Journal of Pathology, Microbiology and Immunology

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ORIGINAL ARTICLE OPEN ACCESS

Microbiological Analysis of Lower Airway Samples: Does It Influence the Outcome in Patients With Community-Acquired Pneumonia?

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Received: 11 November 2024 | **Revised:** 27 February 2025 | **Accepted:** 6 April 2025

Keywords: antimicrobial treatment | community-acquired pneumonia | lower-airway sampling | microbiological findings

ABSTRACT

This prospective randomized trial examined the impact of extensive lower respiratory tract sampling and microbial analysis on treatment decisions and patient outcomes in community-acquired pneumonia (CAP). One hundred and eighteen patients were randomized to rigorous sampling (intervention group, $n = 64$) or standard care (control group, $n = 54$). The primary outcome was antibiotic therapy change based on microbiological results. Secondary outcomes included intravenous therapy duration, hospital stay length, 30-day mortality, and readmission rate. Unexpectedly, many control group patients underwent sampling at the physician's discretion, making the groups equivalent in sampling frequency. Microbiological analysis led to antibiotic therapy changes in 28% of intervention and 30% of control patients. Overall, 34% of sampled patients had treatment adjustments, generally leading to antibiotic broadening. No significant differences were observed between groups in secondary outcomes. While rigorous sampling did not significantly impact overall patient outcomes, microbiological analysis influenced treatment decisions in a substantial proportion of patients. Future studies should evaluate the effects of sampling for CAP diagnosis in settings where broader-spectrum antibiotics are the empirical treatment of choice to determine the impact on treatment decisions.

1 | Introduction

Community-acquired pneumonia (CAP) carries a significant mortality, and successful outcome relies on accurate and swift antimicrobial treatment [1–4]. In Denmark, CAP is associated with 30-day mortality rates ranging from 10% to 15%, underscoring its significant impact on public health. In countries with a restrictive antibiotic policy, like Denmark, national recommendations support the use of narrow-spectrum antibiotics, such as penicillin, to treat CAP. This is based on a high prevalence of susceptibility to penicillin among invasive isolates of pneumococci [5], which is one of the most common respiratory pathogens [6–8]. As patients are hospitalized, however, treatment with broad-spectrum antibiotics may be initiated due to the severity of illness or uncertainty about the causative pathogen. Since pneumonia caused by different pathogens shows similar symptoms, negative diagnostic tests may be used to de-escalate treatment. Conventional culture-based microbiological methods often fail to identify an etiological pathogen in many CAP cases, likely due to a combination of variable quality of sputum samples and the risk that patients are unable to produce good-quality sputum samples, potentially leading to false-negative results. Yet, conventional culturing continues to be the golden standard of microbiological diagnostics [1–4]. A recent study revealed that in a subgroup of patients who underwent lower respiratory tract sampling, the

detection of *Haemophilus influenzae* or *Streptococcus pneumoniae* significantly impacted the narrow-spectrum antibiotic treatment [1–4]. Overall, patients who had a lower respiratory tract sample taken received antibiotics for an average of 10.4 days compared to 9.9 days for patients without sampling. Additionally, the average duration of intravenous antibiotic treatment increased from 3.2 days for patients without sampling to 3.8 days for those with collection. Notably, survival analysis revealed a significantly lower probability of 90-day mortality in patients who underwent lower respiratory tract sampling [1–4]. Molecular diagnostic tests hold promise for optimizing treatment decisions and management of CAP. One study revealed a significant increase in pathogen-directed treatment when using Rapid syndromic PCR testing (BioFire FilmArray Pneumonia plus Panel) compared to the standard-of-care, with 47.4% receiving pathogen-directed treatment within 48 h, compared to only 15.5% with standard-of-care. However, the higher rate of pathogen-directed treatment in the intervention arm suggests that more patients likely had their treatment escalated [1–4]. Another Danish study found no difference between patients diagnosed with either point-of-care PCR or conventional culturing regarding intensive care unit admissions, readmission within 30 days, length of stay, 30 days mortality, and in-hospital mortality [9]. They found no significant difference in prescriptions of no or narrow-spectrum antibiotics at 4 h. The conclusion for the latter study indicated that use of respiratory point-of-care diagnostics was not an effective tool for reducing the use of antibiotics—although this was in a Danish setting with a low level of antibiotic resistance and prudent use of antibiotics [9].

We hypothesize that conventional lower respiratory tract sampling could influence treatment decisions and outcomes for patients with CAP by identifying specific pathogens, allowing for targeted antibiotic therapy, and informing infection control measures.

In the pre-COVID-19 era, we randomized patients admitted with CAP to rigorous lower respiratory tract sampling (intervention group) or to standard care (control group). We wanted to examine whether extensive sampling from the lower respiratory tract and microbial analysis of samples influenced treatment, duration of intravenous therapy, admission length, readmission rates, and survival.

2 | Methods

2.1 | Diagnostic Algorithm and Data Collection

This study was designed as a prospective randomized trial. Patients admitted with CAP to the medical emergency room (ER), Herlev Hospital in Copenhagen, Denmark in the period 1st of April 2017—31st of January 2018, and who were able to give informed consent were eligible for participation. Herlev Hospital has 709 beds, is one of the major emergency hospitals in the Capital Region of Denmark, and 1 of 4 hospitals in the region with 24-h emergency admissions. It serves as an emergency hospital for approximately 425,000 residents, with 153,370 visits to the ER annually.

CAP was defined as a new infiltrate on chest X-ray and at least one of the following symptoms of lower respiratory tract infection: cough, purulent expectoration, fever ($\geq$38.3°C rectally or $\geq$37.8°C tympanic), or pathological lung auscultation. Patients who had uncontrolled bleeding from the upper or lower respiratory tracts and patients who were transferred directly to the intensive care unit were excluded. Treatment with oral antibiotics before the time of diagnosis did not exclude participation. After obtainment of informed consent, participants were randomized either to the intervention group or to the standard care group (control group). The randomization procedure consisted of closed envelopes containing 5 control and 5 intervention assignments in random order. Patients were asked about participation, and in case of acceptance, an envelope was drawn by the assigning physician and the assignment filed. After 10 assignments, another 10 envelopes were placed for the randomization procedure, and so forth.

The patients in the intervention group were asked to produce a sputum sample and if unable to do so, endotracheal aspiration was done by the admitting physician according to local guidelines. In either case, sampling was done before administration of antibiotics. Sampling and microbiological analysis in the control group were not encouraged but were done at the discretion of the treating physician. The primary outcome was a significant change in antibiotic therapy in response to microbiological results. A significant change was defined as: (i) targeting a specific microorganism; (ii) changing to oral treatment; (iii) cancellation of antibiotic treatment. Broadening or narrowing of antibiotic therapy on empirical grounds alone was not considered a significant change.

Secondary outcomes were duration of intravenous therapy, duration of hospital stay, 30-day mortality, and 30-day readmission rate.

Our expectation was an earlier targeting of the antibiotic treatment against a specific microorganism, an earlier change in antibiotic treatment to oral treatment, or an earlier cancellation of antibiotic treatment in response to a microbiological result in the intervention group. We formulated a null hypothesis: the change in antibiotic treatment in the intervention group did not happen earlier than in the control group. We aim to reject the null hypothesis with 80% confidence, and with 95% certainty, that is, with a probability that the findings represent random variation of less than 5% ($p \leq 0.05$). Our pre-study anticipation was a reduction in days on intravenous antibiotic treatment from 5 to 3 days. From this prerequisite, the number of patients in the two groups was calculated to 60 (Power Calculator, Vanderbilt Biostatistics PS).

2.2 | Microbiological Analyses

Airway samples were analyzed at the Department of Clinical Microbiology, Herlev University Hospital. Microbiological analysis included Gram-stain and microscopy to determine the quality of the sample. Respiratory pathogens were identified from conventional culturing in air with 5% carbon dioxide (CO_2) at 35°C for 24 h using 5% blood agar and chocolate agar plates (SSI Diagnostica, Cph, Denmark or Herlev Hospital). Culturing in

ambient air at 35°C for 24 h using blue agar plates with modified Conradi-Drigalski diagnostic substrate, selective for Gram-negative bacteria (SSI Diagnostica, Cph, Denmark), was carried out as well. Cultured species were identified by use of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) (Bruker Daltonics, Billerica, MA, USA). Scores > 2.0 were required for identification at the species level and > 1.7 at the genus level. Atypical bacteria (*M. pneumoniae*, *C. pneumoniae*, *C. psittaci* and *L. pneumophila*) were identified using real-time PCR (DNA extraction by QiaSymphony, Qiagen and RotorGene Q using an in-house protocol) and respiratory viruses were identified using PCR (Influenza A, Influenza B and Respiratory syncytial virus [RSV]) (Panther Fusion Assay, Hologic). For the current study, all cultures were assessed daily.

2.3 | Data Analysis

Data were collected from the electronic patient files: antibiotic treatment history, microbiological findings, clinical course, length of stay, and outcome. Changing antibiotic treatment in response to microbiological findings (i.e., narrowing the antibiotic treatment, targeting a specific microorganism, changing to oral treatment, or cancelation of treatment) was considered a significant action. Some patients were discharged before microbiological results became available, but if the treating physician encountered the result and acted upon it, for example, by treatment change via phone call, it was still considered a significant action. If, on the other hand, results were not acted upon before the patient was readmitted, it was not considered a significant action. Most clinical guidelines recommend re-evaluating the need for further intravenous therapy after 72 h of treatment. Continuing intravenous therapy after microbiological analysis revealed a susceptible microorganism, or changing to oral therapy on pure clinical grounds, was not considered a significant action.

Admission length was calculated as hospital stay in calendar days. Likewise, time to oral treatment was calculated as the number of calendar days with parenteral treatment; thus, initiating parenteral treatment and changing to oral treatment on the same day was, by convention, 1 day of parenteral treatment. Thirty-day mortality was assessed from admission, and patients who died during hospital stay were considered discharged on the day of death.

Each patient was assigned a number in a database. Data were subsequently analyzed as described above by investigators who were unaware of the study group assignment. Analysis of data was done independently by two different physicians. Disagreements between conclusions made by the investigators were discussed in plenum with a third investigator. Consensus was obtained in all cases.

2.4 | Ethics, Informed Consent, National Registry Board

Endotracheal aspiration is a well-recognized clinical procedure performed routinely in patients with pneumonia. Written informed consent was provided by the participants, and each participant could withdraw their consent at any time. The study was approved by the Danish Patient Safety Authority ("*De Videnskabsetiske Komitéer*", record no. H-16046798).

2.5 | Statistical Analysis

Non-parametric statistics with unpaired *t* test was used to compare admission length and length of intravenous therapy between groups. On the assumption that admission length and length of intravenous treatment both fitted into a normal distribution, they were reported as mean values ± SEM. Significance between groups for significant action was calculated with Fisher's exact test on the assumption that intervention would lead to a higher number of treatment changes as compared to no intervention. All *p* values were two-sided; a value ≤ 0.05 was considered significant.

3 | Results

A total of 118 patients were included in the study. There were 64 patients in the intervention group: 35 males and 29 females; median age 74 years; range 20–95 years. Two of the patients (an 81-year-old woman and a 91-year-old man) were included twice 40 and 7 days after the first discharge, respectively. One patient apparently never delivered a sample/had an endotracheal aspiration done despite inclusion in the intervention group. For 1 patient, the sample was not correctly labeled (discarded by the lab), and for 1 patient, the sputum container was leaky (discarded by the lab). For 8 patients, the samples contained upper respiratory tract secretions only (culture results were not reported), while for 48 of 64 patients in the intention-to-treat (ITT) population, the samples were of good quality, that is, contained lower respiratory tract secretions. For 1 patient, sampling was not done before initiation of antibiotic treatment, and for 1 patient, hospital-acquired pneumonia was likely given recent discharge from another hospital department. All 64 patients were included in the ITT analysis.

Thirty-one samples contained a relevant microbiological finding (Table 1) and in 18/64 patients (28%) the microbiological finding led to significant action, that is, changing to another antibiotic, changing to oral treatment, or discontinuing treatment.

Eight patients were discharged with empirical treatment not targeting the recovered microorganism: 5 patients were contacted by phone when microbiological results appeared (1 sample with *Haemophilus influenzae*, 1 sample with *Moraxella catarrhalis*, 1 sample with *Mycoplasma pneumoniae*, and 2 samples with *Legionella pneumophila*). For 2 patients, microbiological findings (*H. influenzae* in both cases) went unnoticed until the time of readmission (5 and 7 days after discharge, respectively), where microbiological findings were reviewed, and appropriate treatment initiated. For 1 patient who was discharged with non-active treatment (phenoxymethylpenicillin) against the cultured microorganisms (*H. influenzae* and *M. catarrhalis*), microbiological results were never acted upon. Two patients continued antibacterial treatment despite only influenza B being recovered from their airway samples. Two patients in the intervention group had *L. pneumophila* infection: 1 had untreated CLL with an unknown source of infection and 1 previously healthy patient

TABLE 1 | Microbiological findings in airway samples from the two groups. In the control group, samples from 23/54 patients (43%) revealed ≥1 or more relevant pathogens. In the intervention group, samples from 27/52 patients (52%) revealed ≥1 relevant pathogen.

Microbiological finding	Control group (ITT; $n=54$)	Intervention group (ITT; $n=64$)
S. pneumoniae	7[a]	5[a]
H. influenzae	11[b]	12[c]
M. catarrhalis	0	2[a]
S. aureus	0	4[d]
P. aeruginosa	0	3[d]
E. coli	0	1[d]
K. oxytoca	0	1[d]
S. marcescens	2[e]	0
Mycoplasma	3[f]	3
Legionella	1	2[g]
Influenza A	1[h]	0
Influenza B	2[i]	2
RSV	0	1[j]

[a]Including 1 sample with concomitant *H. influenzae*.
[b]Including 1 sample with concomitant *S. pneumoniae* and 1 sample with concomitant *S. marcescens*.
[c]Including 1 sample with concomitant *S. pneumoniae*, 1 sample with concomitant *M. catarrhalis*, and 1 sample with concomitant *S. aureus*.
[d]Including 1 sample with concomitant *H. influenzae*.
[e]Including 1 sample from a patient pretreated with amoxicillin/clavulanate prior to sampling, and 1 sample with concomitant *H. influenzae*.
[f]Including a patient where PCR was requested on the initial sample on day 2 of admission due to clinical deterioration.
[g]Including 1 patient who was discharged before the test result appeared and who was readmitted due to deterioration at home; the diagnosis was recognized upon the second admission (PCR was requested with delay on initial sputum sample).
[h]With concomitant *S. pneumoniae*.
[i]One sample with concomitant *S. pneumoniae*.
[j]With concomitant *H. influenzae* and *S. pneumoniae*.

likely contracted *L. pneumophila* while working as an electrician in a building where residual water from abandoned public water-supply tubes sprayed over his head. That patient was discharged after 1 day of admission and on day 3 PCR analysis for *L. pneumophila* came out positive. The patient was readmitted, and anti-legionella treatment initiated.

There were 54 patients in the control group, all unique: 34 males and 20 females; median age 74 years; range 18–90 years. Even though not encouraged by the protocol, respiratory tract sampling was extensively done in the control group as 40/54 patients delivered a sputum or had an endotracheal aspiration done. Four samples contained only upper respiratory tract secretions, 1 sample was analyzed by PCR only and was not evaluated by microscopy, while 35 samples were of good quality; this number was not significantly different from the number in the intervention group. Twenty-two samples revealed a relevant microbiological finding (Table 1) and in 16/54 patients (30%) led to significant action, that is, changing to another antibiotic, changing to oral treatment, or discontinuing treatment. A case of *L. pneumophila* infection in a patient with untreated CLL went unrecognized as the initial sputum sample was not analyzed by PCR. Endotracheal aspiration performed on day 4 of admission tested PCR-positive for *L. pneumophila*. Two patients had concomitant blood stream infection with *S. pneumoniae*, and 1 patient had blood stream infection with both *S. pneumoniae* and *H. influenzae*. For 2 patients, the source of blood stream infection was considered other than lung: 1 with *K. pneumoniae* likely originating in the urinary tract and 1 with *S. anginosus*.

There was no difference between groups in duration of intravenous treatment, admission length, readmission rate, or mortality rate. For the ITT population, the duration of intravenous treatment in the intervention group was (mean ± SEM) 4.40 ± 0.53 days versus 4.39 ± 0.39 days in the control group. Admission length in the intervention group was 5.67 ± 0.63 days versus 6.94 ± 0.76 days in the control group. When considering patients as handled per protocol where sampling was done ($n=101$), the duration of intravenous treatment was 4.41 ± 0.37 days versus 4.38 ± 0.89 days in patients where sampling was not done ($n=17$). Likewise, admission length was 6.26 ± 0.54 days in patients where sampling was done versus 6.44 ± 1.38 days in patients where sampling was not done. Neither difference was statistically significant.

An equal percentage of patients in the intervention group (11/64 patients = 17%) and in the control group (9/54 patients = 17%) were readmitted < 30 days from discharge. A similar percentage of patients in the intervention group (6/64 patients = 9.4%) and in the control group (4/54 patients = 7.5%) died < 30 days from admission.

4 | Discussion

In this study, airway sampling led to significant action in an equal percentage of patients in the intervention group and in the control group (28% vs. 30%). One hundred and one patients in total (61 in the intervention group and 40 patients in the control group) produced a sputum sample or had an endotracheal aspiration done for analysis. Sampling in the control group was not encouraged by the protocol but, for ethical reasons, could not be discouraged, whereby any conclusion about intervention impact was impaired. Thus, airway sampling in the control group (74%) was much higher than usual practice (45%–50%). The intervention and control groups were recruited within the same hospital by the same group of physicians, and we believe that awareness of the study concept encouraged the recruiting physicians to obtain microbiological samples in the control group as well. In favor of this hypothesis, pre-study observations revealed a much lower rate of microbiological sampling in patients with pneumonia (data not shown). Thus, viewing the control group and the intervention group together as a single CAP cohort seems to be the most sober approach. Ideally, the study should have been performed at different hospitals, ensuring a more stringent adherence to protocol [10–12]. Significant action regarding antimicrobial treatment (e.g., de-escalation or broadening) was overall taken in 34 of the 101 sampled patients (34%). Vigilance to pending microbiological results may have led to an even higher number of treatment adjustments, as microbiological analysis

revealed a relevant finding in about half of the cases (54/101 patients = 54%) which agrees with other studies [6, 13].

It is worth noticing that in a Danish context, changing antibiotic treatment in response to a culture result will virtually always mean broadening the treatment. This is because penicillin is the empiric treatment for CAP in Denmark [14] and virtually all other antibiotics have a broader spectrum than penicillin. If only a virus is recovered from the lower airways, ideally, treatment with antibiotics should be interrupted, but clinicians in a hospital setting are reluctant to stop antibiotic treatment. If, for example, only influenza A is found, an antiviral compound typically will not replace antibiotics but will usually be added. If the patient is severely ill, few clinicians will feel comfortable about aborting a cheap and relatively harmless treatment despite no bacterial microorganism being recovered. This practice is understandable given the low negative predictive value of microbiology results for infection. A sensitivity of < 50% has been reported from large clinical studies [15, 16].

Considering the patients where microbiological analysis of the lower airway sample revealed a positive finding (virus or bacterium; $n = 53$), there was a tendency that the time to change to oral treatment was shorter than in the group with no positive sputum/endotracheal sample ($n = 65$): 3.89 ± 0.35 days versus 4.81 ± 0.54 days (95% CI interval −0.42 to 2.27 days; $p = 0.17$). Likewise, the admission length was 5.25 ± 0.49 days in patients with a positive lower airway sample versus 7.09 ± 0.79 days (95% CI interval −0.08–3.77 days; $p = 0.06$) in patients with a negative lower airway sample. The differences, however, did not reach a level of statistical significance. The patients with non-positive lower airway samples comprised a highly heterogeneous group representing diverse medical conditions (data not shown). As part of the inclusion criteria, a new pulmonary infiltrate should be observed on an X-ray film. We did not review the X-ray findings but relied on the initial interpretation by the clinician. Some patients not having CAP may thus have been included in the study, especially in the group with a negative sample.

The most common pathogen recovered was *H. influenzae*, not *S. pneumoniae*, which appears to be a feature of CAP in Denmark [17]. One explanation may be that conjugated pneumococcal vaccination is a part of the national Danish immunization program with 3 doses of a conjugated vaccine given within the first year of living, lowering the prevalence of that pathogen in the community and providing herd immunity to the benefit of elder persons. Next, penicillin, as mentioned, is the drug of choice for CAP in Denmark, and admitted patients may have been selected by pre-hospital treatment with oral penicillin (numbers not assessed in the protocol). Three patients in total had infection with *L. pneumophila*, which might lead to a serious clinical condition with an often-protracted course demanding 2–3 weeks of targeted antimicrobial treatment. This stresses the importance of obtaining a careful history upon admission, but also that details about exposure are sometimes missed; performing upfront microbiological sampling may complement care.

Microbiological results from lower respiratory tract samples in patients admitted with CAP can provide important guidance for antibiotic treatment [9, 12, 18]. Even though our study did not show a significant difference between intervention and control groups, likely due to extensive sampling in both groups, we could speculate that baseline resistance rates influence the outcome. While microbiological findings influenced treatment in about one-third of cases, this did not translate to significant differences in clinical outcomes [12, 15, 18]. This is contradicting to another Danish study presented a different paradox in the management of CAP patients [4, 19]. While lower respiratory sampling was associated with extended antibiotic treatments and longer hospital stays, it also correlated with reduced mortality. The differences in the results of the studies underscores the need for better integration of test results into clinical decision-making to optimize antibiotic use and hospital stay duration [12, 15, 18]. In our study, some relevant microbiological findings went unnoticed or were not acted upon. As seen previously, the association between lower respiratory sampling collection and longer antibiotic treatments (both intravenous and total) raises questions about the interpretation and application of culture results [4, 19]. It is possible that positive cultures lead to prolonged treatment, even when not clinically necessary or that negative cultures in some cases do not lead to ceasing treatment. Additionally, the lower probability of early discharge could be related to the extended antibiotic treatments or might indicate that these patients had more severe infections requiring longer care. Potentially, the use of point-of-care testing or PCR for diagnosing CAP could provide fast and reliable results. Yet, implementing rapid molecular testing systems can be expensive and may require additional resources and training for laboratory staff [9, 19, 20]. The molecular testing systems does come with some limitations as the high sensitivity of these tests may lead to the detection of colonizing organisms or non-viable pathogens, potentially complicating clinical interpretation [9, 19, 20]. This could be a reason that rapid testing so far has shown limited impact on clinical outcomes nor differences in length of hospital stay or readmission rates. Finally, rapid testing does show risk of overtreatment due to the detection of multiple pathogens. However, as the conventional culturing does not seem to decrease treatment length significantly and we likewise found more than one relevant pathogen in 53 of 101 samples (Table 1) there is a continuous need for improving the diagnostic method of CAP [9, 12, 19, 20]. Our results suggest that routine lower respiratory tract sampling could play a crucial role in antibiotic stewardship efforts, potentially reducing unnecessary broad-spectrum antibiotic use. However, the observed paradox of extended treatments despite positive cultures indicates a need for better integration of microbiological results into clinical decision-making processes and for studies to be performed in other locations. Future research should focus on implementing rapid molecular testing alongside conventional methods, improving result interpretation and application, and assessing the impact of this approach in settings with higher antibiotic resistance rates. Additionally, studies should explore strategies to enhance the translation of microbiological findings into improved clinical outcomes and reduced antibiotic use.

Considering patients who died during hospitalization as discharged on the day of death and assessing 30-day mortality from admission ensures a consistent endpoint and standardized timeframe, allowing for uniform length-of-stay calculations and adherence to the ITT principle. However, this approach may underestimate length-of-stay for those who might have stayed longer if they survived and does not distinguish between early

in-hospital and post-discharge deaths, potentially masking differences in immediate treatment effects or the intervention's impact on survivors.

Our study was conducted in Denmark, which has relatively low antibiotic resistance rates compared to most other countries. In areas with high antibiotic resistance rates and the use of broad-spectrum empiric antibiotic treatment, the impact of microbiological sampling as a de-escalation tool may be more pronounced and lead to treatment adjustments. The ITT analysis excluded three cases of CAP due to lost samples, which may have introduced bias and reduced the robustness of our findings. Including these cases could have provided a more comprehensive understanding of the intervention's impact. However, since it was only three cases the impact on the results is expected to be small.

5 | Conclusion

Lower respiratory tract sampling led to treatment modifications in about one-third of sampled patients, demonstrating its potential to optimize antibiotic therapy. In a setting with a low background antibiotic resistance level like Denmark, however, the benefit of systematic lower respiratory tract sampling was not obvious, since narrow-spectrum penicillin is recommended as empirical treatment for CAP. An important exception was the detection of *Legionella* species and of *Mycoplasma pneumoniae*, which would argue for sampling, however only in a minority of patients with CAP. This effect of rigorous sampling should be evaluated in settings different from Denmark, where broader-spectrum antibiotics are the empirical treatment of choice for CAP.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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