Comprehensive Molecular Testing for Respiratory Pathogens in Community-Acquired Pneumonia

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(See the Editorial Commentaries by Musher on pages 824–5 and Jain and Pavia on pages 826–8.)

Background. The frequent lack of a microbiological diagnosis in community-acquired pneumonia (CAP) impairs pathogen-directed antimicrobial therapy. This study assessed the use of comprehensive multibacterial, multiviral molecular testing, including quantification, in adults hospitalized with CAP.

Methods. Clinical and laboratory data were collected for 323 adults with radiologically-confirmed CAP admitted to 2 UK tertiary care hospitals. Sputum (96%) or endotracheal aspirate (4%) specimens were cultured as per routine practice and also tested with fast multiplex real-time polymerase-chain reaction (PCR) assays for 26 respiratory bacteria and viruses. Bacterial loads were also calculated for 8 bacterial pathogens. Appropriate pathogen-directed therapy was retrospectively assessed using national guidelines adapted for local antimicrobial susceptibility patterns.

Results. Comprehensive molecular testing of single lower respiratory tract (LRT) specimens achieved pathogen detection in 87% of CAP patients compared with 39% with culture-based methods. *Haemophilus influenzae* and *Streptococcus pneumoniae* were the main agents detected, along with a wide variety of typical and atypical pathogens. Viruses were present in 30% of cases; 82% of these were codetections with bacteria. Most (85%) patients had received antimicrobials in the 72 hours before admission. Of these, 78% had a bacterial pathogen detected by PCR but only 32% were culture-positive ($P < .0001$). Molecular testing had the potential to enable de-escalation in number and/or spectrum of antimicrobials in 77% of patients.

Conclusions. Comprehensive molecular testing significantly improves pathogen detection in CAP, particularly in antimicrobial-exposed patients, and requires only a single LRT specimen. It also has the potential to enable early de-escalation from broad-spectrum empirical antimicrobials to pathogen-directed therapy.

Keywords. community-acquired pneumonia; bacterial load; viral; molecular testing; PCR.

Community-acquired pneumonia (CAP) is a common infectious disease with an estimated incidence of 2–11 cases per 1000 adults in the developed world and a mortality rate of 2%–14% [1–5]. Due to the range of pathogens responsible for CAP, in moderate or severe infection, broad-spectrum antimicrobial cover should be initiated before de-escalating to narrow-spectrum pathogen-directed agents once a microbiological diagnosis has been made [1, 5]. Unfortunately, de-escalation is uncommon in practice because current diagnostic methods may identify a pathogen in only 30%–40% of patients with CAP [4, 6, 7]. Recent studies have highlighted the need for more timely and sensitive microbiological diagnostic methods in CAP,

particularly for bacteria and in the common scenario of antibiotic administration prior to sampling [4, 8, 9].

The development of multiplex real-time polymerase-chain reaction (PCR) assays currently enables a respiratory specimen to be rapidly screened for a wide range of viral and atypical bacterial pathogens in a small number of reactions [10–15]. However, a similar approach has not yet been adopted for typical bacterial pathogens. It is difficult to interpret PCR results for typical respiratory bacteria in nonsterile samples such as sputum due to the potential for contamination with the same organisms from oropharyngeal flora. However, accurate molecular quantification of bacterial loads may aid in distinguishing infection from contamination in a way that is analogous to the use of quantitative cultures.

Quantitative molecular assays for typical respiratory bacteria have been used previously in patients with pneumonia, but these assays lack a number of key targets, use targets that have been shown to lack specificity, or may be only semiquantitative [16–32]. However, our group recently developed a suitable assay that is capable of quantifying bacterial loads of 8 typical bacterial pathogens from lower respiratory tract (LRT) specimens [33]. This assay was extensively validated on clinical isolates, reference

strains, and clinical specimens such as sputa and bronchoalveolar lavage fluids, and all bacterial targets could be reliably quantified, even when present in mixtures of different concentrations [33].

Our aim in the study was to assess the utility of a comprehensive molecular diagnostic approach encompassing 26 respiratory bacterial and viral pathogens and including bacterial quantification in patients with CAP.

METHODS

Patients and Study Criteria

Patients presented to 2 tertiary care hospitals in Edinburgh, United Kingdom, over an 18-month period between September 2012 and February 2014. Consecutive cases with clinical and radiological evidence of CAP were identified through daily retrospective electronic review of all respiratory samples received by our microbiology laboratory. Inclusion criteria for the study included the following: adult (aged ≥18 years); LRT specimen obtained within 48 hours of admission to the hospital; new radiographic infiltrate as determined by radiologist or consultant respiratory physician; and 3 of more of the following signs or symptoms: new or worsening cough, new or worsening expectoration of sputum, hemoptysis, new or worsening dyspnea, pleuritic chest pain, fever, headache, or abnormalities on chest auscultation or percussion. Exclusion criteria included the following: bronchiectasis, cystic fibrosis, or healthcare-associated pneumonia (hospitalized in an acute care hospital for 2 or more days within 90 days of the infection or resided in a nursing home or long-term care facility) [34]. Radiology reports were reviewed for all patients, and only patients with radiological changes consistent with pneumonia were included. Where there was any uncertainty, the images were reviewed by an experienced respiratory physician (A. T. H.). Clinical data including comorbidities, outcomes, and antibiotic prescriptions were collected retrospectively from patient hospital records using a standard pro forma as part of a clinical audit of pneumonia management (approved by the Quality Improvement Team, Royal Infirmary of Edinburgh). Drug administration prior to hospitalization, including antibiotic prescription, was obtained from the electronic primary care prescription records on admission and later verified by a pharmacist.

Microbiological Culture and Molecular Testing

LRT specimens were cultured according to national standard protocols to detect common respiratory bacteria [35]. Isolates were identified using standard biochemical methods and/or matrix-assisted laser desorption/ionization time-of-flight (Bruker, Coventry, United Kingdom). Specimens with any named bacterial species identified by culture were considered to be culture positive. Following routine culture, LRT specimens were centrifuged, and mucopurulent material was stored at −80°C for up to 15 months. As part of the study protocol, specimens and associated clinical data were collected and

anonymized before molecular testing in accordance with local ethical approval (South East Scotland Scottish Academic Health Sciences Collaboration Human Annotated BioResource reference no. 10/S1402/33). Thawed specimens were homogenized by vortexing with sterile glass beads, and a 200-µL aliquot was treated with lysozyme at 37°C followed by proteinase K at 56°C for 1 hour each. Total nucleic acid was then extracted using the automated nucliSENS easyMAG (BioMérieux, Basingstoke, United Kingdom) instrument with an off-board lysis protocol, including the addition of phocine herpes virus as an internal extraction control. Extracts were stored at 4°C for up to 5 days or longer at −80°C. Fast multiplex real-time PCR was performed using a combination of multiplex assays developed or adapted in-house for the following 26 pathogens: *Streptococcus pneumoniae*; *Haemophilus influenzae*; *Moraxella catarrhalis*; *Staphylococcus aureus*; *Escherichia coli*; *Klebsiella pneumoniae*; *Pseudomonas aeruginosa*; *Acinetobacter baumannii*; *Mycoplasma pneumoniae*; *Chlamydophila pneumoniae*; *Chlamydophila psittaci*; *Legionella pneumophila*; *Legionella* spp.; influenza A; influenza B; respiratory syncytial virus; parainfluenza virus types 1–3; adenovirus; human coronaviruses 229E, HKU1, NL63, and OC43; human metapneumovirus; and rhinovirus [10–15, 33]. As previously described [33], bacterial loads for *S. pneumoniae*, *H. influenzae*, *M. catarrhalis*, *S. aureus*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *A. baumannii* were expressed as colony forming units (CFUs) per milliliter of purulent material and calculated from standard curves generated by target gene plasmid dilution series on each PCR run. Quality control reactions (quantitative real-time PCR for *GAPDH* human gene and qualitative real-time PCR for *gB* phocine herpes virus internal control [IC]) were carried out on all specimens [33]. Quantitative *GAPDH* PCR was used as an indication of the cell content of each specimen because the *GAPDH* gene is present in human DNA. Negative (no template) controls were included on every extraction and PCR run to check purity of reagents. Runs were accepted if the negative controls were negative and the standard curves were linear; runs that failed quality control were repeated. Qualitative results were accepted if the internal control was positive or if at least 1 other target was positive. Quantitative results were accepted if the internal control quantification cycle (Cq) value fell within the range ±1 log Cq difference to negative extraction controls. Outside of this range, quantitative results were not accepted due to the potential for partial PCR inhibition leading to inaccuracy of measurement.

Estimating Impact on Antimicrobial Prescribing

The prescribed empirical therapy for each patient was compared with what antimicrobial(s) would have been appropriate for pathogen-directed therapy, based on the molecular identification result. Appropriate pathogen-directed therapy was determined using UK national guidelines adapted for local antimicrobial susceptibility patterns (Supplementary Table 1) [1].

Statistical Analyses

Categorical variables were compared using Fisher exact test or χ^2 test. Continuous data were assessed using the Shapiro–Wilk W test for nonnormality and analyzed using the Mann–Whitney U test or t test. The McNemar test was used to compare paired proportions. The Bonferroni method was used to correct for multiple comparisons during significance testing. To control for potential confounding factors that affect associations with the outcome of infection, we used logistic regression analysis. For the analysis of the outcome of infection, a combined outcome measure of 30-day mortality and/or intensive care unit (ICU) admission was used because of the infrequent occurrence of either event in our cohort. Data were analyzed using StatsDirect software, version 2.8 0 (Altrincham, United Kingdom).

RESULTS

Patient Characteristics

A total of 323 hospitalized adult patients with CAP were included in the study cohort; 55% were male and the median age was 67 years (Table 1). Critical care admission (ICU or high-dependency unit) was required for 18.6% of patients, with 12.4% admitted specifically to the ICU. Total 30-day mortality was 6.2%.

Pathogen Detection by Molecular and Conventional Testing of LRT Specimens

A total of 323 LRT specimens were received from 323 patients; 310 (96.0%) were sputa and 13 (4.0%) were endotracheal aspirates. Culture-based microbiological testing identified a bacterial pathogen in 127 (39.3%) patients. All specimens were positive by PCR for the human gene target *GAPDH*, with an average load of $1.24 \times 10^5 \pm 1.18 \times 10^5$ gene copies per reaction; all specimens met quality control criteria for qualitative molecular testing.

Bacteria were detected by molecular testing in specimens from 262 (81.1%) patients (Table 2). When a cutoff of $\geq 10^5$ CFU/mL was applied for assays where bacterial load was quantified, bacteria were detected in specimens from 231 (71.5%) patients. Including respiratory virus results, overall pathogen detection by molecular testing rose to 86.7% (n = 280; Table 2). *H. influenzae* and *S. pneumoniae* were the most frequently identified bacteria in patients with CAP, detected in 130 (40.2%) and 115 (35.6%) patients, respectively. Detection of more than 1 bacterial species occurred in 102 (31.6%) patients. *H. influenzae* or *S. pneumoniae* were present in 94 (92.2%) codetections. Viruses were detected in 98 (30.3%) patients (Table 2); rhinovirus was most commonly detected (12.7%, n = 41) followed by influenza A/B (7.1%, n = 23). However, viruses alone were found in only 18 (5.6%) of our patients, with 80 (81.6%) viruses codetected with bacteria (Table 2). The most common combination was rhinovirus with *H. influenzae* and/or *S. pneumoniae* in 30 (37.5%) cases.

PCR detected significantly more *H. influenzae*, *S. pneumoniae*, *M. catarrhalis*, *S. aureus*, *E. coli*, and *K. pneumoniae* than standard culture-based methods (Supplementary Table 2).

Table 1. Characteristics of Included Patients With Community-Acquired Pneumonia (n = 323)

Demographics	N (%)
Male (%)	177 (54.8)
Age, median (interquartile range) years	67, 51–78
Age ≥65 y (%)	182 (56.3)
Age ≥75 y (%)	111 (34.4)
Comorbidity	
Chronic obstructive pulmonary disease	128 (39.6)
Ischemic heart disease or heart failure	91 (28.2)
Immunosuppression[a]	43 (13.3)
Diabetes mellitus	36 (11.1)
Neoplastic disease	31 (9.6)
Cerebrovascular disease	22 (6.8)
Chronic kidney disease	22 (6.8)
Chronic liver disease	6 (1.9)
Severity index	
CURB-65 score[b]	
0	73 (23.1)
1	83 (26.3)
2	84 (26.6)
3	56 (17.7)
4	19 (6.0)
5	1 (0.3)
Pneumonia severity index class[c]	
1	46 (21.2)
2	9 (4.2)
3	18 (8.3)
4	78 (35.9)
5	66 (30.4)
Admission C-reactive protein, mg/L (interquartile range)[d]	149.9 (43–246.5)
Antimicrobial administration	
Received antimicrobials in the 72 h prior to sputum sampling[b]	268 (84.8)
Outcome	
Intensive care unit admission	40 (12.4)
Intubation and ventilation	24 (7.4)
Vasopressor requirement	19 (5.9)
Total 30-day mortality	20 (6.2)

Abbreviation: CURB-65, confusion of new onset, blood urea nitrogen >7 mmol/l , respiratory rate ≥30 breaths per minute, blood pressure <90 mmHg systolic or ≤60 mmHg diastolic, age ≥65 years.

[a] Immunosuppressive drugs, human immunodeficiency virus infection, inherited immunodeficiency syndromes, hematological malignancy.

[b] Information available for 316 patients.

[c] Information available for 217 patients.

[d] Information available for 295 patients.

Of 127 culture-positive specimens, 125 (98.4%) were also PCR positive for the same species of bacteria; the 2 discrepant specimens were *H. influenzae* culture positive but culture negative by PCR. Significantly, PCR was able to detect bacteria in 143 culture-negative specimens. Specimens from 27 (8.4%) patients grew bacteria that were not included in our PCR assays (Supplementary Table 3).

Bacterial Load

Bacterial load quantifications from 29 (9.1%) specimens were excluded from analysis due to the detection of partial inhibition

Table 2. Pathogen Detection in Patients With Community-Acquired Pneumonia Using Molecular Methods (n = 323)

Pathogen	N (%)
Bacteria	
Any bacteria	262 (81.1)
With ≥10⁵ CFU/mL cutoff where quantified	231 (71.5)
Haemophilus influenzae	130 (40.2)
Streptococcus pneumoniae	115 (35.6)
Moraxella catarrhalis	44 (13.6)
Escherichia coli	37 (11.5)
Staphylococcus aureus	33 (10.2)
Klebsiella pneumoniae	13 (4.0)
Pseudomonas aeruginosa	9 (2.8)
Mycoplasma pneumoniae	6 (1.9)
Acinetobacter baumannii	3 (0.9)
Legionella pneumophila	3 (0.9)
Non-*pneumophila Legionella* spp.	3 (0.9)
Chlamydophila psittaci	2 (0.6)
Chlamydophila pneumoniae	0 (0)
Virus	
Any virus	98 (30.3)
Rhinovirus	41 (12.7)
Influenza	23 (7.1)
A	16 (5.0)
B	7 (2.2)
Parainfluenza virus	11 (3.4)
PIV-1	3 (0.9)
PIV-2	6 (1.9)
PIV-3	2 (0.6)
Coronavirus	9 (2.8)
HCoV-OC43	6 (1.9)
HCoV-NL63	2 (0.6)
HCoV-229E	1 (0.3)
HCoV-HKU1	0 (0)
Adenovirus	7 (2.2)
Respiratory syncytial virus	4 (1.2)
Human metapneumovirus	3 (0.9)
Any pathogen[a]	280 (86.7)
With ≥10⁵ CFU/mL cutoff for bacteria where quantified	263 (81.1)

Abbreviation: CFU, colony-forming unit.

[a] Codetection of bacteria and virus in 80 patients.

by the IC assay. A higher bacterial load was detected by PCR in culture-positive specimens in comparison to culture-negative specimens. The mean combined bacterial load was 8.77×10^8 CFU/mL in culture-positive vs 7.03×10^7 CFU/mL in culture-negative specimens ($P < .0001$). Significant differences between culture-positive and culture-negative specimens were also observed when *H. influenzae* ($P < .0001$) and *S. pneumoniae* ($P < .0001$) were considered individually.

Outcomes

Neither culture-positivity nor PCR-positivity was associated with the outcome of infection ($P = .2$; odds ratio [OR] = 1.5; 95% confidence interval [CI], .8–2.9; and $P = .8$; OR = .9; 95% CI, .4–2.6, respectively). Polymicrobial detection was also not

associated with the outcome of infection ($P = .4$; OR = 1.3; 95% CI, .7–2.5). The bacterial load of *S. pneumoniae* was associated with the outcome of infection on univariate analysis, with a higher load present in patients with 30-day mortality and/or ICU admission (4.33×10^8 CFU/mL vs 1.29×10^8 CFU/mL; $P = .009$). However, the CURB-65 score (CURB-65; Confusion of new onset, blood Urea nitrogen >7 mmol/l , Respiratory rate ≥30 breaths per minute, Blood pressure <90 mmHg systolic or ≤60 mmHg diastolic, age ≥65 years) was also associated with outcome on univariate analysis ($P = .01$). When logistic regression was used to correct for CURB-65 score in patients with *S. pneumoniae* infection, bacterial load was no longer significantly associated with outcome ($P = .09$; Supplementary Table 4).

Effect of Antimicrobial Therapy Prior to Microbiological Sampling

Of 316 patients with information available on antimicrobial administration prior to sampling, 268 (84.8%) had received antimicrobials either while in the hospital or in the community during the 72 hours before an LRT specimen was obtained. Prior antimicrobial administration was significantly associated with a patient having a culture-negative LRT specimen ($P < .0001$; OR = 9.1; 95% CI, 4.1–22.4). Of the 268 patients who had received prior antimicrobials, 77.6% (n = 208) had a bacterial pathogen detected by PCR, but only 32.1% (n = 86) were culture positive ($P < .0001$). The mean combined load of all detected bacteria per patient was significantly higher in patients who had not received antimicrobials prior to sampling (5.40×10^9 CFU/mL) compared with those who had received antimicrobials (2.67×10^8 CFU/mL; $P = .0001$).

Estimation of Impact on Antimicrobial Prescribing

Records of antimicrobial treatment during hospitalization were available for 99.1% (n = 320) of patients. Molecular testing had the potential to lead to de-escalation in number and/or spectrum of initial empirical antibiotic agents in 247 (77.2%) patients, escalation in number and/or spectrum of antibiotic agents in 19 (5.9%) patients, and no change in 54 (16.9%) patients (Table 3). The majority of the potential de-escalation events were related to switching of amoxicillin-clavulanate to narrower-spectrum agents such as amoxicillin and doxycycline in cases where *S. pneumoniae* or *H. influenzae* were detected by PCR and to the removal of clarithromycin in cases where atypical bacteria were not detected by PCR.

DISCUSSION

This study demonstrates that the use of a comprehensive multibacterial, multiviral molecular testing approach approximately doubles pathogen detection in patients with CAP from 39.3% to 86.7%, as well as provides valuable information about individual bacterial loads. Testing can be carried out within 1 working day to enable reporting of results in a clinically relevant time-frame, requires only a single LRT specimen, and is not

Table 3. Estimated Potential Impact of Comprehensive Molecular Testing on Antimicrobial Prescribing in Patients With Community-Acquired Pneumonia (n = 320)

Potential Modification	Antibiotic Agent	N (%)
De-escalation		247 (77.2)
Remove 1 agent		113
	CLR	108
	AMC	2
	Other[a]	3
Remove 2 agents		12
	CLR + AMX	6
	CLR + DOX	6
Reduce spectrum of agent		12
	AMC to DOX	8
	AMC to AMX	2
	Other[b]	2
Reduce number and spectrum		110
	AMC + CLR to DOX	61
	AMC + CLR to AMX	22
	AMX + CLR to AMC	12
	AMX + CLR to DOX	5
	CRO + CLR to DOX	4
	AMC + CLR to LEV	2
Other[c]		4
Escalation		19 (5.9)
Add 1 agent		2
	CIP	1
	DOX	1
Increase spectrum of agent		15
	CLR to DOX	6
	CLR to CIP	3
	DOX to AMC	3
	Other[d]	3
Increase number and spectrum		2
	AMX to DOX + CLR	1
	CLR to AMX + CIP	1
No change		54 (16.9)

Abbreviations: AMC, amoxicillin-clavulanic acid; AMX, amoxicillin; AZM, azithromycin; CIP, ciprofloxacin; CLR, clarithromycin; CRO, ceftriaxone; DOX, doxycycline; LEV, levofloxacin.

[a] DOX (1 patient), CRO (1 patient), AMX (1 patient).

[b] DOX to CLR (1 patient), DOX to AMX (1 patient).

[c] AMX + CLR to CIP (1 patient), AMC + CRO + CLR to CIP (1 patient), AMC + AZM to AMX (1 patient), AMC + DOX to AMX (1 patient).

[d] DOX to CIP (1 patient), CLR to AMC (1 patient), AMX to DOX (1 patient).

negatively impacted by antibiotic administration prior to sampling.

Only limited quantitative molecular bacterial testing has been carried out in a well-defined CAP setting [21, 25, 28, 29, 31]. As our study focused on testing sputum by molecular methods, our high level of pathogen detection for typical bacteria may not be directly comparable to results from other recent studies in hospitalized adult CAP. By combining several methodologies such as serology, culture, antigen detection, and PCR on multiple sample types, etiology was determined in 38%–76% of cases [4, 7, 21, 31, 36–41]. Atypical bacteria were uncommon in our cohort (<5% patients), but it was not an epidemic period for *M. pneumoniae* [42]. Viruses were detected in approximately one third of patients, which is in agreement with the 13%–56% range reported in previous studies; influenza was also detected at comparable levels (<10%) [4, 7, 21, 31, 36, 38, 39, 43]. Although we were not able to test controls, viral detections by PCR from the upper respiratory tract have been found to be rare in asymptomatic adults compared with those with CAP in a recent study [43].

One limitation of our study was that it was restricted to CAP patients who could produce a sputum specimen. Also, we did not include blood culture or urinary antigen tests. Sputum is a pragmatic, although imperfect, choice of specimen type for the microbiological investigation of CAP. Microscopy for sputum quality is not routinely carried out locally; however, only mucopurulent material was tested, and the cellular content was quantified by human *GAPDH* gene PCR to rule out salivary specimens. However, sputum that is expectorated from the lower airways will always be at risk of oropharyngeal flora contamination. Despite the limitations, national CAP guidelines [1, 5] recommend sputum investigation for patients admitted to the hospital with moderate to severe CAP, both to aid microbiological diagnosis in order to guide therapy as well as for surveillance. Alternative lower respiratory specimen types such as bronchoalveolar lavage fluids may be expected to be less at risk of contamination, but these require a semi-invasive procedure that is certainly not routine in nonintubated patients.

A key strength of our study was the availability of quantitative bacterial load outputs in addition to qualitative detection results. Quantification of bacterial DNA load may be important in distinguishing infection from oropharyngeal contamination in sputum. Most molecular work done to date has focused on *S. pneumoniae*, and a cutoff of 10^4–10^5 gene copies/mL is typically described as a significance threshold [20, 21, 29, 44]. A cutoff of 10^5 CFU/mL is also generally agreed upon for sputum culture as determined through years of experience. Based on these data, we applied a significance threshold of $\geq 10^5$ CFU/mL for all bacterial loads, and this did not significantly decrease overall pathogen detection. However, it remains unclear whether a single molecular cutoff is relevant for all bacterial species and whether cutoffs for sputum culture are relevant to sputum PCR. Quantitative molecular tools for many bacterial species are new [32, 33], and there is a clear need for further exploration of their role. Determination of thresholds for significant and nonsignificant detection are key targets for future work.

We found a higher mean bacterial load by PCR in culture-positive specimens in comparison to culture-negative specimens. However, the culture-negative group was more frequently antibiotic exposed, and antibiotic exposure was also associated with lower bacterial loads. As PCR is able to detect dead as well as viable bacteria, it is not clear for how long bacterial loads might be detectable after initiation of appropriate antibiotics

in patients with CAP and if monitoring of bacterial load by PCR would be useful in these patients. With the quantitative molecular tools now available for the relevant bacteria, these issues can also be further investigated.

A recent review has noted that interventions to reduce excessive and increase effective antibiotic prescribing in hospitalized patients can have a positive impact on antimicrobial resistance, hospital-acquired infections, and clinical outcomes [45]. However, studies in the pneumonia setting have been small and either focused on atypical bacteria and viruses [46] or nonquantitative molecular detection of bacteria [30], demonstrating 11% and 67% antibiotic modification, respectively. The results of comprehensive molecular testing in our study were not available to the treating physicians due to the anonymized study protocol. However, we estimated that de-escalation and/or reduction in the number of agents might have occurred in three quarters of our CAP patients. This was based on significantly improved bacterial detection capability, high rates of broad-spectrum empirical antibiotic usage in our cohort, and knowledge of local antimicrobial resistance patterns.

Clearly, a large number of additional factors influence antimicrobial selection (eg, severity of illness, concurrent infection at sites other than the lower respiratory tract, drug allergy, antimicrobial susceptibility testing, inflammatory markers), and we do not suggest that sputum PCR testing alone will be sufficient. However, the lack of positive microbiological identification is a significant issue, and it is highly likely that enhancing the detection of pathogens and reporting of bacterial loads would have a major impact on the clinical decision-making process. Therefore, our study illustrates the feasibility of providing the physician with significantly more information on which to base treatment decisions than is currently available and suggests that comprehensive PCR testing including bacterial load quantitation should be one of the inputs to future prospective studies in this area.

Supplementary Data

Supplementary materials are available at http://cid.oxfordjournals.org. Consisting of data provided by the author to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the author, so questions or comments should be addressed to the author.

Notes

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