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Multicentre evaluation of two multiplex PCR platforms for the rapid microbiological investigation of nosocomial pneumonia in UK ICUs: the INHALE WP1 study

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Multicentre evaluation of two multiplex PCR platforms for the rapid microbiological investigation of nosocomial pneumonia in UK ICUs: the INHALE WP1 study

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Keywords: hospital-acquired pneumonia, rapid diagnostics, PCR, antimicrobial stewardship

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Summary

Background

Culture-based microbiological investigation of hospital-acquired or ventilator-associated pneumonia (HAP or VAP) is insensitive, with aetiological agents often unidentified. This can lead excess antimicrobial treatment of patients with susceptible pathogens, whilst those with resistant bacteria are treated inadequately for prolonged periods. Using PCR to seek pathogens and their resistance genes directly from clinical samples may improve therapy and stewardship.

Methods

Surplus routine lower respiratory tract samples were collected from ICU patients about to receive new or changed antibiotics for hospital-onset lower respiratory tract infections at 15 UK hospitals. Testing was performed using the BioFire FilmArray Pneumonia Panel (bioMérieux) and Unyvero Pneumonia Panel (Curetis). Concordance analysis compared machine- and routine microbiology results, while Bayesian latent class (BLC) analysis estimated the sensitivity and specificity of each test, incorporating information from both PCR panels and routine microbiology.

Findings

In 652 eligible samples; PCR identified pathogens in considerably more samples compared with routine microbiology: 60.4% and 74.2% for Unyvero and FilmArray respectively *vs.* 44.2% by routine microbiology. PCR tests also detected more pathogens per sample than routine microbiology. For common HAP/VAP pathogens, FilmArray had sensitivity of 91.7-100.0% and specificity of 87.5-99.5%; Unyvero had sensitivity of 50.0-100.0%%, and specificity of 89.4-99.0%. BLC analysis indicated that, compared with PCR, routine microbiology had low sensitivity, ranging from 27.0% to 69.4%.

Interpretation

Conventional and BLC analysis demonstrated that both platforms performed similarly and were considerably more sensitive than routine microbiology, detecting potential pathogens in patient samples reported as culture negative. The increased sensitivity of detection realised by PCR offers potential for improved antimicrobial prescribing.

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Key Messages

What is the Key Question?

How do the two currently-available automated PCR-based syndromic test systems perform in the microbiological diagnosis of hospital-acquired and ventilator-associated pneumonia (HAP/VAP) in critical care?

What is the Bottom Line?

Bayesian latent class analysis demonstrated that syndromic PCR-based diagnostic tests offer considerably improved sensitivity for the microbiological diagnosis of HAP and VAP compared with standard-of-care routine microbiological culture.

Why Read On?

The improved speed and sensitivity of PCR-based diagnosis of pneumonia has potential to optimise therapy of critically ill patients and to improve antibiotic stewardship.

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Introduction

Pneumonia is differentiated into its community-acquired (CAP), hospital-acquired (HAP) and ventilator-associated (VAP) forms. [1] Even pre-COVID-19, it was the most-frequently-reported infection in intensive care unit (ICU) patients,[2-4] with crude mortality estimated at 30-70% for nosocomial cases (i.e. HAP and VAP).[2] Swift effective antimicrobial therapy after clinical onset is crucial to outcome, with increased mortality among patients receiving delayed antibiotics or those that prove inactive.[5,6]

The bacteria, viruses and (rarely) fungi that cause nosocomial pneumonia cannot be distinguished from clinical symptomology. Rather, microbiological diagnosis is needed, delivering results in 48-72h and meaning that the patient must be treated empirically in the interim. EU, US and UK guidelines advocate broad-spectrum empirical antibiotics owing to the diversity of bacteria that can be responsible and the need to cover the resistances these may carry. [2,4 7,8] Aetiological investigation is by microbiological culture, hereafter termed routine microbiology , which depends upon cultivable bacteria being recoverable and fails to identify a pathogen in up to 50% of cases .[9-11] These patients nonetheless remain sick and mostly continue to receive empirical antibiotics.

The slowness and poor sensitivity of routine microbiology thus combine to promote poor stewardship and prolonged use of broad-spectrum agents, increasing the risk of side effects, including selection of resistant gut bacteria and *Clostridium difficile*.[12] A further hazard, particularly in high-resistance countries, is that the empirical agent proves ineffective against the pathogen, increasing the risk of a poor clinical outcome.

Rapid, accurate, diagnostics provide a route to improving this situation, promoting early refinement of individual patients' therapy. Commercial "sample-in, answer-out" PCR-based pneumonia tests are now available, specifically the Unyvero (Curetis) and BioFire FilmArray (bioMérieux) platforms which have both received FDA-clearance for diagnosis of pneumonia.[13] Both are substantially automated, seek prevalent pathogens and critical resistances and have turnaround times of hours instead of days.[13-16] We evaluated and compared their performance, in respect of pathogen and resistance detection using lower respiratory tract samples from patients clinically diagnosed with HAP or VAP at 15 UK ICUs. As well as providing a manufacturer-independent direct comparison, we sought to choose one test to take forward into a randomized controlled trial (RCT), evaluating outcomes compared with patient management based on routine microbiology. This is now underway (Trial ID: ISRCTN16483855).[17] Note that this study and RCT are distinct from a recently published trial for nebulised amikacin with the same name.[18]

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Materials and Methods

Additional details and methods are described in supplementary data.

Patients and specimens

Between September 2016 and May 2018, surplus routine lower respiratory tract samples were collected from eligible patients with suspected HAP/VAP at the 15 participating ICUs. The sites represented a range of UK hospital types, included tertiary referral (n= 6), district general (n=7), children's (n=1) and private (n=1).

Specimens were included if they had sufficient volume (>400 μl) and were from patients hospitalized ≥48h about to receive a new antibiotic or change in antibiotic for suspected lower respiratory tract infection. Specimens were eligible only when collected within 12h (before or after) of antimicrobial therapy being initiated and then tested (or frozen at -80°C), within 72h of collection. All lower respiratory specimen types were accepted, whereas upper respiratory tract specimens were excluded. Second specimens from the same patient were included only when collected >14 days after the first sample.

Ethical approval

This work had study-specific approval from the UK Health Research Authority (Reference: 16/HRA/3882, IRAS ID: 201977) and the UCL DNA Infection Bank Committee, whose operation is governed by the London Fulham Research Ethics Committee (REC Reference: 17/LO/1530).

Routine microbiology

Each respiratory specimen was initially cultured locally at the laboratory serving the participating hospital. Testing was according to their standard operating procedures (SOPs), all based on the Public Health England (PHE) UK Standard.[19]

PCR Testing

Samples were transported to two central research laboratories (University of East Anglia and University College London) by courier. Upon receipt, each was promptly tested using both the Unyvero Pneumonia Panel (Curetis, Holzgerlingen, Germany) and the BioFire FilmArray Pneumonia Panel (BioFire Diagnostics, Salt Lake City, USA) according to manufacturer's instructions. The tests are described in Table 1.

Data Analysis

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Analyses were carried out using Stata (v 15) and R (v 3.5 or above), and followed a pre-defined, detailed statistical plan. Results from the conventional and PCR tests were described using standard summary statistics. Agreement between results was examined by categorising each sample in terms of concordance of organisms detected by PCR and routine microbiology, then calculating overall concordance with 95% CIs. Definitions of the categories are detailed in Table 2.

Sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV) initially were estimated (with exact 95% CIs) for each PCR target, taking routine microbiology and routine virology as the gold standard. Owing to concerns that routine microbiology provides a poor gold standard[20] which could result in biased estimation of the diagnostic ability of PCR, estimates (with 95% credible intervals) were also calculated using Bayesian Latent Class (BLC) models[21, 22, 23] incorporating results from both PCR tests, and routine microbiology. BLC models do not assume the infallibility of any diagnostic test or combination thereof, instead estimating their accuracies based on the actual infection status (i.e., infected or not) of each patient. Models used non-informative priors for all parameters (although specificities were constrained to be above 0.15 to obtain more stable posterior distributions), and were fitted with and without assuming correlation between tests. The best-fitting models were identified based on Deviance Information Criteria.

Scoring the Overall Performance of PCR-based Diagnostic Tests

At the outset of the study, through expert consensus, a scoring system was developed to assess the suitability of each ‘sample-in, answer-out’ test for progression to the INHALE RCT. Tests were assessed against one essential criterion - *that the incidence of major discordances, meaning failures to detect pathogens found by routine microbiology, must be <5%*, and ten points-based ‘Desirable Criteria’, scoring a total of 150 (Table S1). Criteria i-iii were based on study results, criteria iv-viii on manufacturer’s published information and criteria ix and x on a user questionnaire. The scale was weighted towards accurate detection of pathogens, with implementation-based criteria given a lower weighting.

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Role of the Funding Source

The funder had no role in the study design, nor in the collection, analysis, and interpretation of data or in the writing of the report. The funder appointed an independent research Programme Steering Committee to provide quality assurance and oversight. Membership of the committee is

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listed on the study website (www.ucl.ac.uk/inhale-project/people).The corresponding author had full access to all study data and had final responsibility for the decision to submit for publication.

Results

Specimens Collected

A total of 752 samples, 652 of them eligible, were collected from the 15 participating ICUs (Figure 1). The range of eligible samples per site was 7-141, with 9 sites each providing >20 eligible samples. Most were from adults, with 72 from children; 260 were from patients with suspected HAP and 392 from patients with suspected VAP. Endotracheal aspirates (n=299) were the most numerous sample type; followed by sputa (272 samples) BALs (44 samples) and non-directed BALs (23 samples), with 14 samples in the "other" or "unknown" category. A small majority of samples (n=357) were collected before antibiotic administration.

Routine Microbiology Results

Routine microbiology was performed on all samples at the local laboratories. The median time to a result was 70.2h (interquartile range (IQR) 51.1h-92.1h), including a median of 6.1h (IQR 2.5h-15.4h) transit time from the ICU to laboratory booking-in and 55.5h (IQR 44.8h-76.5h) from sample booking to release of results. The positivity rate was 44.2%, with 35.1% recording one significant organism with 9.1% reporting two or more. The remaining 55.8% of samples were reported variously as 'normal flora', 'non-significant growth', or 'no growth'.

Staphylococcus aureus was the most-frequently-found bacterium (Figure 2), representing 23.6% (83/352) of all organisms reported, followed by *Pseudomonas aeruginosa* (20.7%); Enterobacterales collectively accounted for 38.1% of isolates, with *Klebsiella* spp. and *Escherichia coli* prominent (Figure 3a). Occasionally routine microbiology laboratories reported *Candida* spp., *Enterococcus* spp. and coagulase-negative staphylococci: these were excluded because there is no evidence base for their involvement in pneumonia. Table S2 lists the bacteria detected by all three methods in HAP compared with VAP patients.

Results of standard-of care diagnostic virology were recorded if was performed within 24h of collection of the eligible bacteriology specimen. Only 113 patients, 33 of them children, had virology results meeting this criterion, and, of these, 31 (27.4%) were positive: seven had influenza A, six adenovirus and six cytomegalovirus. The study was undertaken before SARS-CoV2 began to circulate.

PCR Results

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Among the 652 eligible samples, 631 had Unyvero tests and 632 had FilmArray tests within 72h of the sample's collection, or with a frozen sample (Figure 1). Among these eligible tests, 620 generated a result on the FilmArray, whilst 12 failed. Defining failure on the Unyvero is more complex since targets are divided into eight chambers. We considered one sample where >2 chambers failed as a "total failure" along with 24 samples that failed to generate any result, leaving 606 valid results. In 32 of these 606 one or two chambers nonetheless failed. Their data were retained in the analysis, with the proviso that organisms sought by the failed chambers would have been missed. We did not note any user errors for either test; neither machine requires regular service or maintenance.

The overall positivity rate for both machines exceeded routine microbiology, at 60.4% for the Unyvero and 74.2% for the FilmArray (chi-square test: $p < 0.0001$). Most specimens had multiple organisms detected (Figure 2), with this proportion higher for FilmArray than Unyvero. FilmArray found only bacteria in 54.2% of samples and only viruses in 6.9% whereas 13.1% contained both. The principal species detected by PCR, and their relative prevalence were broadly similar to routine microbiology, although *E. coli* and *Klebsiella* spp. were detected relatively more frequent by PCR, whereas *S. aureus* and *P. aeruginosa* were found less frequently (Figure 3b). Among viruses detected by the FilmArray, rhinovirus was the most prominent (n=55), followed by influenza A (n=29) and B (n=25) (see Table S3); Unyvero does not seek viruses.

Performance of PCR Tests

Test performance was compared in several ways to accommodate the fact that routine microbiology is an imperfect 'gold standard' and the fact that the PCR tests seek multiple targets, more than one of which may be present in any sample, confounding simple calculation of overall sensitivity and specificity.

Overall test performance was first measured as concordance with routine microbiology, taken as a gold standard (Table 2). Both PCR tests deliver semi-quantitative outputs: the FilmArray reports bacterial targets as 10^4, 10^5, 10^6 or $\geq 10^7$ copies per ml, whereas the Unyvero reports as +, ++ or +++. In addition to detection at any concentrations, we therefore also undertook further concordance calculations, considering only targets detected at high concentration, defined as 10^6 or $\geq 10^7$ copies/ml for FilmArray and ++ or +++ for Unyvero (Table 2). Around half of the PCR results by each method demonstrated full positive or negative concordance with routine microbiology. Most of the remainder were either partially concordant or had minor discordance. Major discordance was rare, totalling only 4.6% for Unyvero and

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1.8% for FilmArray. Details of results that were discordant between routine microbiology and PCR are shown in Tables S4 and S5. If PCR detections at low concentrations were excluded, full concordance increased for both tests, but major discordance increased unacceptably. A comparison of negative results determined that there was no significant difference in the number of positive PCR detections between samples reported in routine microbiology as "no growth" and "no significant growth" compared with those reported as "normal flora" and "mixed growth" (data not shown). The number of organisms detected per sample did not vary significantly according to sample type (Table S6).

PCR assay sensitivity was >95% for most target bacteria, with NPVs > 98% (Table 3). Specificity and PPVs were lower, due to the PCR tests detecting more organisms per sample and finding more positive samples than routine microbiology. Strikingly, however, both machines often found the *same* organism as each other when routine microbiology failed to record any organism, casting doubt on routine microbiology as a gold standard. Accordingly, Table 4 shows performance estimates obtained from BLC models that make no presumption of one method being the reference. Based on this analysis, routine microbiology was the least sensitive technique, with sensitivity values for individual pathogens ranging from 27.1 % to 68.7%. In contrast, sensitivity values for the PCR tests remained high; FilmArray sensitivity ranged from 89.4% to 99.3 % versus 83.9% to 96.9% (expect *K. aerogenes*, 48.4%) for Unyvero. Specificity and PPV values for both PCR tests increased considerably compared with the values calculated using routine microbiology as a gold standard: in particular, specificity exceeded 99% for Unyvero targets and ranged from 93.9% to 99.9% for FilmArray targets. The PPV range was 62.1% to 99.3% for Unyvero and 56.1% to 96.6% for FilmArray. This BLC analysis omits data from 16S rRNA testing, also performed, as this technique could not distinguish species within several key genera, including *Streptococcus* and *Klebsiella,* reducing granularity. BLC analysis including 16S data is included as Table S9; its numbers differ slightly from Table 4 but support the same conclusions.

We further conducted sub-analyses to investigate factors that might influence the results, such as the timing of the sample in relation to antibiotic administration, fresh vs. frozen samples, or time from sample collection to testing (24h,48h or 72h). None of these factors had a significant impact on the performance of the PCR tests (Tables S10, S1 and data not shown).

Chlamydophila pneumoniae, Legionella pneumophila and *Mycoplasma pneumoniae* were excluded from analysis because they are not ordinarily sought by routine microbiology. Unyvero and FilmArray each detected *M. pneumoniae* once, in the same specimen, from participating sites where it was not sought by local microbiology. Unyvero detected two samples

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with *L. pneumoniae*; FilmArray and routine microbiology found none. *C. pneumophila* was not found by any method. Virology performance is shown in Table S3 for FilmArray only since Unyvero did not seek viruses; confidence intervals are wide due to the small number of routine results available for comparison.

Antimicrobial Resistance and comprehensive culture

All routine microbiology results for antimicrobial susceptibility testing were recorded, and Table S12 shows data for antimicrobials commonly used to treat HAP and VAP against prevalent species. The PCR tests differ from routine microbiology by seeking resistance (as genes) in a whole sample, not in particular bacteria. Assessment of the machines' performance in respect of resistance gene detection is further complicated because routine microbiology often reported no organism for PCR-positive samples. In other cases, we were unable to retrieve routine isolates for genetic investigation. These isolates were supplemented with those recovered by "comprehensive culture" on a sub-set of the discrepant samples (Supplementary methods). In total, comprehensive culture detected 12 additional key resistance genes, the host bacteria of which were not isolated or reported by routine microbiology (Table 5).

Specific resistance gene detections are catalogued in Table S13. We performed concordance analysis for 'high-consequence' resistance genes only, encoding extended-spectrum β-lactamases (ESBLs), carbapenemases or methicillin-resistant *Staphylococcus aureus* (MRSA) phenotypes. Among 17 Enterobacterales with ESBL phenotypes, 12 were from specimens where Unyvero found $bla_{CTX\text{-}M}$ and 17 from those where FilmArray found $bla_{CTX\text{-}M}$. Considered from the opposite perspective, culture found ESBL producers in 12/14 cases where Unyvero found $bla_{CTX\text{-}M}$ and 17/32 cases where FilmArray did so. Fifteen cultured *S. aureus* isolates had an MRSA phenotype, of these 13 were from specimens where Unyvero found *mecA/C* and all 15 from those where FilmArray found *mecA/C*-MREJ. Considered from the opposite perspective, culture found MRSA in 13/25 cases where Unyvero found *mecA/*C in presence of *S. aureus* and 15/32 cases where FilmArray did so. There were only 11 detections of carbapenemase producers by Unyvero (including *Acinetobacter* OXA enzymes) and three by FilmArray, precluding review by enzyme type: culture confirmed a carbapenemase producer in 7/11 samples where Unyvero found a carbapenemase gene and 2/3 where FilmArray did so. Unyvero found a carbapenemase gene in all eight samples that grew an organism with carbapenemase phenotype, whilst FilmArray only found two carbapenamses in these isolates. (Table 5).

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Overall, comprehensive culture was performed on 103 samples, from which 123 potential pathogens were grown. Routine microbiology reported 65 potential pathogens from the same samples. Of the additional pathogens grown by comprehensive culture, 86% were also identified by one or both PCR tests.

Overall Comparison of PCR Tests

Both PCR systems met the essential requirement of having <5% major discordances. Accordingly, we collated performance and implementability data in order to choose which to carry forward to the INHALE RCT. Our scoring (Table S1 and Table 6) weighted performance, but also considered ease-of-use, footprint, turnaround time and overall user experience.

FilmArray scored 105 points versus 68 for Unyvero. Unyvero was more concordant with routine microbiology, but FilmArray had better sensitivity; Unyvero had a broader target panel but more failed tests. FilmArray performed better on characteristics relating to implementation, ease-of-use, turnaround time and user experience. Accordingly, we have preferred the FilmArray Pneumonia Panel for the INHALE RCT, now being undertaken across 12 UK ICUs.

Discussion

We undertook a comprehensive, independent, head-to-head comparison of the two currently available rapid tests for the microbiological investigation of pneumonia. Samples were from ICU patients for whom clinicians prescribed antimicrobials to treat pneumonia.

Both systems were considerably faster than routine microbiology and detected more organisms. This underscores the known poor sensitivity of routine microbiology in pneumonia.[9-11] Crucially, PCR tests tended to detect the *same* additional organisms in a given sample, implying that these additional detections were '*real'* and that PCR may improve microbiological diagnosis of ICU pneumonia, increasing the proportion of patients who potentially could receive targeted antimicrobials. Furthermore, we perfromed comprehensive culture on A confounder is that, unlike the molecular tests, routine microbiology was decentralised, performed across 11 different hospital laboratories, receiving specimens from the 15 ICUs. The main difference between the two PCR tests is that Unyvero seeks *S. maltophilia* whereas FilmArray seeks respiratory viruses as well as bacteria. Early detection of *S. maltophila* might lead to early tailored therapy with co-trimoxazole, whereas fast viral detection may prompt the early cessation or de-escalation of antibiotic therapy.

To analyse test performance, we initially took routine microbiology as a gold standard. Only 56.6 % of Unyvero results and 50.3 % of FilmArray results were fully concordant with

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routine microbiology, with the remaining partial concordances and minor discordances mostly reflecting additional organisms detected by PCR, reflecting increased sensitivity of the latter. Per pathogen sensitivity performance was consistently good (91.7 to 100%) for FilmArray; Unyvero's performance was more variable, with sensitivity <90% for several pathogens. Cases where pathogens represented on the PCR panels were missed by these tests but found by routine microbiology were rare at 4.6% for Unyvero and 1.8% for FilmArray. Sensitivity and specificity values are similar to those reported by others in evaluations of one or other of the two PCR tests.[15,16,24-27]

We initially hoped that 16S rRNA analysis could act as an alternative, molecular, reference, but it proved less sensitive than PCR and was abandoned (See Supplementary methods and data). Instead, the widely acknowledged limitations of routine microbiological culture[20] – confirmed by the frequency with which both PCR tests detected the *same* organism that was missed by routine microbiology - led us to adopt BLC analysis. In brief, this technique uses information from all tests to infer a new, unmeasurable yet underlying (i.e., latent) gold standard result, with no prior assumption about any one test being 'correct'. This method has been recommended and frequently adopted for studies evaluating diagnostics in settings where reference tests are acknowledged to be sub-optimal.[21,22,28,29] BLC analysis showed (i) the sensitivity of routine microbiology was extremely poor and (ii) the specificity and PPV of the PCR tests were considerably higher than those calculated using routine microbiology as the "gold" standard. This suggests that both PCR tests were clearly superior to routine microbiology, and that the latter should perhaps not be considered a gold standard technique. A caveat is that it is perhaps predictable that two similar PCR tests (albeit with different primers and detection methods) should agree better with each other than with a dissimilar culture-based method. A potential concern in respect of PCR-based methods is that they may detect residual nucleic acids rather than viable pathogens requiring treatment. However, this argument is partly countered in the present study by the observation that comprehensive culture methodology was able to grow around 86% viable pathogens that were not reported by routine culture. It is crucial to remember, in context, that all patients in this study were severely-ill, clinically diagnosed with respiratory infection and received contingent antibiotic treatment; it therefore seems more reasonable to consider an organism found by any one method as potentially significant rather than to dismiss those methods that most often recorded a potential pathogen in favour of one that failed to do so simply because it is the 'traditional method'.

If the molecular results are accepted, it becomes possible to identify groups of patients, e.g. those found only to have *S. aureus* pneumonia or *Haemophilus influenzae*, in whom there

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is wide scope to de-escalate from typical empirical therapy for HAP/VAP with e.g. piperacillin/tazobactam or a carbapenem. This supports a potential to deliver improved antimicrobial stewardship along with better targeted, personalized, treatment of pneumonia. A countervailing risk is that the additional organisms found by PCR instead may prompt unnecessary prescribing. Both the present systems offer semi-quantitative detection which might, in theory, assist assessment of the need for therapy. In a sub-analysis, excluding organisms detected at low concentration by PCR, we did observe increased concordance with routine microbiology, but at the price of discounting organisms confirmed by routine microbiology. Ultimately the best approach may be to combine rapid microbiology with measurement of patient biomarkers as a guide to the need for therapy.

The types and relative frequencies of organisms identified were similar for routine microbiology and both PCR tests, without any obvious bias for either approach to miss particular organisms. The species distribution resembled that reported in numerous HAP/VAP studies from Europe and North America, with *S. aureus, P. aeruginosa* and Enterobacterales predominant[7,8]. Comparison of resistance gene detection with resistance phenotypes from routine microbiology is complicated by imperfect genotype / phenotype associations and the fact that phenotypic resistance may arise from unsought mechanisms (e.g., a combination of an ESBL and impermeability may confer carbapenem resistance in Enterobacterales).[30] Moreover, except for *mecA* on the FilmArray, PCR detection of a resistance gene in a clinical sample does not indicate which bacterial species is hosting that gene. We therefore conducted independent genotypic investigation of isolates identified as resistant by routine microbiology and for further organisms recovered by comprehensive culture. Overall, despite all these caveats, 66% of Unyvero gene detections and 51% of FilmArray detections were concordant against a combination of routine microbiology and comprehensive culture results. Crucially, PCR tests identified several key high-consequence resistance genes that had been missed by routine microbiology but which were confirmed by testing bacteria recovered by comprehensive culture. Although the PCR-methods did not provide a full susceptibility profile, they do deliver a swift and sensitive predictor of critical resistance, potentially useful for early identification of patients who should be isolated or have their therapy escalated.

The run times of the machines are measured in hours rather than the days required for routine microbiology. Total turn-around will also reflect the machine's placement in the clinical pathway; this could not be measured here because the tests were run retrospectively under research conditions. However, we established that the median transport time of samples from the ICU to the laboratory was 6h, with longer times when laboratories were remote from the

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hospital site. If the advantages of speed are to be realised, the machine must be placed in, or near to, the ICU.

The decision of whether to adopt a rapid diagnostic into routine clinical practice will depend not only on its performance but also on the practicalities. Here, we evaluated diagnostic accuracy as well as potential for implementation, finding the FilmArray to be more sensitive than the Unyvero, also faster, smaller and easier to use. Accordingly, we have taken the FilmArray Pneumonia panel forward into INHALE's involving an RCT where patients either receive treatment guided by results of FilmArray test, performed in the ICU, or 'standard to care', comprising empirical antibiotics, adapted once microbiology results become available. This trial will determine if the potential of PCR in ICU HAP/VAP can be realised without compromising patient safety.[31]

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Transparency declarations:

DML: Advisory Boards or ad-hoc consultancy Accelerate, Allecra, Antabio, Centauri, Entasis, GSK, Meiji, Menarini, Mutabilis, Nordic, ParaPharm, Pfizer, QPEX, Roche, Shionogi, Summit, T.A.Z., VenatoRx, Wockhardt, Zambon, Paid lectures – Astellas, bioMérieux, Beckman Coulter, Cardiome, Cepheid, Hikma, Merck/MSD, Menarini, Nordic, Pfizer and Shionogi. Relevant shareholdings or options – Dechra, GSK, Merck, Perkin Elmer, Pfizer, T.A.Z, amounting to <10% of portfolio value. He also has nominated holdings in Avacta, Byotrol, Destiny, Diaceutics, Evgen, Faron, Fusion Antibodies, Genedrive, Hardide, Renalytics, Scancell and

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Synairgen (all with research/products pertinent to medical and diagnostic innovation) through Enterprise Investment Schemes but has no authority to trade these shares directly.

VG: Advisory boards or ad-hoc consultancy Gilead, Shionogi, bioMérieux, MSD, Vidya Diagnostics

VE: Speaking honoraria, consultancy fees and in-kind contributions from several diagnostic companies including Curetis GmbH, bioMérieux and Oxford Nanopore.

JOG: JOG: has received speaking honoraria, consultancy fees, in-kind contributions or research funding from Oxford Nanopore, Simcere, Becton-Dickinson and Heraeus Medical.

All other authors: None to declare.

Author Contributions

VE, VG, DML and JOG conceived the study and obtained funding. JB, JH and AMS contributed to study design. VE and JH obtained study approvals. AC built the study database. AA, RB, DRO, HR, CR and BONI managed the machine-based testing, generating the data for analysis. HR, DRO, AA and RB performed the supplementary laboratory analyses. VE and JOG supervised the laboratory work. CR, HR, AA, DRO and VE performed data checks and queries. JB and FR wrote the statistical analysis plan and designed the BLC analysis, FR performed the analyses. VE, VG, DML and JOG interpreted the data and conceived the scoring system for machine evaluation. VE wrote the manuscript with assistance from RB and DML. All authors reviewed the manuscript and approved the final version. VE, FR and RB had full access to the data and could check their validity.

Data availability statement

The dataset for this study is available on request from Norwich Clinical Trials Unit

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Table 1. Features and Target Panels of the Curetis Unyvero Pneumonia Panel and the BioFire FilmArray Pneumonia Panel multiplex PCR tests.

Characteristic	Curetis Unyvero HPN Hospitalised Pneumonia Panel	BioFire FilmArray Pneumonia Panel *plus*
Technology	Automated sample preparation, multiplex PCR and microarray detection of targets	Automated sample preparation and nested PCR
Regulatory status	CE-IVD[1]	CE-IVD & FDA Cleared[2]
Hands-on preparation time	2 min, using a standard pipette to transfer sample to the sample tube. Bacteria are then lysed for 30 min in the 'Lysator' before transfer to the cartridge.	2 min, using a proprietary flock swab to transfer the sample to a sample tube, which is loaded into the test pouch with the aid of a loading station.
Run-time	5h	1h 15 min
Bacteria sought	*Acinetobacter baumannii* complex *Citrobacter freundii* *Enterobacter cloacae* complex *Escherichia coli* *Haemophilus influenzae* *Klebsiella aerogenes* *Klebsiella oxytoca* *Klebsiella pneumoniae* *Klebsiella variicola* *Moraxella catarrhalis* *Morganella morganii* *Proteus* spp. *Pseudomonas aeruginosa* *Serratia marcescens*	*Acinetobacter calcoaceticus-baumannii* complex *Enterobacter cloacae* complex *Escherichia coli* *Haemophilus influenzae* *Klebsiella aerogenes* *Klebsiella oxytoca* *Klebsiella pneumoniae* *Moraxella catarrhalis* *Proteus* spp. *Pseudomonas aeruginosa* *Serratia marcescens* *Streptococcus agalactiae* *Streptococcus pneumoniae*

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	Stenotrophomonas maltophilia *Streptococcus pneumoniae*	*Streptococcus pyogenes*
Atypical organisms and Fungi sought	*Chlamydophila pneumoniae* *Legionella pneumophila* *Mycoplasma pneumoniae* *Pneumocystis jirovecii*	*Chlamydophila pneumoniae* *Legionella pneumophila* *Mycoplasma pneumoniae*
Viruses sought	None	Adenovirus Coronaviruses OD43, NL63, HKU1 and 229E Human metapneumovirus Human rhinovirus/enterovirus Influenza A Influenza B Parainfluenza virus Respiratory syncytial virus MERS Coronavirus
Antimicrobial Resistance Genes sought	*ermB* *mecA* *mecC* bla_{TEM} bla_{SHV} bla_{IMP} bla_{KPC} bla_{NDM} $bla_{OXA\text{-}23}$ $bla_{OXA\text{-}24/40}$ $bla_{OXA\text{-}48}$ $bla_{OXA\text{-}58}$ bla_{VIM} *sul1* *gyrA83* *gyrA87* *mecA/C* and MREJ	bla_{KPC} bla_{NDM} $bla_{OXA\text{-}48\ like}$ bla_{VIM} bla_{IMP} $bla_{CTX\text{-}M}$

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[1]A similar panel, featuring a reduced number of antimicrobial resistance genes, has FDA clearance.

[2] We evaluated the Research Use Only (RUO) version

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Table 2. Concordance-based performance of PCR tests compared with routine microbiology

Category	**Definition**	**All Detections**		**Detections reported at higher concentrations[a]**	
		Unyvero (%, 95% CI)	**FilmArray (%, 95% CI)**	**Unyvero (%, 95% CI)**	**FilmArray (%, 95% CI)**
Full positive concordance	Organisms detected were an exact match	19.3 (16.2 - 22.4)	18.2 (15.2 - 21.3)	22.4 (19.1 - 25.8)	21.1 (17.9 - 24.3)
Full negative concordance	No organisms detected by either method	37.3 (33.4 - 41.1)	32.1 (28.4 - 35.8)	42.1 (38.1 - 46.0)	44.5 (40.6 - 48.4)
Partial concordance	PCR detected the same organism as RM plus additional organism(s)	18.2 (15.1 - 21.2)	21.0 (17.8 - 24.2)	11.6 (9.0 - 14.1)	11.8 (9.2 - 14.3)
Minor discordance	RM was negative but machine found ≥1 organism	20.6 (17.4 - 23.8)	26.9 (23.4 - 30.4)	15.8 (12.9 - 18.7)	14.5 (11.7 - 17.3)
Major discordance	RM found ≥1 organism, at least one of which was on the PCR panel, but not detected	4.6 (2.9 - 6.3)	1.8 (0.7 - 2.8)	8.1 (5.9 - 10.3)	8.1 (5.9 - 10.2)

CI - confidence interval, RM - routine microbiology

[a] Calculated based on semi-quantitative detections Reported as ++ or +++ by Unyvero or 10^6 or $\geq 10^7$ copies/ml by FilmArray

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Table 3. Pathogen-specific performance of PCR tests as compared with routine microbiology as the gold standard. 95% confidence intervals are omitted to aid readability but are included in supplementary Table S7, along with frequencies of detection.

Organism	Unyvero				FilmArray			
	Sensitivity	**Specificity**	**PPV**	**NPV**	**Sensitivity**	**Specificity**	**PPV**	**NPV**
A. baumannii complex	100.0	99.0	45.5	100.0	100.0	99.5	66.7	100.0
C. freundii	100.0	98.7	11.1	100.0	NA**	NA	NA	NA
E. cloacae	100.0	97.5	44.4	100.0	91.7	93.4	21.6	99.8
E. coli	87.8	89.4	37.5	99.0	97.6	87.5	36.3	99.8
H. influenzae	100.0	93.7	36.2	100.0	95.2	88.1	22.0	99.8
K. aerogenes	50.0	99.5	50.0	99.5	100.0	99.2	54.5	100.0
K. oxytoca	90.9	95.0	25.0	99.8	100.0	95.2	27.5	100.0
K. pneumoniae	83.3	94.2	37.0	99.3	92.0	91.4	31.1	99.6
M. catarrhalis	100.0	98.2	26.7	100.0	100.0	96.9	17.4	100.0
M. morganii	100.0	98.3	9.1	100.0	NA	NA	NA	NA
P. aeruginosa	95.3	93.9	64.9	99.4	98.5	93.1	63.1	99.8
S. aureus	87.2	93.2	65.4	98.0	96.2	88.9	56.2	98.2
S. agalactiae	NA	NA	NA	NA	ND	96.5	0.0	100.0
S. maltophilia	92.9	94.4	28.3	99.8	NA	NA	NA	NA
S. marcescens	77.8	98.3	41.2	99.7	100.0	98.2	45.0	100.0
S. pneumoniae	100.0	97.3	27.3	100.0	100.0	94.5	15.0	100.0
S. pyogenes	NA	NA	NA	NA	100.0	98.9	22.0	100.0

*ND – not determined because routine microbiology detected no positives; **NA – not applicable; organism not on test panel

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Table 4. Pathogen-specific performance of routine microbiology and PCR tests estimated using BLC models. 95% credible intervals are omitted to aid readability but are shown in supplementary table S8. Only organisms on both PCR panels are included

Organism	**Routine microbiology**				**Unyvero**				**FilmArray**			
	Sensitivity	**Specificity**	**PPV**	**NPV**	**Sensitivity**	**Specificity**	**PPV**	**NPV**	**Sensitivity**	**Specificity**	**PPV**	**NPV**
A. baumannii complex	57.5	99.9	87.4	99.4	92.6	99.5	70.9	99.0	89.4	99.9	91.3	99.8
E. cloacae	42.9	99.9	94.6	97.2	94.9	99.9	97.2	99.8	94.2	96.4	56.1	99.7
E. coli	38.8	99.7	96.1	88.5	89.6	99.7	98.6	97.8	98.9	98.7	94.2	99.8
H. influenzae	36.3	99.9	96.8	93.5	96.9	99.7	97.1	99.7	95.3	93.8	62.4	99.5
K. aerogenes	68.7	99.9	88.9	99.6	48.4	99.6	62.1	99.3	89.8	99.4	67.8	99.9
K. oxytoca	30.2	99.9	94.3	95.5	92.7	99.2	88.7	99.5	95.2	99.7	95.9	99.7
K. pneumoniae	37.8	99.5	89.3	93.5	88.9	99.8	97.6	98.8	98.1	97.7	82.2	99.8
M. catarrhalis	27.6	99.9	86.7	98.0	89.0	99.9	95.5	99.7	95.7	98.9	71.4	99.9
P. aeruginosa	64.7	99.7	97.3	93.9	95.8	99.9	99.2	99.2	99.2	99.3	96.6	99.9
S. aureus	65.2	99.2	95.2	92.5	91.1	99.8	99.3	98.0	99.3	95.6	83.9	99.8
S. marcescens	48.4	99.9	92.9	98.4	83.9	99.9	95.7	99.5	96.1	99.8	94.2	99.9
S. pneumoniae	27.1	99.9	90.0	97.0	90.8	99.9	96.7	99.6	97.1	97.1	57.9	99.9

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Table 5. Concordance of antimicrobial resistance gene detection by PCR and comparator methodology

Resistance Gene	**Unyvero**		**FilmArray**	
	Concordant detections[a]/total detections by PCR	Found in cultured isolates but missed in PCR testing	Concordant detections[a]/total detections by PCR	Found in cultured isolates but missed in PCR testing
$bla_{CTX\text{-}M}$	12/14	3	17/32	0
Carbapenemase	8/11	0	2/3	1
mecA/mecC (+*MREJ* in FilmArray)	13/25[b]	1	15/32	0

[a]Total concordance, based on results from both routine microbiology and comprehensive culture. Each sample is only counted once in the event of both tests being positive

[b]Only includes detections where *S. aureus* as well as *mecA/C* was also reported by the Unyvero. For total detections see table S13

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Table 6. Scores allocated to PCR tests based on scoring system designed to evaluate overall performance, ease of use and implementability. See table S1 for full details of the scoring system.

	Machine Score			
Criterion	**Curetis Unyvero Pneumonia Panel**		**BioFire FilmArray Pneumonia Panel**	
	Value	**Score**	**Value**	**Score**
Overall concordance (max 45 points)	74.8%	20	71.3%	16
Sensitivity for detection of common pathogens (max 20 points)	3 targets with better performance	6	7 targets with better performance	14
Breadth of panel (max 15 points)	244 unique detections	15	191 unique detections	12
Time to result (max 15 points)	270 min	7	75 min	14
Cost per test (max 15 points)[a]	+++	10	++	15
Failure rate (max 15 points)	9.1%[b]	0	1.9%	11
Footprint (max 5 points)	7.4 sq. ft	1	3.2 sq. ft	5
Customer service (max 5 points)	-	3	-	4
Consumable logistics (max 5 points)[c]	-	0	-	5
Ease of use (max 10 points)	-	6	-	9
Total (Max 150)	**-**	**68**	**-**	**105**

[a] Costs in the range of £150-300/test depending on local purchase conditions. Includes estimates of cost of instrument purchase and operator time.

[b] includes both total and partial failures

[c]Comprised of one point each for space required for storage, storage temperature, delivery cost, delivery timescales and shelf-life

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Figure Legends

Figure 1. Schematic representation of sample eligibility

Figure 2. Number of significant organisms detected per respiratory sample by routine microbiology or PCR.

Figure 3a. Numbers and types of bacteria detected by routine microbiology culture from respiratory samples included in the study.

Figure 3b. Numbers and type of bacteria detected by PCR from respiratory samples included in the study. Unyvero, solid bars, n = 606; FilmArray, hatched bars, n = 620. Species sought by one test only are marked with an asterisk.

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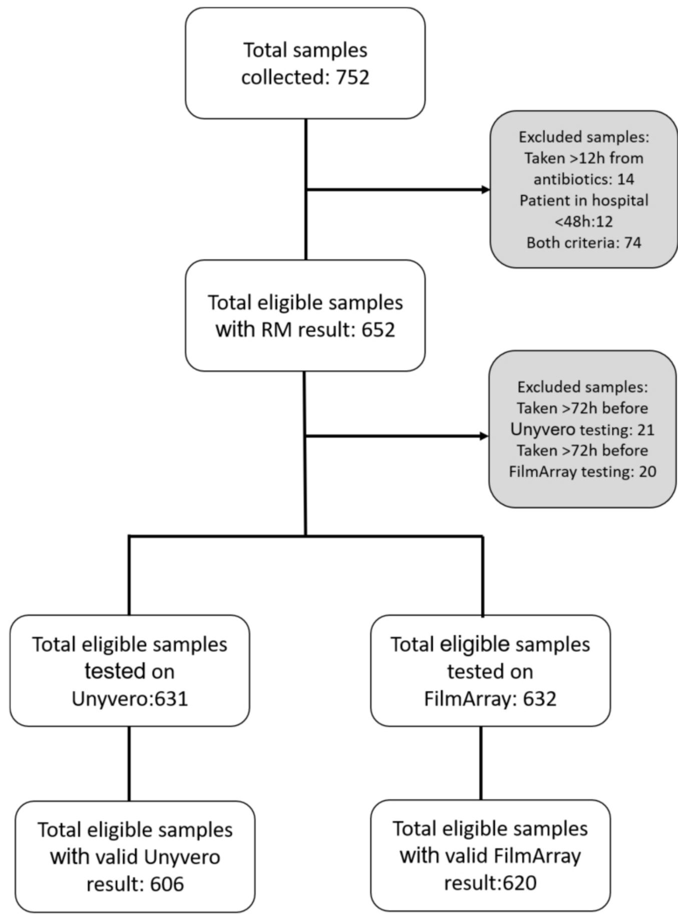

Figure 1. Schematic representation of sample eligibility

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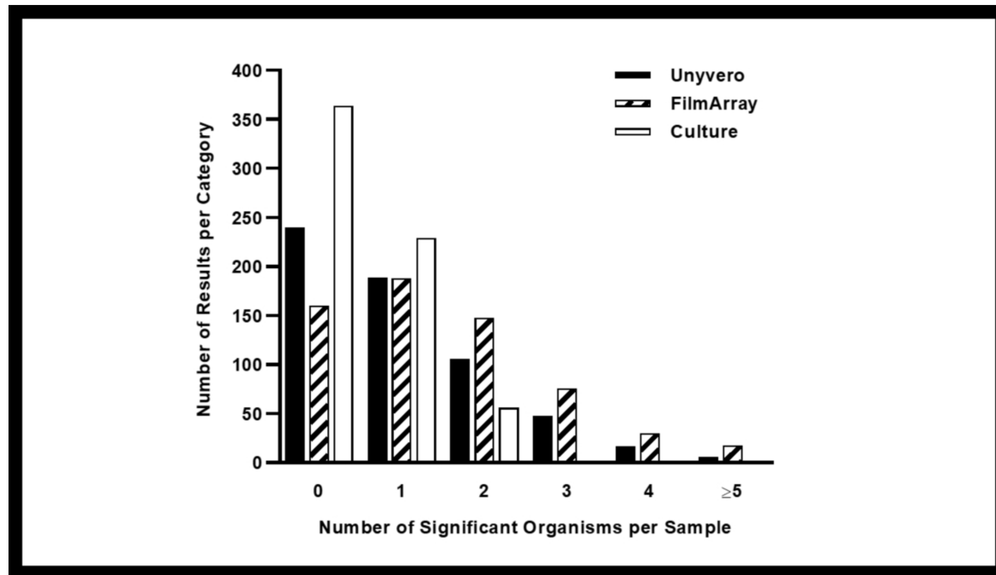

Figure 2. Number of significant organisms detected per respiratory sample by routine microbiology or PCR.

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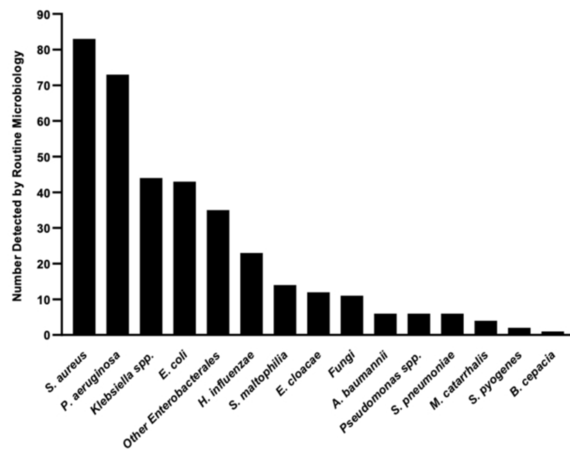

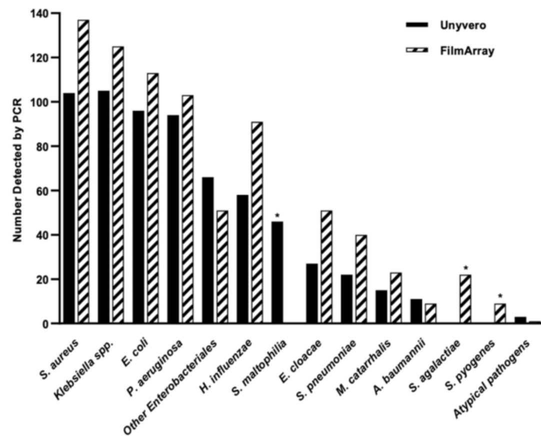

Figure 3. 3a (top) Numbers and types of bacteria detected by routine microbiology culture from respiratory samples included in the study. 3b (bottom) Numbers and type of bacteria detected by PCR from respiratory samples included in the study. Unyvero, solid bars, n = 606; FilmArray, hatched bars, n = 620. Species sought by one test only are marked with an asterisk.

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Supplementary Appendix for:

Multicentre evaluation of two multiplex PCR platforms for the rapid microbiological investigation of nosocomial pneumonia in UK ICUs: the INHALE WP1 study

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Supplementary Methods

Study Sites

The study was conducted at Aintree University Hospital NHS Foundation Trust, Bupa Cromwell Hospital, Chelsea and Westminster Hospital NHS Foundation Trust, City Hospitals Sunderland, Dudley Group NHS Foundation Trust, Great Ormond Street Hospital, Guy's and St Thomas' NHS Foundation Trust, James Paget University Hospitals NHS Foundation Trust, Norfolk and Norwich University Hospitals NHS Foundation Trust, North Middlesex University Hospital NHS Trust, Queen Elizabeth Hospital Kings Lynn NHS Trust, Royal Free Hospital, Royal Liverpool and Broadgreen University Hospitals NHS Trust, University College London Hospitals and University Hospitals of North Midlands. These sites were served by 11 different microbiology laboratories

Conventional culture and susceptibility testing

Each respiratory specimen was initially cultured locally, at the laboratory serving the participating hospital, according to their standard operating procedures (SOPs). These SOPs were all based on the Public Health England (PHE) UK Standard.[1] Prior to culture, specimens underwent quality control checks. Salivary specimens or those with excess epithelial cells were rejected. Except in the case of bronchoalveolar lavage (BAL) specimens, the PHE standard specifies initial homogenisation of the respiratory sample with 0.1% dithiothreitol, followed by a 10^{-5} dilution, and inoculation of the diluted and undiluted specimen onto chocolate agar with bacitracin (incorporated, or as a disc), cysteine lactose electrolyte deficient agar (CLED) or MacConkey agar, along with Sabouraud agar for fungi. Blood agar was added at some sites. In the case of BAL specimens, culture is performed on serial dilutions of a sample that has been concentrated by centrifugation.

Plates are incubated at 35-37°C in the presence of 5% CO_2 (blood and chocolate agar) or in air (MacConkey and CLED agar) for 40-48h, with daily reading of results. Bacterial pathogens are identified to species level by MALDI-TOF or biochemical methods, followed by antimicrobial susceptibility testing using EUCAST or BSAC interpretive standards.

The PHE standards provide guidance on the interpretation of culture results for BAL samples, whereas interpretation and reporting are left to the discretion of individual laboratories for other sample types.

16S rRNA Analysis

All specimens with a sufficient surplus (300 µl) after PCR testing underwent 16S rRNA analysis. Samples were inactivated by incubating for 30 minutes at 99°C, then DNA was extracted using the ZR Viral RNA/DNA kit and ZR BashingBead Lysis Tubes (Zymo Research). Briefly, 300 µl of sample were transferred into a bead tube, homogenized in a bead-beater for 30 seconds at 3,500 oscillations per minute, centrifuged for 1 minute at 21,000 ***g***. Next, 200 µl of the supernatant were transferred to a clean microcentrifuge tube and DNA was extracted following manufacturer's instructions. Illumina 16S rRNA sequencing was then performed according to the manufacturer's protocol (Illumina, 15044223B). The V3-V4 16S rRNA region was amplified on a LightCycler 480 II instrument (Roche) and sequenced on an Illumina MiSeq system. The Illumina BaseSpace

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16S rRNA pipeline was used to analyse the results. Only samples with at least 10,000 total reads were deemed eligible for analysis. For a genus to be considered significant, it had to comprise at least 1% of all reads.

Comprehensive Culture

A sub-set of 103 specimens, selected at random or based on disagreement between culture and PCR for resistance detection, underwent additional culture-based analysis, termed 'comprehensive culture' at the UCL research laboratory, using methodology described previously.[2] Briefly, a sweep of growth was taken across the plate of a fresh primary culture of the specimen on chocolate agar, and stored in Microbank™ vials at -80°C until analysis. Ten microliters of neat sample and a 10^{-5} dilution in 0.9% saline were then plated onto chocolate agar, Columbia blood agar (CBA), Brilliance UTI agar (Oxoid, Basingstoke, UK) and Columbia colistin-nalidixic acid agar (C-CNA) (Oxoid). The CBA, UTI and C-CNA plates were incubated at 37°C in air for 18h; chocolate agar plates were incubated in 5% CO_2 at 37°C for 18h. Representative bacterial colonies of different morphologies on each medium were identified by MALDI-TOF MS (Bruker GmbH, Mannheim, Germany), either directly from colonies or by using formic acid extraction where necessary

Characterisation of Antimicrobial Resistances

Additional investigation of antimicrobial resistances, or the genes responsible, was performed on isolates found resistant in microbiology laboratories or by comprehensive culture, or when either of the two molecular systems detected key resistance genes.

Gram-negative bacteria (i) reported resistant to cephalosporins or carbapenems in routine microbiology, or (ii) found to have ESBL or carbapenemase genes using the PCR systems, or (iii) grown in comprehensive culture were tested for resistance to ceftazidime, cefotaxime, ceftriaxone, ertapenem, meropenem and imipenem (Enterobacterales) or imipenem, meropenem, ceftazidime and piperacillin/tazobactam (*Acinetobacter* spp. and *P. aeruginosa*) by EUCAST disc diffusion methodology.[3] Potential methicillin-resistant *Staphylococcus aureus* (MRSA) were screened for resistance to cefoxitin.

When isolates had phenotypes consistent with the presence of antimicrobial resistance genes, genetic testing was performed. Enterobacterales resistant to a carbapenem or to oxyimino cephalosporins, *P. aeruginosa* resistant to both carbapenems and cephalosporins and *A. baumannii* resistant to imipenem or meropenem were tested with the Check-MDR CTX103XL kit (Checkpoints, Wageningen, the Netherlands) according to manufacturer's instructions, following extraction of total genomic DNA using the Qiagen DNA Mini Kit (Qiagen). *S. aureus* isolates resistant to cefoxitin underwent in-house PCR (primers and conditions described previously)[4,5] for detection of *mecA* and *mecC* using HotStartTaq PCR Mastermix (Qiagen) on DNA extracted with the Qiagen DNA Mini Kit.

Data collection

Routine microbiology data available on the Laboratory Information Management Systems (LIMS) of each participating hospital were collected and managed using REDCap[6] electronic data capture tools hosted at Norwich Clinical Trials Unit. For each included sample, we collected: (i) the culture result as reported to treating clinicians and (ii) details of significant organisms reported, and their full antimicrobial susceptibility

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profile. Any results for relevant respiratory pathogens detected by non-culture-based methods were also included. Hospitals' routine virology data (by other PCR methods) were collected if testing had been performed on the same calendar day as collection of the lower respiratory tract sample for INHALE. We also collected details required to confirm patient eligibility and the times samples were collected, processed and results released. All PCR and supplementary data generated by study staff were also recorded in RedCap. All data were anonymised.

Supplementary Results

16S rRNA Analysis

16S rRNA analysis was originally included to act as an independent molecular reference method. Four-way BLC analysis including 16S rRNA results is shown in Table S5. However, he 16S technique was only able to distinguish organisms to genus level, so PCR and routine microbiology data are likewise grouped to genus level. Streptococci are omitted because of the high density of commensal streptococci found in the respiratory tract and the inability of the 16S method to distinguish these from each other and from pathogenic streptococci, including *S. pneumoniae*[7]. For this analysis only, *Klebsiella aerogenes* was grouped within the genus *Enterobacter* owing to its relatively recent re-classification. The results show that 16S rRNA analysis was less sensitive than PCR and so was not fit-for-purpose as an alternative molecular reference method; nonetheless, it had had greater sensitivity than routine microbiology. Further optimisation might yield better results.

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Table S1. Criteria for HAP/VAP diagnostic test progression to RCT phase of the inhale study (WP3)

Criterion	**Description**	**Point Scoring**	**Maximum available points**
Concordance - essential criterion	Major discordance i.e. failures by the test to find pathogen(s) detected by routine microbiology must account for < 5% of all tests performed.	NA	NA
Overall Concordance	A measure of the overall accuracy of the test compared to the gold standard.	1 point is awarded for every % point over 55% overall concordance	45 points
Sensitivity	Sensitivity for detection of common pathogens (i.e. *P. aeruginosa, S. aureus, K. pneumoniae, K. oxytoca*, *E. coli, E. cloacae, E. aerogenes, A. baumannii, H. influenzae* and *S. pneumoniae*)	2 points for every 'win', i.e. the best sensitivity against a particular pathogen	20 points
Breadth of panel	Each PCR test seeks some targets that the other cannot, principally resistance genes for Curetis and viruses for Biofire.	Maximum points for most detections of unique targets, other tests awarded points as a proportion of unique detection	15 points
Time to Result	Time to Result	1 Point allocated for each 30 min less than 8h, the common dosage interval for antibiotics	15 points
Cost of tests and equipment	Cost per test, A composite measure of both test and equipment cost.	Cheapest test is awarded the maximum points. One point is deducted from others for every 10% increase in price compared to the cheapest.	15 points
Failure rate	Failure rate of test and/or machine, full or partial.	1 point deducted for each 0.5% of failures	15 points
Footprint and space occupied	Amount of space required to host machine	Smallest machine awarded maximum points. Cheapest test is awarded the maximum points. One point is deducted from others for every 10% increase in price compared to the cheapest.	5 points
Customer service	The quality and speed of customer service in the event of breakdown, ordering, installation etc.	. Average score based on assessment from individual users who have dealt with manufacturers during the study.	5 points
Consumable logistics	Space required for storage of consumables, storage temperature, shelf life, delivery speed, delivery cost.	1 point for best performing machine for each criterion	5 points
Ease of use	User perception and experience	Average scored based on assessments from individual users who have operated machines during the study.	10 points
Total			**150 points**

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Table S2. Number of bacterial target organisms detected by routine microbiology or PCR in specimens from patients with HAP or VAP.

Target Organism	Routine Microbiology		Unyvero		FilmArray	
	HAP (n= 260)	VAP (n=392)	HAP (n = 240)	VAP (n = 366)	HAP (n = 247)	VAP (n = 373)
A. baumannii	1	5	3	8	2	7
C. pneumoniae	0	0	0	0	0	0
C. freundii	0	2	4	5	NA	NA
Coliform	2	4	NA	NA	NA	NA
E. cloacae	4	8	9	18	22	29
E. coli	15	28	45	51	51	62
H. influenzae	9	14	20	38	34	57
K. aerogenes	0	6	2	4	2	9
K. pneumoniae	7	19	21	33	31	43
K. oxytoca	3	9	13	27	13	27
K. variicola	0	0	2	3	NA	NA
L. pneumophila	0	0	1	1	0	0
M. catarrhalis	1	3	5	10	9	14
M. morganii	1	0	6	5	NA	NA
M. pneumoniae	0	0	0	1	0	1
Proteus sp.	2	8	13	16	14	17
P. aeruginosa	25	48	37	57	41	62
Pseudomonas sp.	0	6	NA	NA	NA	NA
S. marcescens	1	9	3	14	3	17
S, aureus	31	52	46	58	58	79
S. maltophilia	3	11	15	31	NA	NA
S. agalactiae	0	0	NA	NA	10	12
S. pneumoniae	2	4	9	13	14	26
S. pyogenes	1	1	NA	NA	2	7

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Table S3. Viral detections made by FilmArray (n=620 eligible samples) and sensitivity and specificity compared with routine virology (n = 102 samples with routine virology performed with eligible FilmArray result). 95% confidence intervals are given in brackets.

Virus	Number of Detections	% positive samples	Sensitivity %	Specificity %	PPV %	NPV %
Rhinovirus	55	8.9	100.0 (15.8 - 100.0)	85.6 (77.3 - 91.7)	11.8 (1.5 - 36.4)	100.0 (95.9 - 100.0)
Influenza A	29	4.7	100.0 (59.0-100.0)	97.0 (91.4 - 99.4)	70.0 (34.8 - 93.3)	100.0 (96.2 - 100.0)
Influenza B	25	4.0	100.0 (39.8 - 100.0)	98.0 (93.1 - 99.8)	66.7 (22.3 - 95.7)	100.0 (96.4-100.0)
Parainfluenza	17	2.7	75.0 (19.4-99.4)	99.0 (94.7 - 100.0)	75.0 (19.4-99.4)	99.0 (94.7 - 100.0)
Coronavirus (229E, HKU1, NL63, OC43)	16	2.6	ND*	95.3 (89.3 -98.5)	0.0 (0.0 - 52.2)	100.0 (96.4-100.0)
Adenovirus	7	1.1	50.0 (6.8-93.2)	100.0 (96.4 - 100.0)	100.0 (15.8 - 100.0)	98.1 (93.2 - 99.8)
Respiratory Syncytial Virus	6	1.0	66.7 (9.4 - 99.2)	99.0 (94.7 - 100.0)	66.7 (9.4 - 99.2)	99.0 (94.7 - 100.0)
Human metapneumovirus	5	0.8	100.0 (2.5 - 100.0)	100.0 (96.5 - 100.0)	100.0 (2.5 - 100.0)	100.0 (96.5 - -100.0)
MERS coronavirus	0	0	ND	ND	ND	ND

*ND – not determined because routine virology did not report any positives.

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Table S4. Discordant samples, where one or both PCR tests did not detect potential pathogens which they sought and which were reported by routine microbiology.

Sample designation[a]	Sample type	Routine microbiology result[b]	Unyvero Result[c]	FilmArray result[c]
D001	SPU	***S. aureus***	Negative	*S. aureus*
D014	ETT	*M. catarrhalis*, ***S. aureus***	*M. catarrhalis, H. influenzae, S. pneumoniae*	*M. catarrhalis, S. pneumoniae*
D019	SPU	***P. aeruginosa, S. marcescens***	Negative	*P. aeruginosa, S. marcescens*
D022	SPU	***S. aureus***	Negative	*S. aureus*
D037	ETT	*H. influenzae*, ***S. aureus***	*H. influenzae*	*H. influenzae, S. aureus*
D054	SPU	*S. aureus*, ***H. influenzae***	*H. influenzae, M. catarrhalis, S. aureus*	*M. catarrhalis, S. aureus*
D064	SPU	*M. catarrhalis*, ***K. pneumoniae***	*M. catarrhalis*	*M. catarrhalis, S. pyogenes*
D065	SPU	***E. coli***, *H. influenzae*	*H. influenzae*	*E. coli, H. influenzae*
E005	SPU	*P. aeruginosa*, ***K. pneumoniae***	*P. aeruginosa, S. maltophila*	*P. aeruginosa, S. agalactiae*
F006	Other	***S. aureus***	Negative	*S. aureus*
I012	SPU	*P. aeruginosa*, ***S. aureus***	*K. pneumoniae, P. aeruginosa*	Invalid result
I026	SPU	***P. aeruginosa***	Negative	*P. aeruginosa*
I052	SPU	*P. aeruginosa*, ***S. aureus***, Coliform	*P. aeruginosa*	*P. aeruginosa, S. aureus*
I063	ETT	***K. pneumoniae***	*K. variicola*	*K. pneumoniae*
I076	SPU	***E. aerogenes***, *B. cepacia*	Negative	*E. aerogenes*
J002	ETT	***K. pneumoniae***	*E, coli, K. oxytoca, S. aureus*	*E. cloacae, E. coli, K. pneumoniae, K. oxytoca, S. aureus*
J007	SPU	***P. aeruginosa***, *S. maltophila*	Negative	Negative
K060	ETT	***E. aerogenes***	Negative	*E. aerogenes, Proteus* sp.
K101	BAL	***S. aureus***	Negative	Negative
L002	ND-BAL	***P. mirabilis***	Negative	Negative
L011	ND-BAL	***E. coli***	Negative	*E. coli*
L022	ND-BAL	***E. coli, S. aureus***	Negative	*E. coli*
L034	SPU	***E. coli***, *M. catarrhalis*	*K. pneumoniae, M. catarrhalis*	*K. pneumoniae, M. catarrhalis*
L039	ND-BAL	***K. oxytoca***	Negative	*K. oxytoca*
M055	ETT	***E. cloacae***	*E. cloacae*	Negative
N002	SPU	***E. coli***	Negative	*E. coli, S. aureus, S. pneumoniae*
N018	SPU	***E. aerogenes***, *H. influenzae*	*H. influenzae*	*E. cloacae, E. aerogenes, H. influenzae*
N054	SPU	*E. cloacae*, ***S. aureus***	*E. cloacae*	*E. clocae, S. aureus, S. agalactiae*

[a] The prefix letter is an arbitrary code indicating samples were from the same site.
[b] Discordant pathogen(s) is shown in bold type.
[c] Shading indicates missed detections
BAL, bronchoalveolar lavage; ND-BAL, non-directed bronchoalveolar lavage; ETT, endotracheal tube aspirate; SPU, sputum.

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Table S5. Summary of frequency of potential pathogens reported by routine microbiology that are absent from the panels of one or both of the PCR tests.

	Organism[a]	**Frequency of detection by routine microbiology**	**Sample type (number)**
Organism absent from Unyvero panel	*Streptococcus pyogenes*	2	ETT (1) SPU(1)
Organisms absent from FilmArray panel	*Citrobacter freundii*	1	ND-BAL (1)
	Morganella morganii	1	ETT (1)
	Stenotrophomonas maltophila	12	ETT (6), ND-BAL (1), SPU(4) Other (1)
Organisms absent from both Unyvero and FilmArray panels	*Burkholderia cepacia*	1	SPU (1)
	Citrbacter koseri	5	ETT (3), ND-BAL (1), SPU (1)
	Raoultella ornitholytica	2	ETT (1), SPU (1)
	Achromobacter xylosoxidans	1	ETT (1)
	Corynebacterium striatum	1	ETT (1)
	Enterococcus faecium	1	ETT (1)
	Group G streptococcus	1	ETT (1)
	Pseudomonas putida	1	ETT (1)
	S. pseudopneumoniae	1	ETT (1)

[a]In addition, there were 3 cases (1 x ETT and 2 x SPU) where routine microbiology reported 'coliforms' not identified to species level, 1 (Other specimen type) where it reported *Pseudomonas* spp. and 2 (both SPU) where it reported *Streptococcus* spp. Without a species level identification is impossible to distinguish whether these represent cases where the PCR tests failed to detect organisms that they sought, or cases where the particular species was not sought by these tests.

Table S6. Mean numbers of pathogens per eligible and valid sample detected by PCR tests in relation to sample type

PCR Test	**Sample Type**		
	BAL or ND-BAL	**ETT**	**Sputum**
Unyvero	0.81 ± 0.96 (n = 63)	1.08 ± 1.18 (n = 278)	1. 13 ± 1.19 (n = 251)
FilmArray	1.23 ±1.37 (n = 64)	1.44 ± 1.26 (n = 285)	1.63 ± 1.40 (n = 257)

BAL, bronchoalveolar lavage; ND-BAL, non-directed bronchoalveolar lavage; ETT, endotracheal tube aspirate

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Table S7. Pathogen-specific performance of PCR tests when compared with routine microbiology as the gold standard, including 95% confidence intervals

UNYVERO													
Target Organism	**Number of Detections**	**Sensitivity**			**Specificity**			**PPV**			**NPV**		
		%	**95% CI**		**%**	**95% CI**		**%**	**95% CI**		**%**	**95% CI**	
P. aeruginosa	94	95.3	86.9	99.0	93.9	91.6	95.8	64.9	54.4	74.5	99.4	98.3	99.9
S. aureus	104	87.2	77.7	93.7	93.2	90.7	95.2	65.4	55.4	74.4	98.0	96.4	99.0
K. pneumoniae	54	83.3	62.6	95.3	94.2	91.9	95.9	37.0	24.3	51.3	99.3	98.2	99.8
K. oxytoca	40	90.9	58.7	99.8	95.0	92.9	96.6	25.0	12.7	41.2	99.8	99.0	100.0
E. coli	96	87.8	73.8	95.9	89.4	86.5	91.8	37.5	27.8	48.0	99.0	97.7	99.7
E. cloacae	27	100.0	73.5	100.0	97.5	95.9	98.6	44.4	25.5	64.7	100.0	99.4	100.0
K. aerogenes	6	50.0	11.8	88.2	99.5	98.5	99.9	50.0	11.8	88.2	99.5	98.5	99.9
A. baumannii	11	100.0	47.8	100.0	99.0	97.8	99.6	45.5	16.7	76.6	100.0	99.4	100.0
H. influenzae	58	100.0	83.9	100.0	93.7	91.4	95.5	36.2	24.0	49.9	100.0	99.3	100.0
S. pneumoniae	22	100.0	54.1	100.0	97.3	95.7	98.5	27.3	10.7	50.2	100.0	99.4	100.0
M. catarrhalis	15	100.0	39.8	100.0	98.2	96.8	99.1	26.7	7.8	55.1	100.0	99.4	100.0
S. marcescens	17	77.8	40.0	97.2	98.3	96.9	99.2	41.2	18.4	67.1	99.7	98.8	100.0
C. pneumoniae	0												
L. pneumophila	2												
M. pneumoniae	1												
C. freundii	9	100.0	2.5	100.0	98.7	97.4	99.4	11.1	0.3	48.2	100.0	99.4	100.0
M. morganii	11	100.0	2.5	100.0	98.3	97.0	99.2	9.1	0.2	41.3	100.0	99.4	100.0
S. maltophilia	46	92.9	66.1	99.8	94.4	92.3	96.1	28.3	16.0	43.5	99.8	99.0	100.0

FILMARRAY													
Target Organism	**Number of Detections**	Sensitivity			Specificity			PPV			NPV		
		%	95% CI		%	95% CI		%	95% CI		%	95% CI	
P. aeruginosa	103	98.5	91.8	100.0	93.1	90.7	95.1	63.1	53.0	72.4	99.8	98.9	100.0
S. aureus	137	96.2	89.4	99.2	88.9	85.9	91.4	56.2	47.5	64.7	99.4	98.2	99.9
K. pneumoniae	74	92.0	74.0	99.0	91.4	88.9	93.6	31.1	20.8	42.9	99.6	98.7	100.0
K. oxytoca	40	100.0	71.5	100.0	95.2	93.2	96.8	27.5	14.6	43.9	100.0	99.4	100.0
E. coli	113	97.6	87.4	99.9	87.5	84.6	90.1	36.3	27.4	45.9	99.8	98.9	100.0
E. cloacae	51	91.7	61.5	99.8	93.4	91.1	95.3	21.6	11.3	35.3	99.8	99.0	100.0
K. aerogenes	11	100.0	54.1	100.0	99.2	98.1	99.7	54.5	23.4	83.3	100.0	99.4	100.0
A. baumannii	9	100.0	54.1	100.0	99.5	98.6	99.9	66.7	29.9	92.5	100.0	99.4	100.0
H. influenzae	91	95.2	76.2	99.9	88.1	85.3	90.6	22.0	14.0	31.9	99.8	99.0	100.0
S. pneumoniae	40	100.0	54.1	100.0	94.5	92.3	96.1	15.0	5.7	29.8	100.0	99.4	100.0
M. catarrhalis	23	100.0	39.8	100.0	96.9	95.2	98.1	17.4	5.0	38.8	100.0	99.4	100.0
S. marcescens	20	100.0	66.4	100.0	98.2	96.8	99.1	45.0	23.1	68.5	100.0	99.4	100.0
C. pneumoniae	0												
L. pneumophila	0												
M. pneumoniae	1												
S. agalactiae	22	NA	0.0	100.0	96.5	94.7	97.8	0.0	0.0	15.4	100.0	99.4	100.0
S. pyogenes	9	100.0	15.8	100.0	98.9	97.7	99.5	22.2	2.8	60.0	100.0	99.4	100.0

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Table S8. Pathogen specific performance of routine microbiology and PCR tests using independent BLC modelling, including 95% confidence intervals

ROUTINE MICROBIOLOGY												
Target Organism	**Sensitivity**			**Specificity**			**PPV**			**NPV**		
	%	**95% CI**		**%**	**95% CI**		**%**	**95% CI**		**%**	**95% CI**	
P. aeruginosa	64.7	54.7	73.9	99.7	98.9	100.0	97.3	91.5	99.6	93.9	91.7	95.8
S. aureus	65.2	56.1	74.1	99.2	98.2	99.8	95.2	88.8	98.6	92.5	90.0	94.7
K. pneumoniae	37.8	26.0	51.4	99.5	98.6	99.9	89.3	73.2	97.7	93.5	90.9	95.6
K. oxytoca	30.2	18.3	45.5	99.9	99.3	100.0	94.3	73.0	99.8	95.5	93.7	97.0
E. coli	38.8	29.8	48.2	99.7	98.9	100.0	96.1	86.8	99.5	88.5	85.5	91.1
E. cloacae	42.9	25.6	61.3	99.9	99.3	100.0	94.6	71.6	99.8	97.2	95.5	98.4
K. aerogenes	68.7	32.1	94.7	99.9	99.4	100.0	88.9	54.4	99.6	99.6	98.5	99.9
A. baumannii	57.5	27.1	84.9	99.9	99.4	100.0	87.4	50.8	99.5	99.4	98.5	99.8
H. influenzae	36.3	24.8	49.1	99.9	99.3	100.0	96.8	84.5	99.9	93.5	91.0	95.4
S. pneumoniae	27.1	15.9	46.2	99.9	99.4	100.0	90.0	61.0	99.6	97.0	95.0	98.3
M. catarrhalis	27.6	15.8	50.4	99.9	99.4	100.0	86.7	50.5	99.4	98.0	96.5	99.0
S. marcescens	48.4	27.7	69.7	99.9	99.3	100.0	92.9	67.4	99.7	98.4	97.1	99.2

UNYVERO												
Target Organism	**Sensitivity**			**Specificity**			**PPV**			**NPV**		
	%	**95% CI**		**%**	**95% CI**		**%**	**95% CI**		**%**	**95% CI**	
P. aeruginosa	95.8	89.6	99.0	99.9	99.2	100.0	99.2	95.8	100.0	99.2	98.0	99.8
S. aureus	91.1	82.9	96.1	99.8	99.2	100.0	99.3	96.4	100.0	98.0	95.9	99.2
K. pneumoniae	88.9	73.3	97.5	99.8	99.0	100.0	97.6	90.6	99.9	98.8	96.6	99.8
K. oxytoca	92.7	80.3	98.8	99.2	98.1	99.9	88.7	74.7	98.6	99.5	98.6	99.9

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E. coli	89.6	80.5	96.4	99.7	98.9	100.0	98.6	94.4	99.9	97.8	95.8	99.3
E. cloacae	94.9	74.9	99.8	99.9	99.3	100.0	97.2	86.9	99.9	99.8	98.5	100.0
K. aerogenes	48.4	21.4	80.3	99.6	98.8	99.9	62.1	25.5	93.0	99.3	98.1	99.8
A. baumannii	92.6	66.2	99.7	99.5	98.6	99.9	70.9	39.7	94.6	99.9	99.4	100.0
H. influenzae	96.9	84.8	99.9	99.7	98.8	100.0	97.1	89.3	99.9	99.7	98.2	100.0
S. pneumoniae	90.8	63.2	99.6	99.9	99.3	100.0	96.7	83.8	99.9	99.6	97.8	100.0
M. catarrhalis	89.0	60.6	99.5	99.9	99.4	100.0	95.5	78.1	99.8	99.7	98.5	100.0
S. marcescens	83.9	64.1	95.6	99.9	99.4	100.0	95.7	78.0	99.8	99.5	98.7	99.9

FILMARRAY												
	Sensitivity			**Specificity**			**PPV**			**NPV**		
Target Organism	**%**	**95% CI**		**%**	**95% CI**		**%**	**95% CI**		**%**	**95% CI**	
P. aeruginosa	99.2	95.9	100.0	99.3	98.3	99.9	96.6	91.1	99.6	99.9	99.2	100.0
S. aureus	99.3	96.5	100.0	95.6	93.3	97.5	83.9	76.2	90.7	99.8	99.1	100.0
K. pneumoniae	98.1	91.1	99.9	97.7	95.8	99.4	82.2	69.5	95.6	99.8	99.0	100.0
K. oxytoca	95.2	81.0	99.8	99.7	98.9	100.0	95.9	84.0	99.8	99.7	98.6	100.0
E. coli	98.9	95.3	100.0	98.7	96.8	99.9	94.2	86.1	99.6	99.8	99.0	100.0
E. cloacae	94.2	81.7	99.2	96.4	94.6	97.9	56.1	40.5	72.6	99.7	99.0	100.0
K. aerogenes	89.8	58.5	99.6	99.4	98.4	99.9	67.8	34.3	96.7	99.9	99.2	100.0
A. baumannii	89.4	55.6	99.5	99.9	99.4	100.0	91.3	62.5	99.6	99.8	99.2	100.0
H. influenzae	95.3	87.4	99.2	93.8	91.5	95.8	62.4	51.9	73.8	99.5	98.5	99.9
S. pneumoniae	97.1	85.8	99.9	97.1	95.4	98.8	57.9	40.8	81.7	99.9	99.3	100.0
M. catarrhalis	95.7	80.2	99.8	98.9	97.6	99.8	71.4	47.1	95.0	99.9	99.4	100.0
S. marcescens	96.1	81.8	99.9	99.8	99.2	100.0	94.2	76.3	99.8	99.9	99.3	100.0

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Table S9. Pathogen-specific performance of routine microbiology, PCR tests and 16S rRNA analysis using independent BLC modelling, showing 95% confidence intervals

ROUTINE MICROBIOLOGY

	Sensitivity			**Specificity**			**PPV**			**NPV**		
Target Genus	**%**	**95% CI**		**%**	**95% CI**		**%**	**95% CI**		**%**	**95% CI**	
Acinetobacter	54.2	26.8	82.0	99.9	99.3	100.0	87.5	49.5	99.5	99.2	98.3	99.8
Escherichia	38.7	29.1	48.7	99.6	98.6	99.9	95.3	85.2	99.3	88.3	85.2	91.0
Enterobacter	46.4	30.2	63.3	99.8	99.2	100.0	95.9	79.0	99.9	96.2	93.7	97.8
Haemophilus	30.4	20.2	42.8	99.8	99.2	100.0	96.6	83.7	99.9	90.9	87.7	93.4
Klebsiella	38.0	27.8	48.5	99.4	98.3	99.8	92.1	79.4	98.1	89.6	86.6	92.3
Moraxella	23.9	15.4	43.4	99.9	99.3	100.0	85.7	50.4	99.5	97.5	96.0	98.6
Pseudomonas	75.3	64.8	83.8	99.5	98.5	100.0	96.6	89.8	99.8	95.8	93.7	97.4
Proteus	33.6	18.3	52.2	99.7	98.9	100.0	84.8	57.4	97.7	96.5	94.6	97.8
Staphylococcus	66.3	56.4	75.2	99.6	98.6	99.9	97.5	91.6	99.7	92.5	89.6	94.8
Serratia	51.8	30.3	73.7	99.8	99.2	100.0	92.4	65.7	99.7	98.4	97.1	99.3

UNYVERO

	Sensitivity			**Specificity**			**PPV**			**NPV**		
Target Genus	**%**	**95% CI**		**%**	**95% CI**		**%**	**95% CI**		**%**	**95% CI**	
Acinetobacter	92.9	67.9	99.7	99.7	98.8	100.0	83.0	51.9	98.0	99.9	99.3	100.0
Escherichia	95.6	89.4	99.0	99.8	99.1	100.0	99.2	96.0	100.0	99.1	97.7	99.8
Enterobacter	80.4	60.6	94.3	99.8	99.2	100.0	97.6	87.7	99.9	98.6	96.4	99.6
Haemophilus	80.5	67.5	92.0	99.7	98.8	100.0	97.1	90.6	99.8	97.3	95.0	99.0
Klebsiella	90.7	81.8	96.6	99.0	97.6	99.8	94.2	86.5	98.8	98.3	96.5	99.4
Moraxella	80.0	56.0	95.3	99.9	99.3	100.0	95.2	76.8	99.9	99.3	98.2	99.9
Pseudomonas	95.7	89.4	99.0	99.8	99.2	100.0	99.1	95.2	100.0	99.3	98.1	99.8

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Proteus	96.6	83.5	99.8	99.7	99.0	100.0	94.7	82.5	99.7	99.8	99.0	100.0
Staphylococcus	92.6	85.2	97.1	99.8	99.1	100.0	99.2	96.1	100.0	98.2	96.3	99.3
Serratia	85.1	64.5	96.3	99.9	99.3	100.0	95.5	78.0	99.8	99.5	98.7	99.9

FILMARRAY												
	Sensitivity			Specificity			PPV			NPV		
Target Genus	**%**	**95% CI**		**%**	**95% CI**		**%**	**95% CI**		**%**	**95% CI**	
Acinetobacter	83.2	53.5	98.4	99.9	99.2	100.0	91.4	61.8	99.7	99.7	99.0	100.0
Escherichia	99.2	95.9	100.0	97.8	96.0	99.0	90.9	83.7	95.9	99.8	99.1	100.0
Enterobacter	95.1	84.6	99.4	96.8	94.6	98.7	68.5	51.9	86.8	99.6	98.8	100.0
Haemophilus	96.8	89.8	99.5	95.5	92.9	97.6	75.8	63.2	86.7	99.5	98.5	99.9
Klebsiella	96.4	90.0	99.7	97.8	95.9	99.1	88.9	79.7	95.8	99.3	98.0	99.9
Moraxella	96.1	81.2	99.9	99.1	97.9	99.8	77.7	54.3	95.8	99.9	99.3	100.0
Pseudomonas	98.5	93.4	99.9	99.0	97.8	99.7	94.4	88.5	98.2	99.7	98.8	100.0
Proteus	96.9	84.9	99.9	99.3	98.3	99.9	88.9	74.1	97.8	99.8	99.1	100.0
Staphylococcus	99.3	96.4	100.0	95.6	93.2	97.5	84.5	76.4	91.0	99.8	99.1	100.0
Serratia	96.0	80.7	99.8	99.7	98.9	100.0	91.1	72.2	99.1	99.9	99.3	100.0

16S rRNA Analysis												
	Sensitivity			Specificity			PPV			NPV		
Target Genus	**%**	**95% CI**		**%**	**95% CI**		**%**	**95% CI**		**%**	**95% CI**	
Acinetobacter	83.2	55.1	97.6	97.8	96.2	98.8	38.9	19.4	62.3	99.7	99.0	100.0
Escherichia	73.3	64.0	81.8	99.2	97.9	99.8	94.9	88.2	98.5	94.5	92.1	96.4
Enterobacter	21.1	15.3	34.9	86.5	83.2	89.4	10.6	6.1	18.6	93.7	90.8	95.9
Haemophilus	85.2	74.8	93.0	88.0	84.6	90.8	50.5	40.5	60.6	97.6	95.6	99.0

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Klebsiella	66.8	56.0	77.2	95.7	93.5	97.4	74.0	63.5	83.6	94.0	91.4	96.1
Moraxella	58.0	34.5	78.4	99.9	99.2	100.0	93.1	69.7	99.7	98.6	97.3	99.4
Pseudomonas	89.0	81.0	94.5	94.7	92.3	96.5	74.5	65.1	82.5	98.0	96.4	99.0
Proteus	54.6	36.1	71.7	99.9	99.2	100.0	95.5	77.4	99.8	97.6	96.0	98.7
Staphylococcus	82.8	74.3	89.2	77.9	73.8	81.8	47.4	40.2	55.0	95.0	92.1	96.9
Serratia	90.6	72.7	98.6	87.8	84.8	90.5	19.8	12.1	29.8	99.7	98.8	100.0

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Table S10. Pathogen-specific performance of PCR tests compared with routine microbiology according to whether the sample was taken before or after antibiotic administration.

UNYVERO								
	Before Antibiotics (n = 329)				**After Antibiotics (n = 277)**			
Target organism	**Sensitivity**		**Specificity**		**Sensitivity**		**Specificity**	
	%	**95% CI**	**%**	**95% CI**	**%**	**95% CI**	**%**	**95% CI**
P. aeruginosa	100.0	89.1 - 100.0	93.3	89.8 - 95.8	90.6	75.0 - 98.0	94.7	91.1 - 97.1
S. aureus	91.5	79.6 - 97. 6	93.3	89.7 - 95.9	80.6	62.5 - 92.5	93.1	89.2 - 95.1
K. pneumoniae	83.3	51.6 - 97.9	94.6	91.6 - 96.8	83.3	51.6 - 97.9	93.6	89.9 -96.2
K. oxytoca	100.0	59.0 - 100.0	93.5	90.2 - 95.9	75.0	19.4 - 99.4	96.7	93.8 - 98.5
E. coli	81.0	58.1 - 94.6	89.9	86.0 - 93.1	95.0	75.1 - 99.9	88.7	84.2 - 92.3
E. cloacae	100.0	54.1 - 100.0	97.2	94.8 - 98.7	100.0	54.1 - 100.0	97.8	95.2 - 99.2
K. aerogenes	50.0	11.8 - 88.2	99.4	97.8 - 99.9	ND	ND	99.6	98.0 - 100.0
A. baumannii	100.0	15.8 - 100.0	98.8	96.9 - 99.7	100.0	29.2 - 100.0	99.3	97.4 - 99.9
H. influenzae	100.0	71.5 - 100.0	93.1	89.7 -95.6	100.0	69.2 -100.0	94.4	90.9 - 96.8
S. pneumoniae	100.0	47.8 - 100.0	97.8	95.6 - 99.1	100.0	2.5 - 100.0	96.7	93.9 - 98.5
M. catarrhalis	100.0	29.2 - 100.0	98.8	96.9 - 99.7	100.0	2.5 - 100.0	97.5	94.8 - 99.0
S. marcescens	85.7	42.1 - 99.6	98.4	96.4 - 99.5	50.0	1.3 - 98.7	98.2	95.8 - 99.4
C. freundii	100.0	2.5 - 100.0	97.9	95.7 - 99.1	ND	ND	99.6	98.0 - 100.0
M. morganii	100.0	2.5 - 100.0	98.5	96.5 - 99.5	ND	ND	98.2	95.8 - 99.4
S. maltophila	100.0	71.5 -100.0	93.1	89.7 - 95.6	66.7	9.4 - 99.2	96.0	92.9 - 98.0

ND - no detections

FILMARRAY								
	Before Antibiotics (n = 337)				**After Antibiotics (n = 283)**			
Target organism	**Sensitivity**		**Specificity**		**Sensitivity**		**Specificity**	
	%	**95% CI**	**%**	**95% CI**	**%**	**95% CI**	**%**	**95% CI**
P. aeruginosa	100.0	89.1 - 100.0	92.5	88.9 - 95.2	97.1	84.7 - 99.9	94.0	90.3 - 96.6
S. aureus	100.0	92.6 -100.0	88.6	84,3 - 92.0	90.6	75.0 - 98.0	89.2	84.7 - 92.8
K. pneumoniae	84.6	54.6 - 98.1	92.3	88.8 - 94.9	100.0	73.5 - 100.0	90.4	86.3 - 93.6
K. oxytoca	100.0	59.0 - 100.0	93.6	90.4 - 96.0	100.0	39.8 - 100.0	97.1	94.4 - 98.8
E. coli	95.5	77.2 - 99.9	88.6	84.5 - 91.9	100.0	83.2 - 100.0	86.3	81.6 - 90.2
E. cloacae	83.3	35.9 - 99.6	92.7	89.4 - 95.3	100.0	54.1 - 100.0	94.2	90.8 - 96.7
K. aerogenes	100.0	54.1 - 100.0	98.8	96.9 - 99.7	ND	ND	99.6	98.0 - 100.0
A. baumannii	100.0	29.2 - 100.0	99.4	97.9 - 99.9	100.0	29.2 - 100.0	99.6	98.0 - 100.0
H. influenzae	100.0	71.5 - 100.0	88.0	84.0 - 99.4	90.0	55.5 - 99.7	88.3	83.9 - 91.8
S. pneumoniae	100.0	47.8 - 100.0	96.4	93.8 - 98.1	100.0	2.5 - 100.0	92.2	88.4 - 95.0
M. catarrhalis	100.0	29.2 - 100.0	97.9	95.7 - 99.2	100.0	2.5 - 100.0	95.7	92.7 - 97.8
S. marcescens	100.0	59.0 - 100.0	98.5	96.5 - 99.5	100.0	15.8 - 100.0	97.9	95.4 - 99.2
S. agalactiae	ND	ND	97.3	95.0 - 98.8	ND	ND	95.4	92.3 - 97.5
S. pyogenes	ND	ND	98.2	96.2 - 99.3	100.0	15.8 - 100.0	99.6	98.0 - 100.0

ND - no detections

All samples had to be taken within 12h of antibiotic adminstration

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Table S11. Pathogen-specific performance of PCR tests compared with routine microbiology in relation to whether samples were fresh or had been frozen prior to PCR testing

UNYVERO								
	Fresh Samples (n = 456)				Frozen Samples (n = 150)			
Target organism	Sensitivity		Specificity		Sensitivity		Specificity	
	%	95% CI	%	95% CI	%	95% CI	%	95% CI
P. aeruginosa	98.0	89.6 - 100.0	93.8	91.0 - 96.0	84.6	54.6 - 98.1	94.2	88.8 - 97.4
S. aureus	84.0	70.9 - 92.8	92.6	89.6 - 95.0	92.9	76.5 - 99.1	95.1	89.6 - 98.2
K. pneumoniae	77.8	52.4 - 93.6	94.3	91.7 - 96.3	100.0	54.1 - 100.0	93.8	88.5 - 97.1
K. oxytoca	88.9	51.8 - 99.7	94.9	92.4 - 96.7	100.0	15.8 100.0	95.3	90.5 - 98.1
E. coli	87.9	71.8 - 96.6	90.1	86.8 - 92.7	87.5	47.3 - 99.7	87.3	80.7 - 92.3
E. cloacae	100.0	54.1 - 100.0	96.9	94.8 - 98.3	100.0	54.1 - 100.0	99.3	96.2 - 100.0
K. aerogenes	25.0	0.6 - 80.6	99.3	98.1 - 99.9	100.0	15.8 - 100.0	100.0	97.5 - 100.0
A. baumannii	100.0	47.8 - 100.0	98.9	97.4 - 99.6	ND	ND	99.3	96.3 - 100.0
H. influenzae	100.0	75.3 - 100.0	95.0	92.6 - 96.9	100.0	63.1 -100.0	89.4	83.2 - 94.0
S. pneumoniae	100.0	2.5 - 100.0	97.4	95.4 - 98.6	100.0	47.8 - 100.0	97.2	93.1 - 99.2
M. catarrhalis	100.0	39.8 - 100.0	98.2	96.5- 99.2	ND	ND	98.0	94.3 - 99.6
S. marcescens	83.3	35.9 - 99.6	98.2	96.5 - 99.2	66.7	9.4 - 99.2	98.6	95.2 - 99.8
C. freundii	100.0	2.5 - 100.0	98.7	97.2 - 99.5	ND	ND	98.7	95.3 - 99.8
M. morganii	100.0	2.5 - 100.0	98.0	96.3 - 99.1	ND	ND	99.3	96.3 - 100.0
S. maltophila	100.0	69.2 -100.0	95.3	92.9 - 97.1	75.0	19.4 - 99.4	91.8	86.1 - 95.7

FILMARRAY								
	Fresh Samples (n = 476)				Frozen Samples (n = 144)			
Target organism	Sensitivity		Specificity		Sensitivity		Specificity	
	%	95% CI	%	95% CI	%	95% CI	%	95% CI
P. aeruginosa	98.1	89.9 - 100.0	92.9	90.0 - 95.2	100.0	75.3 - 100.0	93.9	88.3 - 97.3
S. aureus	94.4	84.6 - 98.8	87.4	83.9 - 90.4	100.0	86.8 - 100.0	94.1	88.2 - 97.6
K. pneumoniae	89.5	66.9 - 98.7	91.9	89.0 - 84.2	100.0	54.1 - 100.0	89.9	83.6 - 94.3
K. oxytoca	100.0	66.4 - 100.0	94.6	92.2 - 96.5	100.0	15.8 - 100.0	97.2	92.9 - 99.2
E. coli	97.0	94.2 - 99.9	88.0	84.6 - 90.9	100.0	66.4 - 100.0	85.9	78.9 - 91.3
E. cloacae	100.0	54.1 - 100.0	92.6	89.8 - 94.8	83.3	35.9 - 99.6	96.4	91.7 - 98.8
K. aerogenes	100.0	39.8 - 100.0	98.9	97.5 - 99.7	100.0	15.8 - 100.0	100.0	97.4 - 100.0
A. baumannii	100.0	54.1 - 100.0	99.6	98.5 - 99.9	ND	ND	99.3	96.2 - 100.0
H. influenzae	92.9	66.1 - 99.8	89.6	86.5 - 92.2	100.0	59.0 - 100.0	83.2	75.9 - 89.0
S. pneumoniae	100.0	2.5 - 100.0	95.2	92.8 - 96.9	100.0	47.8 - 100.0	92.1	86.3 - 96.0
M. catarrhalis	100.0	39.8 - 100.0	96.6	94.6 - 98.1	ND	ND	97.9	94.0 - 99.6
S. marcescens	100.0	54.1 - 100.0	98.1	96.4 - 99.1	100.0	29.2 - 100.0	98.6	95.0 - 99.8
S. agalactiae	ND	ND	96.6	94.4 - 98.1	ND	ND	95.8	91.2 - 98.5
S. pyogenes	100.0	2.5 - 100.0	99.6	98.5 - 99.9	100.0	2.5 - 100.0	96.5	92.0 - 98.9

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Table S12. Antimicrobial resistance (%) to selected agents as determined by routine microbiology

	Antimicrobial										
Organism	**n [a]**	**AMC**	**3GC**	**CIP**	**MAC**	**METH**	**GEN**	**MEM**	**TZP**	**GLYC**	**MDR**
P. aeruginosa & Pseudomonas spp.	79	-	21.6[b]	17.3	-	-	10.8	23.6	24.3	-	20.3
S. aureus	83	-	-	18.9	25.0	14.6	10.0	-	-	0.0	13.3
E. coli	43	47.5	21.4	25.6	-	-	10.3	0.0	16.2	-	37.1
H. influenzae	23	22.2	-	0.0	41.7	-	-	-	-	-	13.0
Klebsiella spp.	44	30.8	20.7	10.5	-	-	7.7	3.3	13.5	-	15.9

- : Drug inherently inactive against species group

Antimicrobial abbreviations: AMC – amoxicillin/clavulanate, 3GC, third-generation cephalosporin (meaning ceftazidime, cefotaxime or ceftriaxone, as tested), CIP – ciprofloxacin, MAC – macrolide (erythromycin and clarithromycin depending on local laboratory), METH – detection of methicillin resistance (agent tested may be cefoxitin, flucloxacillin and oxacillin, depending on local laboratory), GEN – gentamicin, MEM – meropenem, TZP- piperacillin-tazobactam, GLYC, glycopeptide (vancomycin and teicoplanin depending on local laboratory)- MDR – multi-drug resistant, defined as resistant to ≥ 3 classes according rules described in Magiorakos et al.[8]

[a] Refers to the total number of isolates in the data set. The number tested for any given drug may be fewer.

[b] Ceftazidime only considered for these species.

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Table S13. Frequency of resistance gene detections by PCR tests among eligible samples (n = 606 for Unyvero, n = 620 for FilmArray)

Resistance Gene Target	Unyvero	FilmArray
Carbapenemases		
bla_{IMP}	0	1
bla_{KPC}	1	1
bla_{OXA-23}	5	NA
$bla_{OXA24/40}$	0	NA
bla_{OXA-48}	0	0
bla_{OXA-58}	0	NA
bla_{NDM}	2	0
bla_{VIM}	3	1
Other genes relevant to resistance to β-lactams		
bla_{CTX-M}	14	32
bla_{SHV}	55	NA
bla_{TEM}	108	NA
mecA	92	NA
mecC	3	NA
mecA/C and MREJ	NA	32
Miscellaneous		
ermB	68	NA
E. coli gyrA83	29	NA
P. aeruginosa gyrA87	35	NA
sul1	67	NA

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