Fast multiplex bacterial PCR of bronchoalveolar lavage for antibiotic stewardship in hospitalised patients with pneumonia at risk of Gram-negative bacterial infection (Flagship II): a multicentre, randomised controlled trial

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Summary

Background PCR-based testing has transformed the management of suspected respiratory viral infections. We aimed to determine whether multiplex bacterial PCR of bronchoalveolar lavage fluid aids antibiotic stewardship in patients with pneumonia.

Methods This investigator-initiated, multicentre, randomised controlled trial was conducted at two tertiary care centres in Switzerland (University Hospital of Basel and Kantonsspital St Gallen). Patients aged 18 years or older who were admitted to hospital with suspected pneumonia, had a clinical indication for bronchoscopy with bronchoalveolar lavage, and were at risk of Gram-negative bacterial infection were included. Patients were randomly assigned (1:1) to either the multiplex bacterial PCR group or the conventional microbiology control group using a random allocation sequence. Treating physicians were not masked, but the committee panel was masked to patient randomisation. All patients underwent bronchoscopy with bronchoalveolar lavage and samples were assessed by conventional microbiological culture (and additionally, in the PCR group, by multiplex bacterial PCR for Gram-negative rods using the Unyvero Hospitalized Pneumonia [HPN] Cartridge; Curetis, Holzgerlingen, Germany). Patients received empirical antibiotic therapy as clinically indicated by the treating physician. In the PCR group, a recommendation regarding antibiotic therapy was made approximately 5 h after taking the sample. The primary outcome was the time in hours on inappropriate antibiotic therapy from bronchoscopy to discharge or to 30 days after bronchoscopy. This trial was registered with the International Clinical Trials Registry Platform, ISRCTN95828556.

Findings Between May 31, 2017, and Sept 25, 2019, 740 patients with pneumonia were screened for eligibility and 208 were included and randomly assigned to the PCR group (n=100) or conventional microbiology control group (n=108). The mean age of patients was 65·9 years (SD 14·0) and 135 (65%) were male. After daily follow-up until hospital discharge or for a maximum of 30 days, the duration of inappropriate antibiotic treatment was significantly shorter by 38·6 h (95% CI 19·5–57·7) in the PCR group than in the control group (adjusted mean 47·1 h [34·7–59·5] *vs* 85·7 h [78·8–95·6]; p<0·0001), which translates as a decrease in the duration of inappropriate antibiotic therapy of 45·0% (37·9–52·1). Adverse events due to antimicrobial therapy occurred in nine patients (five [5%] in the PCR group *vs* four [4%] in the control group) and due to bronchoscopy occurred in four patients (two [1%] *vs* two [1%]). There were eight (8%) deaths in the PCR group and 11 (10%) in the control group. All in-hospital deaths were attributed to a respiratory cause.

Interpretation Multiplex bacterial PCR examination of bronchoalveolar lavage decreases the duration of inappropriate antibiotic therapy of patients admitted to hospital with pneumonia and at risk of Gram-negative rod infection. This approach warrants further consideration in future antibiotic stewardship strategies.

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Introduction

Despite advances in diagnostics and treatment, pneumonia remains one of the leading causes of morbidity and mortality worldwide.[1,2] Determining the causative infectious agent is pivotal in the prognosis and management of pneumonia.[3–6] Irrespective of the investments in prevention, hospital-acquired pneumonia accounts for more than 20% of nosocomial infection.[7] Hospital-acquired pneumonia is caused by Gram-negative pathogens in around a third of cases, which has prompted guidelines to recommend empirical coverage of Gram-negative rods.[4] Unsuccessful antimicrobial targeting of such infectious agents has been linked to increased mortality.[8] Similarly, risk factors (such as

Research in context

Evidence before this study

Currently, the identification of bacterial pathogens relies mainly on microbiological cultures, which offer results after 24–48 h. Multiplex bacterial PCR assays are emerging as a complementary method that offers shorter turnaround times when detecting microorganisms that cause lower respiratory tract infections. Retrospective studies suggest that multiplex bacterial PCR could increase and accelerate the rates of antibiotic de-escalation, as the main goal of antibiotic stewardship. Nevertheless, multiple issues, such as positive PCR results because of colonisation and an absence of tailored antibiotic therapy recommendations, have precluded faster diagnostic turnaround times and affected clinical outcomes. We searched PubMed from Jan 1, 1990, to Sept 29, 2021, with no language restrictions using the terms "bacterial multiplex PCR" AND "pneumonia" AND "bronchoalveolar lavage". We identified 43 studies, none of which were randomised controlled trials. Our study addresses the unmet need for high-quality evidence to support diagnostic algorithms based on bacterial molecular testing.

Added value of this study

To our knowledge, this is the first prospective, multicentre, randomised controlled trial assessing the safety and efficacy of antibiotic stewardship using a Gram-negative targeted multiplex bacterial PCR of bronchoalveolar lavage in patients admitted to hospital with pneumonia. We have shown that inappropriate antibiotic therapy can be substantially reduced by complementing conventional microbiology with a multiplex bacterial PCR-guided antibiotic therapy recommendation. Moreover, the refinement of antibiotic therapy was achieved without compromising essential outcomes, such as time to clinical stability, length of hospital stay, intensive care admission, or mortality.

Implications of all the available evidence

Trials in the past 30 years addressing the viability of PCR testing at improving antibiotic stewardship programmes have mainly focused on viral agents and meticillin-resistant *Staphylococcus aureus*. Our study provides strong evidence that additional targets, such as Gram-negative rods, could help to achieve the goals of antibiotic stewardship. Should these results be reproduced in subsequent trials, multiplex bacterial PCR opens up new possibilities for curbing the increase in bacterial resistance.

previous admission to hospital or exposure to antibiotic therapy) are associated with increased risk of infection by Gram-negative rods resulting in community-acquired pneumonia,[6,9–12] which is associated with higher mortality.[9,11] Broad-spectrum empirical therapy, similar to that for hospital-acquired pneumonia, is recommended for patients with such risk factors.[3] The emergence and spread of antibiotic resistance in Gram-negative bacteria is of particular concern, affecting both patient outcomes and cost efficiency.[12,13]

Conventional microbiological testing does not routinely deliver a rapid diagnosis for patients with a suspected bacterial infection. Therefore, treatment remains empirical in most cases. A viable strategy, endorsed by current guidelines,[3] includes consistent sampling and broad antimicrobial coverage with antibiotic de-escalation at 48–72 h if cultures remain negative. Alternatively, the potential of accurate and targeted pathogen testing has prompted medical societies, such as the Infectious Diseases Society of America[14] and American Thoracic Society,[3] to advocate further research into new molecular diagnostic tests. Although the efficacy of viral PCR-based diagnostic tests in reducing unnecessary antibiotic use through antimicrobial stewardship programmes is established,[6,15] the promising efficacy of multiplex bacterial PCR is still unclear. Despite its higher sensitivity than microbiological culture,[16] the results of molecular testing have not yet been translated into improved patient outcomes.[14,17] Based on the fact that Gram-negative coverage can be de-escalated using PCR multiplex tests,[14,18] we hypothesised that analysis by multiplex bacterial PCR of bronchoalveolar lavage fluid from patients admitted to hospital with pneumonia and at risk of Gram-negative rod infection would lead to shorter duration of inappropriate antibiotic therapy than analysis by conventional microbiology.

Methods

Study design and participants

This investigator-initiated, multicentre, randomised controlled trial was conducted at two tertiary care centres in Switzerland (University Hospital of Basel and Kantonsspital St Gallen), with 24 h availability of respiratory care specialists and access to a bronchoscopy. Patients aged 18 years or older who were admitted to hospital with suspected pneumonia, had a clinical indication for bronchoscopy with bronchoalveolar lavage, and were at risk of Gram-negative bacterial infection were included. The suspicion of pneumonia was on the basis of new pulmonary infiltrate seen on chest x-ray or CT scan and at least one of the following: new or increased cough with or without sputum; fever or systemic inflammation, such as abnormal white blood cell count showing leukocytosis ($>10{\cdot}0\times10^9$/L) or leukopenia ($<4{\cdot}0\times10^9$/L); or C-reactive protein (≥10 mg/L) or procalcitonin (≥0·1 μg/L) values higher than the local upper limit of normal.

Participants enrolled in the study had a working diagnosis of hospital-acquired pneumonia, defined as pneumonia occurring 48 h after hospital admission,[4] or community-acquired pneumonia with concomitant risk factors for a Gram-negative rod infection (details,

particularly for Enterobacteriaceae and non-fermenters, are shown in the appendix p 1), as suggested by guidelines.[3,6,19,20] Patients with neutropenia, defined by an absolute blood neutrophil count of less than $0·5×10^9$/L, were excluded. Full details of eligibility criteria are provided in the appendix (p 1). The study material was standardised, and procedures were run equivalently at both sites.

All patients provided written informed consent. An independent ethics committee approved the study (Ethikkommission Nordwest- und Zentralschweiz, Basel, Switzerland; EKNZ 2017–00043), which was carried out in accordance with the Declaration of Helsinki, Guidelines for Good Clinical Practice, the Swiss Law, and Swiss regulatory authority requirements.

Randomisation and masking

Patients were randomly assigned (1:1) to either the multiplex bacterial PCR group or the conventional microbiology control group using a prespecified computer-generated random allocation sequence. The randomisation list was concealed using a centralised password-secure website and managed by an external company (Particletree, Basel, Switzerland), independently of the sponsor and study personnel. Participants were enrolled by trained study physicians and nurses. The treating physicians were not masked to patient randomisation. The committee panel was masked to patient randomisation until the question regarding change of therapy arose, according to PCR results.

Procedures

All patients underwent bronchoscopy with bronchoalveolar lavage after assessment by a respiratory physician. Patients were asked to expectorate spontaneous sputum for later analysis. Nurses trained in endoscopy performed conscious sedation[21–23] and the bronchoscopy was performed transnasally or transorally with the patients in a semi-recumbent position. Selection of the bronchoalveolar lavage site was guided by previous radiological findings and the procedure was performed by introducing 150 mL of sterile 0·9% NaCl into the affected lobe or segment. All patients received standard periprocedural care.[21]

All bronchoalveolar lavage samples were assessed by conventional microbiological investigations. In the PCR group, bronchoalveolar lavage samples were additionally assessed for Gram-negative bacteria by multiplex bacterial PCR using the Unyvero Hospitalized Pneumonia [HPN] Cartridge, as per manufacturer's instructions (Curetis, Holzgerlingen, Germany).[24] This molecular diagnostic system detects pneumonia-causing pathogens and markers of antibiotic resistance. The application integrates sample preparation, nucleic acid extraction and purification, amplification, specific detection, and analysis.[25,26] The HPN panel can identify two Gram-positive bacteria (*Staphylococcus aureus* and *Streptococcus pneumoniae*),[14] Gram-negative bacteria (including *Legionella pneumophila*; appendix p 1), two atypical bacteria (*Mycoplasma pneumoniae* and *Chlamydophila pneumoniae*), and one fungus (*Pneumocystis jirovecii*). Only the results for Gram-negative rods were disclosed to the attending physician. For the control group, multiplex bacterial PCR was performed in a batch after the study ended and had no influence on patient treatment or care. All bronchoalveolar lavage samples were also analysed using a second, commercially available multiplex PCR test designed for identification of multiple respiratory viral and atypical bacterial agents (appendix p 4). The University Hospital Basel used MAGPIX (Luminex, 's-Hertogenbosch, Netherlands) until Nov 30, 2018, and RespiFinder-22 (RF-22, PathoFinder, Maastricht, Netherlands) from Dec 1, 2018, and the Kantonsspital St Gallen used the Seegene Allplex Respiratory Panel (Seegene, Seoul, South Korea).

Results of the assays for Gram-positive and Gram-negative bacteria were considered by the adjudication board for all cases. The adjudication board, a panel of at least three physicians (a respiratory physician, infectious disease specialist, and internal medicine specialist) retrospectively assessed appropriateness, the choice and duration of antibiotic therapy regarding Gram-negative pathogens, and the reason for changing antibiotic agents based on a predefined standardised form (appendix p 5). Medical records, radiological examinations, pathology reports, microbiological and multiplex bacterial PCR analysis of the bronchoalveolar lavage, and all other biological specimens (sputum, nasopharyngeal swabs, blood, and urine) were available to the committee.

Patients received empirical antibiotic therapy as clinically indicated by the treating physician. Prescriber education was carried out at the start of the study by a team of respiratory care, infectious disease, and internal medicine specialists. Written guidance for local empirical therapy (appendix p 2) and infectious disease consultations were available for treating physicians during the study. In the PCR group, a recommendation regarding antibiotic therapy was made in writing and by telephone according to the results approximately 5 h after taking the sample. The decision to follow this recommendation was at the physician's discretion. The antibiotic recommendation was standardised, defined a priori, and specified the antibiotic agents of choice for each Gram-negative pathogen included in the test panel. It also considered local resistance rates, which are low for bacteria such as *Haemophilus influenzae* and some Enterobacteriaceae species.[27] If all Gram-negative PCR results in the Unyvero panel were negative, the recommendation was to switch antibiotic therapy from an empirical broad-spectrum antibiotic to a narrow-spectrum antibiotic. Patients allergic to all recommended antibiotics received alternative antibiotic therapy, as prespecified by an infectious disease specialist (appendix p 3).

See Online for appendix

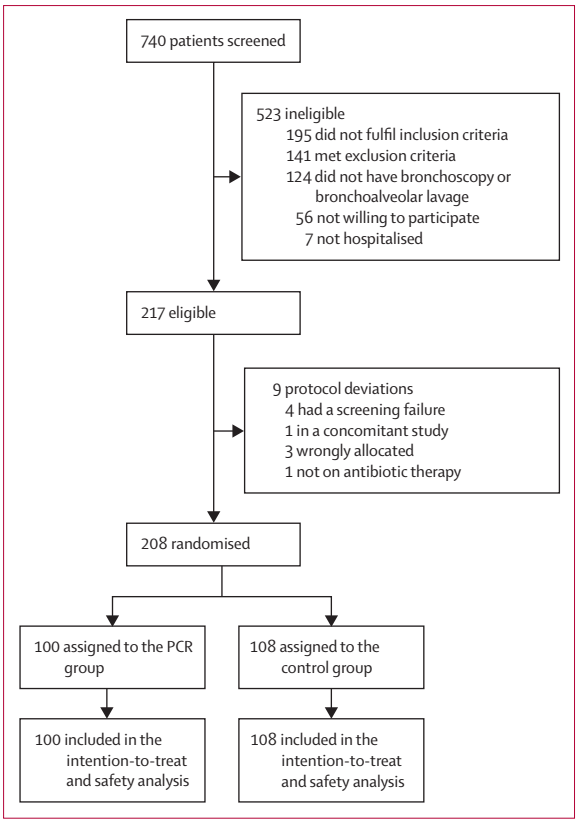

Figure 1: **Trial profile**

Participants were followed up once per day until hospital discharge or for a maximum of 30 days and were asked to report any new hospital admissions to the study team within 30 days after a bronchoscopy. Patients discharged earlier were additionally assessed by telephone interview on day 30 after randomisation. Vital signs were collected from medical records and, if necessary, a daily visit was carried out to obtain clinical parameters (ie, temperature, heart rate, respiratory rate, blood pressure, oxygen saturation, mental status, and the ability of oral intake) and monitor antibiotic therapy, irrespective of the indication. Additional measures included drug therapy, adverse events, radiological examinations, and laboratory tests.

Bronchoalveolar lavage fluid was analysed by microscopy (cytology) and conventional microbiology (blood cultures, urine cultures, antigen tests, and sputum cultures). Specifically, 1 µL of bronchoalveolar lavage fluid was added to culture plates (Columbia sheep blood agar, Colistin Nalidixic Acid blood agar for Gram-positive bacteria, *Haemophilus* chocolate agar, and MacConkey agar for Gram-negative rods). The incubation times and conditions varied according to specific pathogens. Positive bacterial cultures were assessed and quantified in colony-forming units per mL. Identification and susceptibility testing was carried out in concordance with standard methods.[28] Urine samples were analysed using the Sofia *Legionella* Fluorescent Immunoassay (Quidel, San Diego, CA, USA) or BinaxNOW *Legionella* Urinary Antigen Card (Abbott, Chicago, IL, USA) and Sofia *S pneumoniae* Fluorescent Immunoassay or BinaxNOW *S pneumoniae* Antigen Card (to detect *L pneumophila* serogroup 1 antigen and *S pneumoniae* cell wall polysaccharide antigen). All microbiological analyses were performed by well trained and certified personnel.[29] Additionally, some bronchoalvealar lavage specimens, depending on differential diagnosis, were analysed with the Monofluo immunofluorescent antibody test kit (Bio-Rad Laboratories, Hercules, CA, USA) for detection of *P jirovecii*, Fungiqual A fluorochrome stain (Drs Reinehr and Rembold, Kandern, Germany) for detection of fungal agents, and Aerospray TB automated stainer (ELITech, Puteaux, France) for acid-fast bacilli.

Outcomes

The primary outcome was the time in hours on inappropriate antibiotic therapy from bronchoscopy to discharge or to 30 days after bronchoscopy of patients, measured in the intention-to-treat population. The main criteria for defining appropriateness were microbiological efficacy, spectrum coverage, and duration of therapy. Pathogens detected by multiplex bacterial PCR or culture, or both, were considered relevant for the appropriateness evaluation. Inappropriateness was predefined as antibiotic therapy that is inactive based on in vitro susceptibility testing, with known intrinsic resistance, or a spectrum too broad for the identified pathogen (appendix p 6). Only resistance patterns detected by culture were considered. Antibiotic therapy for pneumonia exceeding 7 days after a bronchoscopy and bronchoalveolar lavage was always deemed inappropriate. When no pathogen or no particular antibiotic resistance was identified precluding spectrum narrowing, antibiotic therapy for Gram-negative rods was considered too broad. Per protocol, antibiotic therapy directed against atypical microorganisms was not informed or modified by study procedures and was therefore noted but not adjudicated regarding its appropriateness. Similarly, antibiotic therapy prescribed for extrapulmonary infection was documented but not adjudicated regarding appropriateness.

Secondary outcomes were time to clinical stability, length of hospital stay in days, mortality at 30 days, adverse events (safety), and diagnostic performance of the multiplex bacterial PCR (assessed for bronchoalveolar lavage because of the low number of sputum samples) compared with conventional microbiological testing. A complete list of the criteria used to define clinical stability, all of which had to be met simultaneously, is provided in the appendix (p 4).[19]

Statistical analysis

Assuming a 12 h decrease in the mean time on inappropriate antibiotic therapy between groups with a between-patient SD of 24 h, an α error of 0·05, and a

power of 0·8, we planned to include a total of 128 patients (64 per group). As per protocol, an analysis of factual variance of antibiotic therapy duration was performed for all participants after enrolment of 100 patients. The sample size was reassessed using the same assumptions, but with the new variance (blinded analysis). With this procedure, the type I error was protected.[30] The analysis showed a variance higher (SD 100 h) than projected and informed the continuation of this study up to the predefined sample size of 200 patients. Sample size calculations were based on a two-sided *t* test for independent samples.

Group comparisons of continuous parameters were performed using the Mann-Whitney *U* test and categorical parameters were compared using the χ^2 test. Survival plots for the freedom from inappropriate antibiotic therapy and mortality were obtained using the Kaplan-Meier method. The respective risks were calculated using Cox proportional hazard regression. Mixed linear regression models were used to adjust mean treatment duration for the patient effect (included as a random effect since a patient could have more than one antibiotic course). SAS (version 9.4) was used for the statistical analysis. This trial was registered with the International Clinical Trials Registry Platform, ISRCTN95828556.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Between May 31, 2017, and Sept 25, 2019, 740 patients with pneumonia were screened for eligibility and 208 were included and randomly assigned to the PCR group (n=100) or conventional microbiology control group (n=108; figure 1). 523 patients were ineligible and nine deviated from the protocol. Baseline demographics were similar between the two groups (table 1). The mean age of patients was 65·9 years (SD 14·0) and 135 (65%) were male. 117 (56%) of 208 patients were immunocompromised, with 64 (55%) of these receiving immunosuppressive drugs and 41 (35%) receiving chemotherapy (appendix p 6). The most prevalent comorbidities were arterial hypertension (89 [43%] of 208), solid organ cancer (64 [31%]), and chronic obstructive pulmonary disease (COPD; 57 [28%]; appendix p 6). X-ray or CT (or both) was performed on all patients and consolidation was detected in 187 (90%) participants. The working diagnosis at the time of inclusion was community-acquired pneumonia in 157 (75%) of 208 patients, hospital-acquired pneumonia in 48 (23%), and acute exacerbated COPD in three (1%). Most participants had symptoms such as cough or dyspnoea. The bronchoscopy was performed after a mean length of stay of 4·1 days (SD 6·3; median 2 days [IQR 1–5]). A total of 136 patients (65%) of 208 were already receiving antibacterial agents at the time of bronchoscopy (68 [68%] of 100 in the PCR group *vs* 68 [63%] of 108 in the control group), with a mean duration of 33·2 h (SD 49·2; appendix p 6). After bronchoscopy, 190 (91%) patients received antibiotic treatment (92 [92%] *vs* 98 [91%]).

	Control group (n=108)	PCR group (n=100)
Age, years	65·1 (13·9)	66·8 (14·1)
Sex		
Male	64 (59%)	71 (71%)
Female	44 (41%)	29 (29%)
Smoking status		
Current smoker	27 (25%)	18 (18%)
Past smoker	48 (44%)	53 (53%)
Never smoker	32 (30%)	29 (29%)
Pack years*	38·4 (23·2)	36·7 (23·9)
Immunosuppression†	61 (57%)	56 (56%)
Vaccination		
Influenza	42 (39%)	46 (46%)
Pneumococcal	12 (11%)	7 (7%)
Symptoms		
Duration of symptoms before bronchoscopy, days	17·1 (39·7)	11·2 (13·9)
New or increased cough	83 (77%)	71 (71%)
Fever (>38·3°C) or hypothermia (<36·0°C)	50 (46%)	46 (46%)
Dyspnoea	62 (57%)	44 (44%)
Clinical parameters and vital signs		
Respiratory rate, breaths per min	20·8 (5·3)	21·8 (5·0)
Oxygen saturation, % breathing room air	95·2 (3·2)	94·2 (3·9)
Systolic blood pressure, mm Hg	122·2 (22·2)	126·2 (19·6)
Heart rate, beats per min	85·8 (14·1)	82·4 (14·5)
Systemic inflammation	89 (82%)	92 (92%)
White blood cell count, × 10^9/L	10·4 (6·9)	10·4 (5·1)
C-reactive protein, mg/L	138·9 (105·6)	136·7 (113·8)
Procalcitonin, µg/L‡	0·4 (1·1)	1·0 (3·4)
Prognostic scores		
Charlson Comorbidity Index	3·3 (2·8)	3·5 (2·6)
CURB-65 score	1·0 (0·8)	1·1 (0·9)
Imaging		
Chest x-ray performed	32 (30%)	40 (40%)
Consolidation	28 (26%)	29 (29%)
Interstitial pattern	12 (11%)	15 (15%)
Pulmonary cavitation	1 (<1%)	2 (2%)
Chest CT scan performed	97 (90%)	83 (83%)
Consolidation	84 (78%)	77 (77%)
Interstitial pattern	27 (25%)	25 (25%)
Pulmonary cavitation	1 (<1%)	1 (1%)
Diagnosis at inclusion		
Community-acquired pneumonia	80 (74%)	77 (77%)
Hospital-acquired pneumonia	26 (24%)	22 (22%)
Chronic obstructive pulmonary disease exacerbation	2 (2%)	1 (1%)

(Table 1 continues on next page)

	Control group (n=108)	PCR group (n=100)
(Continued from previous page)		
Patients with both community-acquired pneumonia and risk factors for Gram-negative infection§		
Suspicion or diagnosis of chronic alcoholism	5 (5%)	6 (6%)
Chronic oral steroid or other immunosuppressive drugs	32 (30%)	23 (23%)
Underlying chronic bronchopulmonary disease	41 (38%)	36 (36%)
Aspiration	2 (2%)	3 (3%)
Recent or frequent antibiotic use in the past 3 months	31 (29%)	32 (32%)
Chemotherapy within the past 3 months	18 (17%)	12 (12%)
Immunocompromising condition¶	30 (28%)	21 (21%)

Data are n (%) or mean (SD). *Pack years quantified by multiplying packs of cigarettes smoked per day by the number of years smoking. †Cause of immunosuppression is provided in the appendix (p 6). ‡Procalcitonin was measured in 75 patients. §Full list of risk factors is provided in the appendix (p 1; n=157 patients with community-acquired pneumonia). ¶Immunocompromising condition included haematological disease, HIV, haemodialysis, solid organ transplantation, and stem cell transplantation.

Table 1: **Baseline characteristics of patients**

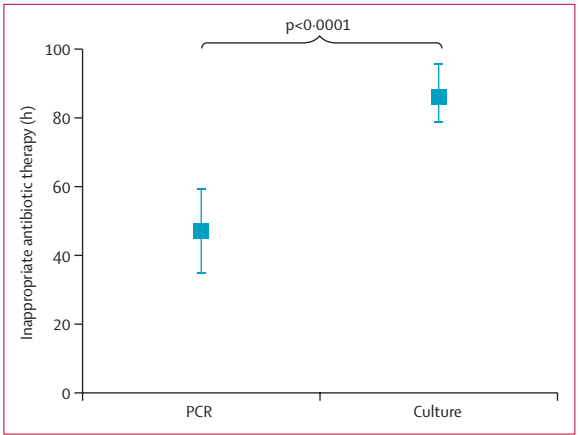

Figure 2: **Duration of inappropriate antibiotic therapy**
Bars indicate 95% CIs.

The duration of inappropriate antibiotic treatment was significantly shorter, by 38·6 h (95% CI 19·5–57·7), in the PCR group than in the control group (adjusted mean 47·1 h [34·7–59·5] *vs* 85·7 h [78·8–95·6]; p<0·0001; figure 2), which translates as a decrease in the duration of inappropriate antibiotic therapy of 45·0% (37·9–52·1). Patients in the PCR group had a higher probability of freedom from inappropriate therapy than those in the control group (HR 3·16 [95% CI 2·23–4·76]; p<0·0001; figure 3). The antibiotic therapy in the PCR group was 22·5% shorter than in the control group (127·2 h [SD 88·3] *vs* 161·3 h [124·1]; p=0·054; data not shown). Post-hoc analyses of antibiotic therapy duration are shown in the appendix (p 7).

There was no significant difference between the PCR and control group in the proportion of patients reaching clinical stability or being discharged from hospital, or both (90 [90%] *vs* 99 [92%]; p=0·90; figure 4). Additionally, time to clinical stability or discharge was similar between the groups (2·5 days [SD 2·6] *vs* 2·4 days [3·4]; p=0·60).

Individual criteria for clinical stability were similar between groups (appendix p 10). Length of stay in the hospital (14·4 days [SD 10·4] in the PCR group *vs* 16·0 days [11·2] in the control group; p=0·29) and hospital readmission rate (37 [40%] of 92 *vs* 31 [32%] of 96; p=0·26) did not differ between the groups. Similarly, no difference was found in the number of admissions to the intensive care unit (21 [21%] of 100 *vs* 21 [19%] of 108; p=0·78) or deaths (eight [8%] *vs* 11 [10%]; p=0·64; data not shown) between the groups. All in-hospital deaths in this study were attributed to a respiratory cause.

Adverse events due to antimicrobial therapy occurred in nine patients, with no significant difference between the groups (five [5%] in the PCR group *vs* four [4%] in the control group; p=0·74; appendix p 7). Adverse events due to bronchoscopy were also balanced between the groups (two [1%] *vs* two [1%]; p=0·99; appendix p 7).

During this study, 399 antibiotic regimens for pneumonia were reviewed and 65 (16%) of these targeted atypical microorganisms, including *P jirovecii*. Of the 334 remaining regimens, 196 (59%) were deemed to be inappropriate, mostly because treatment was unnecessarily broad spectrum (157 [81%]) and because of extensive prescription duration (23 [12%]). There were 83 (46%) of 179 inappropriate antibiotic regimens in the PCR group and 113 (73%) of 155 in the control group (p<0·0001). Penicillin with β-lactamase inhibitors was the most prescribed drug class (242 [72%] of 334; appendix p 11). Antibiotic therapy for indications other than pneumonia was administered on 91 occasions with a balanced distribution between the groups (45 [45%] of 100 patients in the PCT group *vs* 46 [43%] of 108 in the control group; p=0·88).

Post-hoc exploratory analysis showed that of 411 microbiological samples collected after bronchoscopy in the 30-day follow-up, 69 (17%) identified a pathogen. There was numerically a higher prevalence of resistant microbes in the control group (ten [9%] of 108 *vs* five [5%] of 100 in the PCR group; p=0·24). The final diagnosis was community-acquired pneumonia in 113 (54%) of 208 patients, 26 (13%) with hospital-acquired pneumonia, and 16 (8%) with viral pneumonia. All other final diagnoses are provided in the appendix (p 7).

In the PCR group, an indication to change the initial antibiotic regimen according to the PCR results occurred for 61 (66%) of 92 patients who received antibiotics. The attending physician adjusted antibiotic therapy according to recommendations on the basis of multiplex bacterial PCR results in 46 (75%) of these 61 patients. Of the 15 patients for whom non-adherence to therapy recommendation was observed, five were admitted to the intensive care unit and three died versus eight and two of those who were adherent to the protocol. In two patients in the PCR group, escalation of antibiotic therapy was made on the basis of PCR results (from amoxicillin or clavulanic acid to piperacillin or tazobactam after detection of *Pseudomonas aeruginosa,* and from

amoxicillin or clavulanic acid to cefepime after detection of *Enterobacter cloacae* complex).

Of 3120 individual PCRs included in the multiplex bacterial PCR panel and analysed, six had a technical failure and provided no valid result. Similarly, one bacterial culture of the bronchoalveolar lavage did not reach the laboratory. Growth of any microorganism was reported in 150 (72%) bronchoalveolar lavage samples, with identification of a potentially pathogenic bacterial agent in 39 (19%) bronchoalveolar lavage cultures. Gram-negative rods were detected by PCR in 39 (19%) patient samples and by conventional microbiological culture in 30 cases (14%; table 2). Concurrence with the results of routine culture was observed in 16 cases.

Four pathogen species (*Achromobacter xylosoxidans*, *Enterococcus faecium*, *Streptococcus agalactiae*, and *Streptococcus mitis*), including one Gram-negative bacteria (*A xylosoxidans*), were detected by conventional microbiological methods but were not present in the Unyvero HPN panel (appendix p 8).

The multiplex bacterial PCR was positive in 48 (23%) of 206 samples. Accordingly, 162 (79%) of 205 samples concurred with the results of routine culture; 141 (87%) of 162 concordant samples revealed no pathogen.

The multiplex bacterial PCR had a sensitivity of 55·6% and specificity of 86·6% at detecting Gram-negative bacteria, compared with conventional microbiological culture as the reference standard. The negative predictive value of multiplex bacterial PCR was 92·8%, the positive predictive value was 38·5%, and the concordance reached 82·5%. Additional results of the multiplex bacterial PCR and ancillary conventional testing are shown in the appendix (p 9).

Discussion

To our knowledge, this study is the first multicentre, randomised controlled trial showing that results from a multiplex bacterial PCR panel of bronchoalveolar lavage incorporated into antibiotic stewardship translate into less inappropriate antibiotic therapy. Accordingly, the duration of inappropriate antibiotic therapy was reduced in the PCR group with no compromise in clinical outcomes, including time to clinical stability, length of stay in the hospital, and mortality.

Antimicrobial stewardship is a multifaceted concept that encompasses goals such as averting the spread of antibiotic resistance and preserving efficacy of therapeutic agents without compromising or, better still, resulting in improved clinical outcomes.[31] This notion emerged in the late 1990s, calling for administrative regulation alongside clinical trials and educational programmes.[32] The fact that antimicrobial stewardship faces challenges is obvious considering the terminology used in infectious diseases currently, such as multidrug-resistant organisms, extensively drug-resistant organisms, and pandrug-resistant organisms.[33]

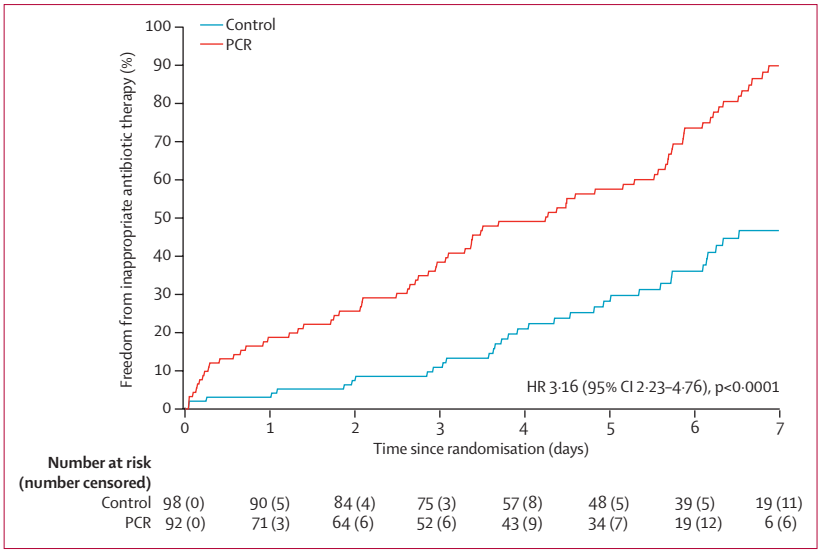

Figure 3: **Freedom from inappropriate antibiotic therapy**

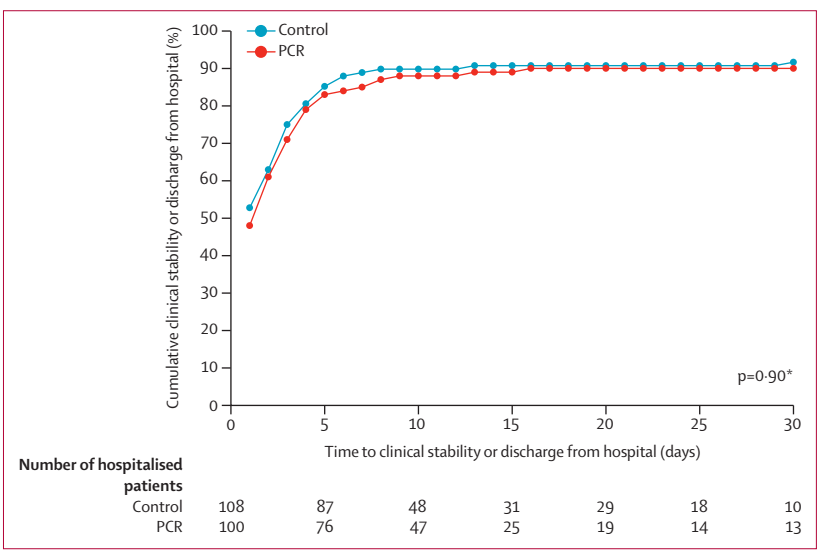

Figure 4: **Time to clinical stability**
*Difference between groups was assessed using the Mann-Whitney *U* test.

Although there is continuous development of modern diagnostic methods (including multiplex PCR),[34] the therapeutic options are slowly depleting, making precision medicine in infectious diseases an urgent goal. In 2018, WHO further stratified this objective by developing a priority list of 20 bacteria, unambiguously focused on Gram-negative rods.[35] Such causative agents, even in community-acquired pneumonia with specific risk factors, are associated with poorer prognosis and higher mortality than Gram-positive and atypical bacteria,[10] compelling international organisations to recommend a broader empirical therapy, with causative agents taking precedence over the dichotomic concept

	Unyvero PCR		Conventional microbiology	
	Control group (n=108)	PCR group (n=100)	Control group (n=107)*	PCR group (n=100)
Citrobacter freundii	1 (<1%)	0	0	0
Escherichia coli	2 (2%)	3 (3%)	1 (<1%)	2 (2%)
Enterobacter cloacae complex	0	2 (2%)	2 (2%)	4 (4%)
Enterobacter aerogenes	1 (<1%)	0	0	2 (2%)
Proteus spp	1 (<1%)	2 (2%)	2 (2%)	2 (2%)
Klebsiella pneumoniae	1 (<1%)	1 (1%)	2 (2%)	0
Klebsiella oxytoca	0	0	0	0
Klebsiella variicola	0	0	1 (<1%)	0
Serratia marcescens	1 (<1%)	0	1 (<1%)	1 (1%)
Morganella morganii	0	2 (2%)	0	1 (1%)
Moraxella catarrhalis	1 (<1%)	1 (1%)	1 (<1%)	0
Pseudomonas aeruginosa	5 (5%)	4 (4%)	5 (5%)	0
Acinetobacter baumannii complex	0	0	0	1 (1%)
Stenotrophomonas maltophilia	2 (2%)	0	0	0
Haemophilus influenzae	10 (9%)	5 (5%)	4 (4%)	0

*One bronchoscopy and bronchoalveolar lavage sample was not assessed by culture.

Table 2: **Results of multiplex bacterial PCR and conventional microbiology of Gram-negative bacteria**

of community-acquired versus hospital-acquired pneumonia, leading to more personalised treatment.

In the past 30 years, PCR diagnostic methods have emerged as the new standard in viral infections,[14] gaining popularity during the COVID-19 pandemic. Nucleic acid amplification testing is accurate at detecting viral infections,[36] and can also play a role in guiding antibiotic stewardship—for instance, in targeting meticillin-resistant *S aureus*.[15,37–40] The immediate challenge is to determine a setting in which broader multiplex bacterial PCR testing could improve antibiotic stewardship and patient outcomes. Retrospective studies[41,42] showed promising results, with hypothetical rates of de-escalating antibiotics as high as 40% and faster administration of appropriate antibiotic therapy with the help of multiplex bacterial PCR. In our trial, multiplex bacterial PCR of bronchoalveolar lavage fluid with a focus on Gram-negative bacteria supported antibiotic de-escalation in 66% of patients. This approach might be helpful in counteracting the perpetual cycle between increased resistance rates and the erroneous use of broad-spectrum antibiotics.

Timely initiation of antibiotic therapy in patients with pneumonia is associated with superior clinical outcomes,[43] and a 4 h threshold for initiation of antibiotics has been suggested.[44] Multiplex bacterial PCR offers faster turnaround times than standard culture that fall within the optimal therapy threshold, thus creating opportunities for novel treatment algorithms. Improved sensitivity and prompt results could shift the current strategy from initial broad-spectrum antibiotics followed by de-escalation to a tailored therapeutic approach from the beginning.

Results of multiple microbiological assessments of diagnostic performance of the Unyvero multiplex bacterial PCR vary in sensitivity (57–97%) and specificity (14–99%) depending on the sample origin, clinical setting, and whether the first-generation or an improved version of the multiplex bacterial PCR panel was used.[25,45–49] In our study, Unyvero multiplex bacterial PCR performed similarly with a sensitivity of 55·6% and specificity in 86·6% at targeting Gram-negative rods. Notably, the accuracy of bacterial PCR is usually measured using culture as the reference standard, although microbiological culture is far from being an optimal gold standard due to its diagnostic performance. We showed that multiplex bacterial PCR has a higher sensitivity than conventional microbiological culture when clinical syndrome or imaging is used as the reference standard.[17,50] However, the absence of an additional confirmatory analysis of discordant results between culture and multiplex bacterial PCR is a limitation of our study. Nevertheless, the shortcomings of multiplex bacterial PCR were considered in the study design, thus excluding Gram-positive bacteria and genetic resistance markers in the therapy recommendation. Many genetic resistance markers that are detected by multiplex bacterial PCR are highly prevalent among commensal bacteria, therefore making the interpretation of a positive result difficult.[51] Although the study by Personne and colleagues[51] restricted the analysis of resistance markers to *Escherichia coli* and *P aeruginosa*, a large number of discrepancies in resistance detection was noted. Although modest in identifying Gram-positive bacteria such as *S pneumoniae*, the multiplex bacterial PCR had improved sensitivity and a positive likelihood when only Gram-negative pathogens were analysed.[50]

This study has some limitations. Because patients were treated in tertiary hospitals with above-average diagnostic options, including CT, bronchoscopy, and multiplex bacterial PCR, caution should be used when generalising these results to less advantageous health-care systems. Currently, guidelines strongly appeal for a conjunction of objective scores and physician-determined subjective factors.[3] Although advantageous in decreasing the number of hospitalised patients, the sole reliance on severity of illness scores is considered hazardous.[3] The decision for point-of-care in pneumonia relies also on the presence of comorbid illnesses.[52] Although our patient population had a CURB-65 score of 1·05 (SD 0·8), the decision for admission to hospital might have been on the basis of comorbidity severity, as suggested by the Charlson Comorbidity Index (3·4 [SD 2·7]), which limits the generalisability of our study. Nevertheless, new data suggest that in patients hospitalised with community-acquired pneumonia, the Charlson Comorbidity Index might be a superior predictor of mortality than CURB-65.[53] Similarly, the 30-day mortality in our study concurred with assessments by Charlson Comorbidity Index rather than by CURB-65.[54,55] Pneumonia severity scores offer invaluable support, but a holistic approach

to medical decision making should be aspired to, as suggested by current guidelines.[3]

The high comorbidity burden in patients with pneumonia is also reflected in the high number of immunosuppressed patients in whom community-acquired pneumonia was suspected. Bronchoscopy is recommended by guidance for pneumonia treatment in such patients and offers an alternative to non-invasive sampling in patients with hospital-acquired pneumonia who are unable to produce sputum.[3,19,56] Although considered safe, bronchoscopy increases treatment costs and might not be generally available, which is another limitation of our study. Additionally, the decision to perform invasive sampling can be individualised and other academic centres might opt for empirical therapy and non-invasive studies where feasible. Perhaps the duration of inappropriate treatment in this patient population could be decreased by antibiotic stewardship interventions that encourage adherence to community-acquired pneumonia guidelines.[3] The adherence to local and international antibiotic treatment guidelines, as a cost-effective and widely available instrument, remains paramount in antibiotic stewardship and might be supported by accurate and rapid diagnostic methods.[3,57] Due to the nature of the illness and sequence of events, treating physicians could not be masked to random assignment of patients. Furthermore, the decision to implement the antibiotic recommendation on the basis of PCR results was at the discretion of the attending physician. Even though the antibiotic recommendation in the PCR group was implemented in two-thirds of patients, the primary endpoint reached statistical significance. Nevertheless, physician adherence to the treatment algorithm was similar to published data, highlighting the complexity of therapeutic decision making.[58] As suggested by recent findings,[59] a standardised antibiotic stewardship programme incorporating bacterial and viral multiplex PCR in addition to conventional microbiology might deliver better results. A prerequisite would be to establish clear management algorithms on the basis of these results.

In conclusion, our study shows that the therapeutic management of patients with pneumonia admitted to hospital can be refined using a multiplex bacterial PCR panel of bronchoalveolar lavage fluid. This approach warrants further consideration in future antibiotic stewardship strategies.

Contributors

DS and MT conceptualised and designed the study. DS and MirO wrote the study protocol. DS acquired funding for the study. DS, MT, AMD, KJ, MicO, SB, WCA, and MB were responsible for oversight of the study at their respective sites and contributed to the recruitment of participants. DS, AMD, MT, KJ, MicO, NK, WCA, and MB collected the data and interpreted the results. LG and DS did the statistical analysis. AMD and DS wrote the manuscript. DMS provided logistical support. HH provided interpretation of the viral assays. All authors contributed to the manuscript review and approved the submitted draft. All authors had full access to all the data in the study and accept responsibility for the decision to submit for publication. DS, AMD, and LG have accessed and verified all the data in the study. All authors are accountable for all aspects of this study and ensure that questions related to the accuracy or integrity of the data are appropriately investigated and resolved.

Declaration of interests

DS reports an unrestricted grant from Curetis; grants from AstraZeneca, Weinmann, ResMed, and Boston Scientifics; and honoraria from and participation on data safety monitoring or advisory boards for CSL Behring, Berlin-Chemie Menarini, Novartis, GlaxoSmithKline, AstraZeneca, Vifor, Merck, Chiesi, and Sanofi. MT reports participation on data safety monitoring or advisory boards for Indorsia and GlaxoSmithKline. WCA reports grants from Gottfried and Julia Bangerter-Rhyner Stiftung, Fungal Infection Network of Switzerland, Swiss National Science Foundation, and Kantonsspital St Gallen; honoraria from Pfizer and Medscape; and participation on advisory boards for Merck, Pfizer, and Sanofi. KJ reports honoraria from Schwabe, Curetis, Vertex, and Actelion; support for attending meetings from Vifor and Actelion; and participation on data safety monitoring or advisory boards for Vifor and OM Pharma. HH reports consulting fees from Roche Diagnostics and Molecular Partners; and honoraria from Gilead and Biotest. All other authors declare no competing interests.

Data sharing

Individual participant data will not be made available.

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