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The Unyvero P55 ‘sample-in, answer-out’ pneumonia assay: A performance evaluation

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ABSTRACT

Background: O’Neill’s recent Review on Antimicrobial Resistance expressed the view that by 2020 high-income countries should make it mandatory to support antimicrobial prescribing with rapid diagnostic evidence whenever possible.

Methods: Routine microbiology diagnosis of 95 respiratory specimens from patients with severe infection were compared with those generated by the Unyvero P55 test, which detects 20 pathogens and 19 antimicrobial resistance markers. Supplementary molecular testing for antimicrobial resistance genes, comprehensive culture methodology and 16S rRNA sequencing were performed.

Results: Unyvero P55 produced 85 valid results, 67% of which were concordant with those from the routine laboratory. Unyvero P55 identified more potential pathogens per specimen than routine culture (1.34 vs. 0.47 per specimen). Independent verification using 16S rRNA sequencing and culture (n = 10) corroborated 58% of additional detections compared to routine microbiology. Overall the average sensitivity for organism detection by Unyvero P55 was 88.8% and specificity was 94.9%. While Unyvero P55 detected more antimicrobial resistance markers than routine culture, some instances of phenotypic resistance were missed.

Conclusions: The Unyvero P55 is a rapid pathogen detection test for lower respiratory specimens, which identifies a larger number of pathogens than routine microbiology. The clinical significance of these additional organisms is yet to be determined. Further studies are required to determine the effect of the test in practise on antimicrobial prescribing and patient outcomes.

1. Introduction

We are entering a new era in the diagnosis of infectious diseases, where rapid, molecular-biology based methods sit alongside traditional culture based techniques. Fast and accurate diagnosis of infections is crucial if we are to achieve evidence-based antimicrobial prescribing and improved antimicrobial stewardship. The recent O’Neill Review on Antimicrobial resistance recommended that by 2020, antimicrobial prescriptions in high-income countries should be made only when supported by rapid diagnostic evidence, where such tests are available [1]. The past five years have seen an explosion in DNA-based tests for bacterial infections, many of which run on ‘sample-in, answer-out’ platforms requiring minimal hands on time and user training. While many such tests have been applied to the diagnosis of blood-stream

infections [2–4], a number of tests for other clinical syndromes such as respiratory tract infections [5] or gastrointestinal infections [6] also exist. Some manufacturers offer not only pathogen detection, but also detection of selected antimicrobial resistance determinants, such as *mecA* or carbapenemases [2,7]. These tests provide much faster turn around times than conventional methods and prompt possibilities for improved patient outcomes and better antimicrobial stewardship [8,9]. Many tests now detect not only bacterial pathogens but also selected antimicrobial resistance genes. However, studies designed to measure impact on such outcomes remain rare.

One such test is the recently introduced Curetis Unyvero P55 pneumonia cartridge [10]. Pneumonia is responsible for significant mortality and morbidity world-wide [11] and is the second most common cause of adult hospitalization in the US after child birth [12]

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Table 1

Pathogens and resistance markers detected by Unyvero P55. Resistance markers considered during our analyses are in bold.

Gram-positive Bacteria	Gram-Negative Bacteria	Fungus	Resistance genes
<i>Staphylococcus aureus</i>	<i>Acinetobacter baumannii</i> , <i>Escherichia coli</i> , <i>Haemophilus influenzae</i> , <i>Klebsiella oxytoca</i> , <i>Klebsiella pneumoniae</i> , <i>Klebsiella variicola</i> , <i>Moraxella catarrhalis</i> , <i>Morganella morganii</i> , <i>Pseudomonas aeruginosa</i> , <i>Serratia marcescens</i> , <i>Stenotrophomonas maltophilia</i> , <i>Legionella pneumophila</i> , <i>Mycoplasma pneumoniae</i> , <i>Enterobacter cloacae</i> complex, <i>Enterobacter aerogenes</i> , <i>Proteus</i> spp.	<i>Pneumocystis jirovecii</i>	<i>bla</i> _{CTX-M} , <i>ermB</i> , <i>GyrA83</i> , <i>GyrA87</i> , <i>bla</i> _{KPC} , <i>bla</i> _{oxa-23} , <i>bla</i> _{oxa-24} , <i>bla</i> _{oxa-48} , <i>bla</i> _{oxa-58} , <i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>mecA</i> , <i>mecC</i> <i>sul1</i>
<i>Streptococcus pneumoniae</i>	<i>Citrobacter freundii</i>		<i>bla</i> _{IMP} , <i>bla</i> _{VIM} , <i>bla</i> _{NDM}

as well as a leading indication for antimicrobial prescriptions [13]. It can be either community-acquired (CAP) or hospital-acquired (HAP) with a subset of HAP patients suffering from ventilator-associated pneumonia (VAP). The P55 test uses multiplex PCR and microarray detection to identify 20 causative agents of severe lower respiratory tract infections (LRTIs) and 19 antibiotic resistance determinants straight from clinical specimens (Table 1). The turn-around time is around 5 h, with minimal hands on time (approx. 2 min).

Here, we evaluated the performance of the Unyvero P55 test on 95 specimens from hospitalized patients with severe LRTIs and compared the results to routine microbiology. Verification of a subset of results was carried out using 16S rRNA sequencing and a more comprehensive culture based method.

2. Materials and methods

2.1. Ethics

We adhered to a Governance framework with an overarching ethics agreement for the UCL Infection DNA Bank (Reference: 12/LO/1089), relating to the use of patient specimens surplus to clinical needs and anonymised patient data.

2.2. Specimen collection and analysis

We collected anonymised respiratory specimens surplus to clinical requirements from adult in-patients with pneumonia at the Royal Free hospital (RFH) and University College London Hospital (UCLH), from June to September 2015. Duplicate specimens from the same patient were excluded unless collected > 6 days apart or different aetiology was found [14]. Fresh specimens (< 48 h old) with radiological confirmation of pneumonia, were processed with the Unyvero P55 Pneumonia assay as per manufacturer's instructions. Briefly, 180 µL of specimen are placed in the sample tube and inserted into the Lysator instrument, which performs mechanical and chemical sample lysis and homogenisation in approximately 30 min. The sample tube is then transferred into a test cartridge, which is inserted into the Analyzer module, which performs DNA purification, multiplex PCR and microarray detection of amplicons. Results are available in approximately 4h30 min. Detailed information of the system and method can be found on the manufacturer's website [10].

2.3. Microbiological analysis

Results were compared to those obtained by the routine clinical microbiology laboratories of the two participating hospitals as previously described [15]. A more comprehensive analysis, as described previously [15], was also performed in order to verify the presence of resistance genes and study discrepancies between routine culture and Unyvero P55. Briefly, a sweep of growth was taken from the primary culture of the specimen on chocolate agar (CHOC), stored in Microbank™ vials at -80 °C, and both 10 µL of the undiluted broth medium and a 10⁻⁵ dilution in saline solution, were cultured onto CHOC, Columbia Blood agar, Brilliance UTI agar and Columbia colistin-nalidixic

acid agar (Oxoid). All colony types of different morphologies were identified and analysed using MALDI-TOF (Biotyper version 3.1 software).

Susceptibility to beta-lactam antibiotics was evaluated using the disc diffusion method on Mueller-Hinton agar following EUCAST recommendations [16]. Cefoxitin (30 µg) discs were used for identification of potential methicillin resistant *S. aureus* (MRSA). Ciprofloxacin susceptibility testing was performed on *P. aeruginosa* and *E. coli*, using the gradient diffusion method (Etest®, Biomérieux), interpreted according to EUCAST guidelines. Double disc diffusion for detection of beta-lactamases was performed using ROSCO Diagnostica kits according to manufacturer's instructions.

2.4. 16S rRNA sequencing

For nucleic acid extractions, 400 µL of specimen were heat killed at 95 °C for 30 min, 300 µL were transferred to a GeneOhm™ Lysis bead tube (BD) and bead beaten in a Biospec Bead Beater at 3500 oscillations per min for 30s. Nucleic acids were extracted from 200 µL of recovered supernatant using ZR Viral RNA/DNA kit (Zymo research) following manufacturer's instructions, and eluted in 50 µL of sterile water.

Nucleic acid extracts were quantified using a Qubit 2.0 fluorometer (ThermoFisher Scientific, US) and diluted to 20 µg/L. Samples were amplified using LightCycler 480 ProbesMaster (Roche) PCR mastermix with forward (5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGC CTACGGGNGGCWGCAG 3') and reverse (5' GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC 3') primers (Sigma-Aldrich) designed to amplify hypervariable regions V3 and V4 of the bacterial 16S gene while also adding sequencing adapters.

The PCR products were cleaned as per manufacturer's instructions using Agencourt AMPure XP beads (Beckman Coulter) and then underwent further limited PCR (8 cycles) to attach Illumina sequencing adapters and Nextera Indexing before a final clean up step. The final PCR products underwent further analysis using 2200 TapeStation bioanalyser (Agilent Technologies, US) to assess amplicon purity and size (approx. 630 bp) and were quantified using Qubit 2.0 fluorometer. The molar concentration of each sample was calculated and a 4 nM final concentration was used.

Sequencing was performed using the Illumina MiSeq Platform according to manufacturer's instructions (Illumina, US) [17] and output was analysed using the 16S rRNA sequencing analysis pipeline in BaseSpace (Illumina, US). Identified organisms were reported to the genus level and those that contributed to > 0.5% of the total number of matches were considered in the analysis.

2.5. Molecular detection of resistance mechanisms

For molecular detection of resistance genes as described previously [15], DNA extraction of resistant bacteria was performed using QIAamp DNA Mini Kit (Qiagen) following manufacturer's instructions. Isolates with suspected presence of beta-lactamases were tested using the Check-MDR CT103XL test (Checkpoints, NL) for molecular detection and identification of genes encoding carbapenemase, AmpC and ESBL enzymes. All suspected ESBL and carbapenemase positive isolates were

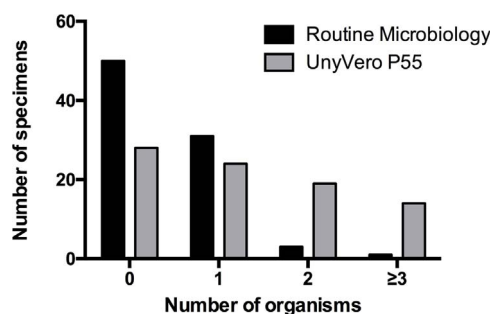


Fig. 1. Distribution of the number of micro-organisms detected per specimen.

confirmed by PCR (HotStar 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 [15].

3. Results

A total of 95 respiratory clinical specimens from hospital inpatients with pneumonia were collected from June to September 2015 from two tertiary hospitals in London. Specimens were tested using the Unyvero P55 Pneumonia assay (Unyvero P55) and results were compared to routine clinical microbiology tests. Ten specimens did not generate a valid result and were excluded from further analysis; 9 were due to test failures (1 machine failure and 8 partial system failures where one or more chambers failed) and 1 where there was a problem with the mastermix. The remaining 85 specimens consisted of 52 sputa, 31 endotracheal tubes (ETT) aspirates and 1 bronchoalveolar lavage (BAL); the vast majority of the specimens came from patients with HAP

(n = 44), while 14 and 27 specimens were from VAP and CAP patients respectively.

3.1. Pathogen detection

The mean turnaround time from specimens received to results from the routine laboratory was 53 h (range 21h–166 h). One or more significant organisms were reported by routine microbiology for 35 specimens (41%) while 'normal respiratory flora' (NRF), 'non-significant growth' (NSG) or 'mixed growth of doubtful significance' (MGODS) were reported for 47 specimens (55%), and 3 specimens produced no growth. On the other hand, Unyvero P55 identified at least one pathogen in 57 specimens (67%) and was negative for 28 specimens including the 3 that produced no growth (Fig. 1). Overall, routine microbiology reported 0.47 significant organisms/specimen whereas Unyvero P55 detected 1.34. The most common organisms reported by the culture laboratory were *P. aeruginosa* (n = 11), *H. influenzae* (n = 7) and *S. maltophilia* (n = 5) whereas the most common organisms detected by Unyvero P55 were *P. aeruginosa* (n = 23), *S. maltophilia* (n = 20), and *E. coli* (n = 15) (Table 2).

Results from Unyvero P55 and standard microbiology culture were concordant in 57 specimens (67%) (Fig. S1). Of these, Unyvero P55 identified the same organism (s) as routine culture in 12 specimens, and the same pathogen and at least one additional organism in 18 specimens. Negative results given by both tests were concordant in 27 specimens. Discordant occurred in the remaining 28 specimens, including 24 reported as NRF, NSG or MGODS by the microbiology laboratory where Unyvero P55 detected specific organisms. Unyvero P55 produced four false-negatives, specifically *S. pneumoniae* in 2 cases, *H. influenzae* in one, and a further single specimen containing *S. maltophilia* by culture but reported by Unyvero P55 as *E. coli*. Approximately 55% of samples were reported to contain normal respiratory flora by the clinical laboratory whereas Unyvero P55 reported an organism in the majority of samples, leading to 'false positive' results. We calculated overall average sensitivity for Unyvero P55 as 88.8% and overall average specificity as 94.9% (Table 2).

Table 2

Frequency of organisms detected by routine microbiology and Unyvero P50 (n = 85 specimens). Negative specimens include those classified by routine microbiology as NRF, NSG, MGODS or no growth.

Group	Target Organism	Routine laboratory	UnyVero P55	True Positive (Routine and Unyvero P55)	False Positive (Unyvero P55 only)	False Negative (Routine only)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	
Gram-positive	<i>S. aureus</i>	4	9	4	5	0	100.0	93.8	44.4	100.0	
	<i>S. pneumoniae</i>	3	3	1	2	2	33.3	97.6	33.3	97.6	
Non-fermenters	<i>A. baumannii</i>	0	3	0	3	0	–	96.5	–	100.0	
	<i>P. aeruginosa</i>	11	23	11	12	0	100.0	83.8	47.8	100.0	
	<i>H. influenzae</i>	7	12	6	6	1	85.7	92.3	50.0	98.6	
	<i>M. catarrhalis</i>	1	3	1	2	0	100.0	97.6	33.3	100.0	
	<i>S. maltophilia</i>	5	20	4	16	1	80.0	80.0	20.0	98.5	
	<i>E. cloacae</i>	0	4	0	4	0	–	95.3	–	100.0	
Enterobacteriaceae	complex										
	<i>E. aerogenes</i>	0	1	0	1	0	–	98.8	–	100.0	
	<i>E. coli</i>	3	15	3	12	0	100.0	85.4	20.0	100.0	
	<i>K. pneumoniae</i>	3	7	3	4	0	100.0	95.1	42.9	100.0	
	<i>K. oxytoca</i>	0	2	0	2	0	–	97.6	–	100.0	
	<i>K. variicola</i>	0	0	0	0	0	–	100.0	–	100.0	
	<i>M. morgani</i>	0	0	0	0	0	–	100.0	–	100.0	
	<i>Proteus spp</i>	0	2	0	2	0	–	97.6	–	100.0	
	<i>S. marcescens</i>	1	7	1	6	0	100.0	92.9	14.3	100.0	
	<i>C. koseri</i>	1	N/A	N/A	N/A	N/A	N/A	N/A	–	–	
	<i>C. freundii</i>	0	1	0	1	0	–	98.8	–	100.0	
	Atypical bacteria	<i>L. pneumophila</i>	0	0	0	0	0	–	100.0	–	100.0
		<i>M. pneumoniae</i>	0	0	0	0	0	–	100.0	–	100.0
Fungi	<i>C. albicans</i>	1	N/A	N/A	N/A	N/A	N/A	N/A	–	–	
	<i>P. jirovecii</i>	0	2	0	2	0	–	–	–	100.0	
	Negative	50	28								
Overall (average)							88.8	94.9			

3.2. Detection of antimicrobial resistance

The routine clinical microbiology laboratories reported a total of 39 organisms (Table S2), 31% of these were fully susceptible while 69% were resistant to one or more antimicrobial classes and 38% multi-resistant (MDR) (defined as resistance to ≥ 3 antimicrobials of different classes) [18]. The Unyvero P55 test is capable of detecting 17 antibiotic resistance markers with many of them highly prevalent among both pathogenic and commensal bacterial populations¹⁹ resulting in 72% of specimens reported to contain at least one resistance marker, including 14 specimens where no organism was detected.

We therefore restricted our analysis to ESBLs, carbapenemases, presumptive MRSA, and fluoroquinolone resistance (FQ^R) among *E. coli* and *P. aeruginosa* only (Table 3), as these are clinically significant regardless of host (ESBLs and carbapenemases) or can be attributed to a particular organism with reasonable reliability (MRSA and FQ^R). Therefore, *bla*_{TEM}, *bla*_{SHV}, *ermB* and *sulI* were excluded from further analysis. Unyvero P55 identified 18 occurrences of relevant resistance markers (resistance marker associated with a relevant organism), whilst routine microbiology identified relevant resistance phenotypes in 10 isolates (Table 3).

Four *P. aeruginosa* isolates resistant to carbapenems were not detected by Unyvero P55. The routine laboratory reported fluoroquinolone resistance for one *E. coli* and three *P. aeruginosa* isolates whereas Unyvero P55 identified 5 *E. coli* and 7 *P. aeruginosa* with *gyrA* mutations. Supplementary analysis of 3 *E. coli* isolates and 4 *P. aeruginosa* isolates (some of which were retrieved using the comprehensive culture method) confirmed the presence of fluoroquinolone resistance and *gyrA* mutations in all but one of the *P. aeruginosa* studied. *parC* mutations were also identified by PCR in the three *E. coli* and two *P. aeruginosa* isolates (Table 3) The remaining 2 *E. coli* and 3 *P. aeruginosa* could not be retrieved for further study. Four putative MRSA positive specimens (*S. aureus* + *mecA*) were detected by Unyvero P55, two of which were reported as MSSA by the routine laboratory, 1 as *K. pneumoniae* and 1 as NRF. During further analyses, *S. aureus* isolates were recovered from all four specimens but lacked the MRSA phenotype and were PCR negative for *mecA*. Unyvero P55 identified two specimens with *bla*_{CTX-M}, one specimen containing *K. pneumoniae* (confirmed as *bla*_{CTX-M-15} positive using Checkpoints) and one specimen containing *E. coli* that routine microbiology reported as containing *S. maltophilia*. A further *bla*_{CTX-M} producing *E. coli* isolate identified by routine microbiology and confirmed by Checkpoints microarray was not detected by Unyvero P55.

3.3. Additional analysis by 16S rRNA sequencing and comprehensive culture

We used additional methods in an attempt to resolve discordance between Unyvero P55 and results from routine microbiology. 16S rRNA (V3-V4) sequencing direct from sputum specimens was performed on 14 randomly selected specimens to study the diversity and relative abundance of bacteria within the specimens (Table 4, Fig. S2). Those where a single pathogen was reported by routine culture tended to have single dominant pathogen detected by 16S rRNA sequencing. Those reported as normal flora by routine culture consisted of a diverse mixture of bacteria according to the sequencing data as expected. This

suggested broad agreement of NGS with routine culture rather than Unyvero P55. Ten of the 14 also underwent comprehensive culture analysis whereby all organisms growing on the primary chocolate agar plate were systematically catalogued and identified. Considering these 10 specimens, routine culture detected 5 pathogens, Unyvero P55 detected 17 and 16S and comprehensive culture detected 10 each. Five pathogens detected by Unyvero P55 were not confirmed by any other method. They included three instances of *S. maltophilia* detection, one *A. baumannii* complex and one *S. marcescens*.

4. Discussion

The laboratory diagnosis of lower respiratory tract infections is primarily culture-based, producing slow and possibly insensitive results. This is the first study to assess the performance and potential clinical utility of the Curetis Unyvero P55 Pneumonia test, a point-of-care sample-in answer-out test, with a turnaround time of 5 h. This is significantly faster than the average turn around time by the routine laboratories (53 h in this study).

The Unyvero P55 test replaces the previously evaluated P50 assay [15], with a revised panel and improved performance for some individual assays. The composition of the resistance panel for P55 is in our opinion much improved compared to P50, making interpretation of results simpler. However, we observed a relatively high failure rate (10.5%), with one total machine failure, 8 partial failures (i.e. when 1 or more chambers fail) and 1 reagent failure. This is similar to the failure rate of 12.6% observed in our hands for the P50 test [15] and thus remains an area for the manufacturer to improve upon.

Sensitivity of pathogen detection was good, with all but 4 of the pathogens reported by routine microbiology detected by P55. The number of positive samples was too low to enable us to comment on the performance of individual assays. As expected of a molecular test, the P55 had considerably more pathogen detection calls than routine culture, both in specimens reported as normal flora only and specimens containing organisms considered significant. Interestingly, two of the additional detections were *P. jirovecii*, a recognized pathogen not routinely tested unless specifically requested by the clinician. However, the clinical relevance of these results remains to be determined as these infected patients have an atypical presentation being able to produce sputum [20]. *P. jirovecii*'s potential for causing disease in the immunosuppressed is clear; this organism, however, is also known to be present in lower numbers as a coloniser immunocompetent individuals, where it causes no disease whatsoever [21]. In both cases where this result was obtained, further investigation revealed immunosuppression in the patients concerned but no clear indications for requesting a *P. jirovecii* test.

Additional detection of organisms can be expected for a PCR amplification based test when applied to a clinical specimen from a non-sterile site such as the respiratory tract or the GI tract, and has been reported in other studies. [6,22] However, it can be difficult to determine whether additional organisms detected by molecular assays are significant or not. We performed further tests on a small number randomly selected specimens in an attempt to determine their true microbiological composition and verify whether additional Unyvero P55 detections resulted from a genuine presence of these organisms. We tested for both viable organisms (comprehensive culture) and presence

Table 3

Number of potentially significant resistance mechanisms detected by routine microbiology versus Unyvero P55.

	ESBL producer	MRSA	Fluoroquinolone resistance	Carbapenem resistance
Routine Microbiology + Checkpoints/PCR	n = 2 <i>bla</i> _{CTX-M}	none detected	n = 4 1 x <i>E. coli</i> , 3 x <i>P. aeruginosa</i>	n = 4 4 x <i>P. aeruginosa</i> , no enzyme detected
Unyvero P55	n = 2 <i>bla</i> _{CTX-M}	n = 4 ^a (<i>mecA</i> + <i>S. aureus</i>)	n = 12 7 x <i>P. aeruginosa</i> 5 x <i>E. coli</i>	n = 0
Concordance	1/2	0/4	3/12	no

^a We presumed potential presence of MRSA when both *S. aureus* and *mecA* or *mecC* were detected in the specimen.

Table 4

Comparison of Unyvero P55 results of 14 random specimens with routine culture, 16S rRNA sequencing and comprehensive culture. For 16S rRNA sequencing results, pathogenic genera accounting for more than 0.5% of individual reads are reported individually, for commensals, genera accounting for more than 10% of reads are reported. Unyvero P55 results + / + + / + + + readings by the instrument, which give an indication of quantity, are reported. ETT – endotracheal tube aspirate; SPU – sputum.

Specimen number & type	Routine Culture Result	Unyvero P55 Result	Comprehensive Culture	16S rRNA Sequencing Results
346 ETT	<i>P. aeruginosa</i>	<i>P. aeruginosa</i> (+ + +) <i>S. marcescens</i> (+ + +) <i>S. aureus</i> (+ +) <i>S. maltophilia</i> (+ +)	<i>P. aeruginosa</i> <i>S. marcescens</i> <i>S. aureus</i>	<i>Pseudomonas</i> spp. 38.4% <i>Streptococcus</i> spp. (not including <i>S. pneumoniae</i>) 17.1% <i>Neisseria</i> spp. 16.4% <i>Serratia</i> spp. 12.5% <i>Stenotrophomonas</i> spp. 1.6% Others 14.0%
347 ETT	<i>P. aeruginosa</i>	<i>P. aeruginosa</i> (+ + +)	<i>P. aeruginosa</i>	<i>Pseudomonas</i> spp. 79.6% Others 20.4%
348 SPU	Non significant growth <i>K. pneumoniae</i> (+) <i>M. catarrhalis</i> (+) <i>Proteus</i> spp. (+)	<i>E. coli</i> (+ + +) <i>P. aeruginosa</i> (+ +)	Not tested	<i>Streptococcus</i> spp. (not including <i>S. pneumoniae</i>) 27.7% <i>Lactobacillus</i> spp. 20.1% <i>Pseudomonas</i> spp. 5.1% <i>Escherichia</i> spp. 1.3% Others 45.8%
349 SPU	No growth	Negative	Not tested	Commensal genera only
350 SPU	Normal respiratory flora	<i>A. baumannii</i> complex (+)	<i>Bacillus cereus</i> <i>Rothia muciliginosa</i>	<i>Streptococcus</i> spp. (not including <i>S. pneumoniae</i>) 50.0% <i>Rothia</i> spp. 13.7% Others 36.3%
351 ETT	Normal respiratory flora	Negative	Negative	<i>Pseudomonas</i> sp. 0.81% Mixed commensal genera
353 ETT	<i>S. maltophilia</i>	<i>S. maltophilia</i> (+ + +) <i>E. coli</i> (+ + +) <i>H. influenzae</i> (+)	Not tested	<i>Stenotrophomonas</i> spp. 95.7% <i>Pseudomonas</i> sp. 0.71% Others 4.3%
355 ETT	Normal respiratory flora	Negative	<i>S. haemolyticus</i>	<i>Neisseria</i> spp. 22.5% <i>Prevotella</i> spp. 13.5% <i>Aggregatibacter</i> spp. 11.6% <i>Haemophilus</i> spp. 11.0% Others 41.4%
357 SPU	<i>K. pneumoniae</i>	<i>K. pneumoniae</i> (+ + +)	Not tested	<i>Klebsiella</i> spp. 57.5% <i>Veionella</i> spp. 13.6% Others 28.9%
358 ETT	<i>P. aeruginosa</i>	<i>P. aeruginosa</i> (+ +) <i>S. maltophilia</i> (+ +)	<i>P. aeruginosa</i>	<i>Pseudomonas</i> spp. 77.2% <i>Stenotrophomonas</i> spp. 0.6% Others 22.8%
360 ETT	Normal respiratory flora	<i>S. maltophilia</i> (+ +)	<i>S. maltophilia</i>	<i>Acinetobacter</i> spp. 3.4% <i>Pseudomonas</i> spp. 1.2% Mixed commensal genera
374 ETT	Normal respiratory flora	<i>S. aureus</i> (+ + +) <i>S. maltophilia</i> (+ +)	<i>S. aureus</i>	<i>Staphylococcus</i> spp. 35.5% <i>Lactobacillus</i> spp. 43.8% Others 20.7%
376 SPU	<i>P. aeruginosa</i>	<i>P. aeruginosa</i> (+ +) <i>S. maltophilia</i> (+ +)	<i>P. aeruginosa</i>	<i>Pseudomonas</i> spp. 77.5% <i>Stenotrophomonas</i> spp. 1.6% Others 20.9%
377 ETT	<i>P. aeruginosa</i>	<i>P. aeruginosa</i> (+ + +) <i>S. maltophilia</i> (+ +) <i>S. marcescens</i> (+ +) <i>S. aureus</i> (+)	<i>P. aeruginosa</i> <i>S. aureus</i> <i>E. faecium</i>	<i>Pseudomonas</i> spp. 38.7% <i>Streptococcus</i> spp. (not including <i>S. pneumoniae</i>) 10.8% <i>Acinetobacter</i> spp. 1.0% Others 49.5%

of DNA (16S rRNA sequencing), and were able to corroborate 58% of additional detections compared to routine culture. On the whole, both 16S rRNA sequencing results and comprehensive culture results more closely resembled routine microbiology results than those produced by Unyvero P55. Three of the five unconfirmed detections were *S. maltophilia*. Indeed, among all specimens, Unyvero P55 detected 20 instances of *S. maltophilia*, while the routine laboratory reported only 5, suggesting an issue with the specificity of this assay. These results are preliminary but suggest that 16S rRNA sequencing is a good supplementary molecular technique for resolving discrepancies between sputum culture and molecular results and may also prove to be a good test for the diagnosis of LRTIs in its own right.

The ability of the Unyvero P55 test to detect antimicrobial resistance was variable. For ease of interpretation and clinical significance, four of the markers on the P55 panel were excluded from analysis, as they are ubiquitous among both pathogens and commensal flora of the respiratory tract [19]. For ESBLs, Unyvero P55 detected two instances of the *bla*_{CTX-M}, one of which was missed by routine culture. Routine microbiology on the other hand detected another *bla*_{CTX-M} that was not picked up by Unyvero P55. Four specimens contained *mecA* and

S. aureus which suggested presence of MRSA but all were confirmed as MSSA; as the *mecA* assay of Unyvero P55 is not species specific, it is likely that detected *mecA* originated from coagulase-negative staphylococci instead [23]. Therefore this assay should not be used for a definitive diagnosis but may be a useful tool for identifying patients who should undergo an MRSA screen. Unyvero P55 reliably detected fluoroquinolone resistance in *E. coli* and *P. aeruginosa*. Routine culture identified four fluoroquinolone resistant organisms, three of which were confirmed by Unyvero P55, which in addition identified a further 9 instances of FQ^R in specimens where significant *E. coli* or *P. aeruginosa* were not identified by routine culture. No carbapenemases were detected in this set of specimens using either method; however, routine culture identified four carbapenem-resistant *P. aeruginosa*, which did not produce carbapenemase. It is likely that resistance in these isolates was porin and/or efflux related, frequent resistance mechanisms in this species [24]. To the best of our knowledge, rapid molecular tests to detect such mutations are not currently available. This prompts reason for caution for interpreting molecular resistance results for *P. aeruginosa*, as lack of carbapenemase genes does not imply phenotypic susceptibility.

5. Conclusions

It is usually recognized that up to 70% culture tests fail to identify an aetiological agent due to existence of unknown pathogens, lack of accuracy and sensitivity in testing methods and failure of organisms to recover from antimicrobial therapy [25,26]. Our results showed that a causative agent was identified by routine microbiology in 41% of the specimens as opposed to more sensitive molecular tests such as Unyvero P55 where 67% were had an organism identified. This by definition challenges the concept of a culture-based “gold standard” by which any alternative detection technology might be judged. On the whole the test detects pathogens in a much larger proportion of specimens than routine culture, however, the clinical significance of these findings remains to be determined. Confirmatory testing failed to support the presence some additional organisms, pointing to possible specificity errors. Interpretation of antimicrobial resistance genes detection is complicated by uncertainty surrounding the host organism of the resistance genes, and discrepancies between phenotypic and genotypic resistance. This brief laboratory evaluation suggests larger evaluation studies are needed, incorporating systematic additional verification tests such as 16S rRNA sequencing in order to control for the imperfect gold standard posed by routine microbiology. If promising, such studies should be followed with randomized controlled clinical trials to assess the impact of Unyvero P55 on antimicrobial prescribing and patient outcomes.

Conflicts of interest

None to declare.

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