'Sample-in, answer-out'? Evaluation and comprehensive analysis of the Unyvero P50 pneumonia assay

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ARTICLE INFO

ABSTRACT

This study aimed to evaluate the performance of the Unyvero P50 pneumonia assay, the first 'sample-in, answer-out' system for rapid identification of pathogens and antibiotic resistance markers directly from clinical specimens. Overall, Unyvero P50 displayed very good sensitivity (>95%); however, specificity was low (33%) mainly because 40% of the specimens were reported as normal flora. Specifically, one or more pathogens were identified in 28 of them. From a detailed analysis of 42 specimens selected at random, 76% of the additionally reported pathogens were confirmed present in primary specimens. Detection of selected resistance markers was compared to routine phenotypic susceptibility testing, supplemented with Checkpoints microarray system, PCR and sequencing. Concordance was mixed, primarily due to issues with panel's choice of markers and detection of some intrinsic beta-lactamases. Finally, we offer a critical analysis of the assay's microbial panel and resistance markers and provide suggestions for improvement.

1. Introduction

Pneumonia is defined as consolidative infection of the lower respiratory tract causing significant morbidity and mortality worldwide. In the UK, (infectious and non-infectious) respiratory diseases account for 20% of deaths (Hubbard, 2006) and in 2006, the British Thoracic Society reported that pneumonia alone accounted for over 1/3 of these (Hubbard, 2006). Pneumonia can be categorised as community-acquired (CAP) if acquired outside of the healthcare setting, or as hospital-acquired (HAP), when the onset of disease/clinical presentation occurs >48 h after hospital admission (Anand and Kollef, 2009). In the clinical setting, of particular concern are patients undergoing intensive or critical care, who develop HAP or ventilator-associated pneumonia (VAP), often as a consequence of aspiration and prolonged hospital stay, or related to mechanical ventilation (Rotstein et al., 2008). This prolonged stay along with the use of empirical broad-spectrum antibiotics may result in infection with multi-drug resistant organisms often associated with high mortality (Brusselaers et al., 2011).

Pneumonia can be caused by a wide variety of bacteria, viruses or fungi that cannot easily be distinguished by clinical presentation (Enne et al., 2014). Current routine diagnostic methods are mainly culture-based, which are limited by low sensitivity and unsuitability for detecting atypical pathogens. At present, turnaround times for

routine culture and antimicrobial susceptibility testing range from 48 to 72 h; in the meantime, the patient receives empirical antimicrobial therapy (Masterton et al., 2008). Such empirical therapy may be compromised by antimicrobial resistance or be used unnecessarily to treat infections caused by viruses or susceptible bacteria, thus driving the development of antimicrobial resistance (Cooke et al., 2014; Kollef, 2004). Hence, a rapid test for detecting microorganisms and their associated susceptibility profiles to direct therapy in pneumonia is urgently needed; both for better prognosis of patients (Niederman, 2006) and improved antimicrobial stewardship (Davies, 2013).

Although there has been an emergence of real-time PCR assays targeted towards respiratory diagnosis, a single method available for rapidly identifying the variety of pathogenic causes of pneumonia is lacking. Accordingly, we evaluated the Curetis Unyvero P50 Pneumonia assay, the first 'sample-in and answer-out' system capable of diagnosing pneumonia aetiology directly from clinical specimens. This test combines automated sample preparation with multiplex PCR for selected targets and microarray hybridisation for amplicon detection. It promises to detect 16 bacteria and one fungus as well as 18 antibiotic resistance markers in around 5 h (Table 1).

2. Materials and methods

2.1. Specimen collection and analysis

We collected anonymised respiratory specimens surplus to clinical requirements from adult in-patients with suspected pneumonia at

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Table 1
Pathogens and resistance markers detected by Unyvero P50. Resistance markers considered during our analyses are in bold.

Gram-positive Bacteria	Gram-Negative Bacteria	Fungus	Resistance genes
Staphylococcus aureus *Streptococcus mitis* group	*Acinetobacter baumanii,* *Escherischia coli, Haemophilus influenzae, Klebsiella oxytoca,* *Klebsiella pneumoniae,* *Moraxella catarrhalis,* *Morganella morganii,* *Pseudomonas aeruginosa,* *Serratia marcescens, Stenotrophomonas maltophilia,* *Chlamydophila pneumoniae,* *Legionella pneumophila,* *Enterobacter* spp, *Proteus* spp	*Pneumocystis jirovecii*	**bla_{CTX-M}, bla_{DHA}, bla_{EBC},** $ermA$, $ermB$, $ermC$ **GyrA83, GyrA87, ParC** **bla_{KPC}, bla_{OXA-51}** bla_{TEM}, bla_{SHV}, $mefA$, $msrA$, **mecA**, $sul1$ $int1$

two tertiary care hospitals in London: the Royal Free (RFH) and University College London Hospitals (UCLH), from December 2014 to June 2015. Duplicate specimens from the same patient were excluded unless collected >6 days apart. Fresh specimens from patients with radiological confirmation of pneumonia were stored at 4 °C until processing (within 48 h). Curetis Unyvero P50 Pneumonia assay was run as per manufacturer's instructions with a turnaround time of approximately 5 h (30 min for mechanical and chemical sample lysis and homogenisation followed by 4 h 30 min for DNA purification, multiplex PCR and microarray detection). Detailed information of the system and method can be found on the manufacturer's website (www.curetis.com).

2.2. Routine clinical microbiology

Results were compared to those released by the routine clinical microbiology laboratories of the two participating hospitals. For the RFH, this comprised 1:1 v/v dilution with dithiothreitol, semiquantitative cultures onto three agar plates (Columbia Blood Agar (CBA), Colombia agar with chocolated horse blood (CHOC) and cystine lactose electrolyte deficient agar (CLED)); identification MALDI-TOF MS (Bruker Microflex™ LT) and antimicrobial susceptibility testing (AST) with the BD Phoenix system or by disc diffusion following EUCAST guidelines (Matuschek et al., 2014). For UCLH, undiluted specimens were cultured onto CBA, CHOC and CLED, organisms were identified using MALDI-TOF or the BioMerieux VITEK2 system and AST was performed using the VITEK 2 or BSAC (British Society for Antimicrobial Chemotherapy) standardised disc susceptibility testing.

Atypical species *Chlamydophila pneumoniae*, *Legionella pneumophila* and *Mycoplasma pneumoniae* are screened using an in house qPCR assay at RFH and by antigen testing or serology at UCLH. MycAssay® Pneumocystis (Myconostica) is used to detect *Pneumocystis jirovecii* at RFH, at UCLH it is detected by Grocott-Gomori's methenamine silver stain.

2.3. Comprehensive microbiological analysis

For a full comprehensive analysis, 42 specimens were chosen at random. A cross-sectional sweep of growth was taken from a fresh primary culture of the specimen on CHOC and stored in Microbank™ vials at −80 °C until analysis. Ten microliters of neat and a 10^{-5} dilution in saline solution were plated onto CHOC, CBA, Brilliance UTI agar (UTI) and Columbia colistin-nalidixic acid agar (C-CNA) (Oxoid). CBA, UTI and C-CNA plates were incubated at 37 °C in air for 18 h while CHOC plates were incubated in 5% CO_2 at 37 °C for 18 h. Representative bacterial colonies of different morphologies on each medium were identified using MALDI-TOF MS.

For bacterial isolates identified during the comprehensive microbiological analysis, susceptibility to beta-lactam antibiotics was evaluated using the disk diffusion method on Mueller-Hinton agar following EUCAST recommendations (Matuschek et al., 2014). The following antibacterial agents (Oxoid) were tested: Aztreonam (30 μg), Piperacillin-

tazobactam (10-6 μg), Ceftazidime (10 μg), Imipenem (10 μg), Meropenem (10 μg), Temocillin (30 μg) for Enterobacteriaceae, *Acinetobacter spp.* and *Pseudomonas spp.*; Ertapenem (10 μg), Ampicillin (10 μg), Amoxiclav (20-10 μg), Cefoxitin (30 μg), Cefotaxime (5 μg) were also tested for Enterobacteriaceae. Cefoxitin (30 μg) discs were used for identification of potential methicillin resistant *Staphylococcus aureus* (MRSA). Ciprofloxacin susceptibility testing was performed on *P. aeruginosa* and *Escherichia coli* using the gradient diffusion method (Etest®, Biomérieux), interpreted according to EUCAST guidelines (http://www.eucast.org/clinicalbreakpoints/). Both laboratories report predominant growth of potentially pathogenic species equivalent to 10^5 CFU/ml or above.

Double disc diffusion for detection of beta-lactamases was performed using ROSCO Diagnostica kits. KPC/metallo-beta-lactamase and OXA-48 Confirm Kit; KPC/MBL in *P. aeruginosa*/Acinetobacter and Total ESBL + AmpC Confirm kits were used according to manufacturer's instructions.

2.4. Sequence-based detection of resistance mechanisms

We extracted DNA from resistant bacteria using QIAmp DNA Mini Kit (Qiagen) following manufacturer's instructions. The Check-MDR CT103XL test (Checkpoints, NL) was used for molecular detection and identification of genes encoding carbapenemase, AmpC and ESBL enzymes according to manufacturer's instructions. All suspected ESBL, AmpC and carbapenemase positives were confirmed by PCR (HotStart Taq Mastermix, Qiagen). The presence of *mecA* among suspected MRSA and the quinolone resistance-determining regions (QRDR) of the *gyrA* and *parC* genes from fluoroquinolone resistant *E. coli* or *P. aeruginosa* were amplified by PCR. All PCR amplicons were sent for DNA sequencing using the Sanger method at Beckman Coulter Genomics and analysed using BioNumerics (Applied Maths) software and NCBI's BLAST. All primers used in this study are listed in Table S1.

2.5. Data analysis

The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and positive and negative likelihood ratios were calculated using MedCalc for Windows. Overall sensitivity and specificity were calculated considering a test result as true positive when both routine cultures reported an organism and Unyvero P50 identified the same organism, regardless of additional organisms that may have been identified by Unyvero P50. False positives were specimens where one or more organisms detected by Unyvero P50 were not found by routine microbiology. False negatives were specimens where routine microbiology detected an organism that the Unyvero P50 missed and true negatives were specimens where neither method reported significant organisms.

During analysis of resistance determinants, only genes considered potentially significant (Table 1) were included; *mecA* was only considered significant when detected simultaneously with *S. aureus*, in such