-time PCR could be improved by increasing the diagnostic yield or decreasing the cost of the assay. An increased diagnostic yield could be achieved by adding more pathogens to the test panel, such as the recently discovered human metapneumovirus and coronaviruses [10, 34], or by performing real-time PCR on sputum samples rather than on nasopharyngeal or oropharyngeal swab specimens. However, only 49% of patients in our study cohort produced adequate sputum samples. Because the duration of symptoms at the time of PCR was inversely related to the likelihood of having positive test results, performance of real-time PCR earlier during the course of LRTI might also increase the diagnostic yield. In The Netherlands, most patients are first seen by their general practitioner, and only those patients who have a more severe clinical presentation or who are not responding to empirical treatment are referred to the hospital. Whether real-time PCR will be cost-beneficial in outpatient clinics, in general-practice populations, or in regions where patients are not initially seen by general practitioners remains to be determined.

Alternatively, costs might be decreased by reducing the numbers of selected pathogens in the test panel or by establishing selection criteria for the patients to be examined. For example, patients admitted in the winter season, with a recent onset of symptoms, low C-reactive protein levels, and absence of infiltrates on chest radiographs were most likely to have viral pathogens detected. Limiting real-time PCR tests to these high-risk patients may increase cost-effectiveness. Finally, physicians were hesitant to discontinue antibiotic treatment even if PCR detected a virus, possibly because bacterial culture results were available only after PCR results were available. It is, therefore, doubtful that more rapid reporting of results, which may be possible in the near future with more expensive automated systems, will lead to improved cost-effectiveness.

Real-time PCR might have been more cost-effective if clinicians would have been less reluctant to change clinical management on the basis of test results. Studies with protocol-based and more-rigorous patient management are needed to address this issue.

In conclusion, although rapid detection of respiratory viruses by means of real-time PCR increased the number of detected pathogens considerably, test results hardly influenced clinical management of patients and did not reduce additional diagnostic procedures, antibiotic use, antibiotic costs, or duration of hospital stay.

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