Rapid syndromic molecular testing in Pneumonia: the current landscape and future potential

S. Poole, T.W. Clark

PII: S0163-4453(19)30372-X
DOI: https://doi.org/10.1016/j.jinf.2019.11.021
Reference: YJINF 4393

To appear in: Journal of Infection

Accepted date: 29 November 2019

Please cite this article as: S. Poole, T.W. Clark, Rapid syndromic molecular testing in Pneumonia: the current landscape and future potential, Journal of Infection (2019), doi: https://doi.org/10.1016/j.jinf.2019.11.021

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2019 Published by Elsevier Ltd on behalf of The British Infection Association.
Highlights

- Syndromic molecular tests for pneumonia have greater sensitivity and detect many more pathogens than conventional culture
- The greatest potential benefit of these tests is rapidly directed antibiotic use
- Currently there are two commercially available syndromic molecular tests for pneumonia
- No interventional trials have assessed the clinical impact of these tests and are urgently needed

Rapid syndromic molecular testing in Pneumonia: the current landscape and future potential
Poole S 1*, Clark TW 2

1 Research Fellow in Infectious Diseases, NIHR Southampton Biomedical Research Centre, University Hospital Southampton NHS Foundation Trust Southampton, UK
2 Associate Professor in Infectious Diseases, School of Clinical and Experimental Sciences, University of Southampton and NIHR Southampton Biomedical Research Centre, University Hospital Southampton NHS Foundation Trust, Southampton, UK

*Corresponding author
Address: LF100, Faculty of medicine, Mailpoint 801, South Academic Block, Southampton General Hospital, Tremona Road, Southampton, SO16 6YD, United Kingdom
Tel: +44 (0) 2381208209
E-mail address: S.Poole@soton.ac.uk

Keywords
Pneumonia, Hospital acquired pneumonia (HAP), ventilator associated pneumonia (VAP), community acquired pneumonia (CAP), molecular diagnostics, rapid diagnostics, point-of-care testing.

Abstract

Rapid syndromic molecular tests for pneumonia
Community acquired pneumonia (CAP), hospital-acquired pneumonia (HAP) and ventilator associated pneumonia (VAP) are all associated with significant mortality and cause huge expense to health care services around the world. Early, appropriate antimicrobial therapy is crucial for effective treatment. Syndromic diagnostic testing using novel, rapid multiplexed molecular platforms represents a new opportunity for rapidly targeted antimicrobial therapy to improve patient outcomes and facilitate antibiotic stewardship. In this article we review the currently available testing platforms and discuss the potential benefits and pitfalls of rapid testing in pneumonia.

Introduction

Lower respiratory tract infections were accountable for an estimated 2.7 million deaths in 2015, making them the third most common cause of death worldwide. Community acquired pneumonia (CAP) caused nearly 30,000 deaths in England and Wales in 2015 and costs Europe around €10 million annually. It is estimated that 25 per 10,000 adults are hospitalised with pneumonia each year.

Hospital acquired pneumonia (HAP) is defined as occurring >48 hours after admission to a healthcare facility. It is caused by a different spectrum of more antibiotic resistant bacterial pathogens than those occurring in the community. Ventilator associated pneumonia (VAP) is defined as occurring >48 hours after intubation for invasive artificial ventilation. The two entities combined (HAP and VAP) are the most common nosocomial infection in the developed world with HAP complicating around 2% of hospital admissions.

The incidence of VAP in intubated patients is around 10% and is associated with mortality of around 10%. A retrospective matched cohort study by Kollef et al found patients who developed VAP were intubated for longer, spent longer on ICU, and were in hospital for a greater period of time. They estimated the additional cost of VAP from to be $40,000 per patient.

Large amounts of empirical ‘broad spectrum’ antibiotics are used to treat pneumonia which inadvertently promote antimicrobial resistance (AMR): a problem identified by the WHO as one of
the leading threats to global health today. The O’Neill report, commissioned by the UK government in 2014, has highlighted the need for developed nations to take a lead in tackling AMR. As part of this there is a specific recommendation that all antibiotic prescriptions should be supported by diagnostic tests where available by 2020\textsuperscript{10}. The UK government recently published a five-year action plan for tackling AMR, which emphasised the need for improved diagnostics to support antibiotic prescribing. This included a target to be able to report the percentage of antimicrobial prescriptions which are supported by a diagnostic test or decision making tool by 2024\textsuperscript{11}.

Timely administration of appropriate antibiotics is a central tenant of care for patients with pneumonia\textsuperscript{12,13} and yet the gold-standard for microbiological diagnosis remains traditional, slow, culture based methods. These take greater than 24 hours to identify an organism and often greater than 72 hours to provide phenotypic antibiotic sensitivity data. Culture is insensitive, only detecting a pathogen in 23-40\% of patients with clinically diagnosed pneumonia\textsuperscript{4,14–16} and an even smaller proportion after the administration of antibiotics.

In recent years several rapid syndromic molecular tests for pneumonia have been developed. These offer the potential to revolutionise treatment by providing information to clinicians in ‘real-time’ on the pathogens present and their likely antibiotic sensitivity by also detecting genotypic markers of resistance. Multiple studies have demonstrated the superior diagnostic accuracy of PCR based platforms for detecting bacterial pathogens in the sputum compared with standard culture\textsuperscript{16–19}. This review will discuss the commercially available syndromic molecular panels for pneumonia, their potential clinical impact and the challenges to implementing them as a ‘front line’ diagnostic test.

**Potential clinical impact of rapid pathogen detection in pneumonia**

**Directed antibiotic use**

The greatest potential clinical benefit of a rapid syndromic test for pneumonia is being able to better utilise antibiotics. The superior diagnostic yield of multiplex PCR means that a pathogen is detected
rapidly in a much greater proportion of patients, so therapy can be quickly tailored to the responsible organism. In some situations, this will allow narrowing of antimicrobial therapy: for example, identification of *Streptococcus pneumoniae* facilitating a change of antibiotics to penicillin, in geographical areas with a low prevalence of penicillin resistant *S. pneumoniae*. In other cases, it may facilitate a change or escalation of antimicrobial therapy: for example, the identification of methicillin resistant *Staphylococcus aureus* (MRSA) which would not be covered by empirical regimens in many areas. The absence of detection is also helpful: the sensitivity when compared to culture of molecular assays is very high so can reassure clinicians that organisms are not present and so support decisions to stop unnecessary antibiotics or to deescalate antibiotics that were used empirically to cover organisms subsequently not detected.

The impact of this improved use of antibiotics are twofold: firstly, earlier appropriate antibiotics should improve clinical outcomes including mortality and length of stay. Secondly, it prevents unnecessary broad-spectrum antibiotic use, which facilitates antibiotic stewardship and reduces antibiotic related adverse events.

The aetiology of CAP and HAP/VAP are highly variable between different regions and times, and this is reflected in studies of causative microbial agents as identified by culture. Patients with underlying lung diseases, for example chronic obstructive pulmonary disease, can be colonised with microbial flora which are more typical pathogens of HAP. As a result, they may develop community acquired infections caused by these agents.

*S. pneumoniae, Haemophilus influenzae, S. aureus, Moraxella catarrhalis* and ‘atypical’ organisms including *Mycoplasma pneumoniae* and *Legionella pneumophila* are all cultured from the sputum of patients with CAP. Many of these organisms have predictable resistance patterns when interpreted with local epidemiological data. Gadsby et al developed and internally validated their own syndromic molecular assay for pneumonia. They used this to test sputum samples of 323 adults admitted to hospital with CAP. Their assay detected a pathogen in 87% of patients (as opposed to
39% of patients using only routine culture). As a result, they proposed that 77% of antibiotic prescriptions in CAP could have been de-escalated based on results from multiplex PCR testing. The majority of these potential interventions involved stopping clarithromycin when atypical organisms were not detected or ‘narrowing’ antibiotics when a likely sensitive pathogen had been detected.

In HAP and VAP, frequently cultured bacterial pathogens include *S. aureus*, *Pseudomonas aeruginosa*, *Klebsiella* species, *Escherichia coli*, *Acinetobacter* species and *Enterobacter* species\(^{20}\). Empirical regimens are therefore broad spectrum and large numbers of antibiotics are consumed. The absence of certain organisms (for example *P. aeruginosa*) could facilitate a narrowing of the antimicrobial spectrum with a knock-on effect of reducing antibiotic related adverse effects and improving stewardship. Furthermore, common Gram negative isolates are increasingly resistant in pneumonia surveillance studies\(^{21}\). Rapid molecular detection of these resistance genes should facilitate earlier initiation of effective antibiotics and this should lead to better outcomes.

**Treatment of other infective agents**

In adults, respiratory viruses are found in approximately one third of community acquired pneumonia cases\(^4,22\). One study found that 36% of patients admitted to intensive care with pneumonia were positive for a respiratory virus, with a broad range of viruses detected\(^23\). Detection of certain viruses such as influenza and adenovirus which are known to cause pneumonia, coupled with the absence of detection of bacteria and low levels of serum biomarkers such as procalcitonin (which is elevated in patients with bacterial infection), could support decisions to stop or use an abbreviated course of antibiotics. The ResPOC trial was a pragmatic randomised controlled trial that tested patients with community acquired acute respiratory illness using the BioFire Respiratory Panel (which tests comprehensively for respiratory viruses and atypical bacteria) at the point-of-care. It found that patients who were tested with the FilmArray were significantly more likely to receive a single dose or shorter course of antibiotics\(^24\) than those who were not. It also found a
significant reduction in length of hospital stay in the intervention group along with improved use of neuraminidase inhibitors (NAI) in patients with influenza.

Currently there are no licenced antiviral agents for respiratory viruses other than influenza. The benefit from NAI treatment is greatest when they are started within 48 hours of symptom onset but there is evidence in adults to suggest ongoing benefit when started beyond this time\textsuperscript{25} and a recent study suggests that treatment earlier in admission to hospital improves outcome irrespective of overall duration of illness\textsuperscript{26}. As such, timely identification and treatment is critical. Antiviral treatments for other respiratory viruses, including respiratory syncytial virus (RSV) are in development.

**Infection control and public health**

Since the 1990s infection control methods including patient source isolation and deep cleaning with targeted decolonisation have been highly successful at reducing the spread of MRSA. Enhanced infection control practices are recommended for a number of pathogens that may be present in patients with pneumonia. Early identification of these should reduce the spread of these organisms, especially in hospitalised patients. Some examples of these which are found on commercially available molecular tests are extended spectrum beta lactamases (ESBLs), carbapenemase producing enterobacteriaceae (CPEs), MRSA, Influenza and RSV.

In the UK there is a mandatory requirement to report certain infectious diseases to Public Health England, so they can be investigated. *L. pneumophilia* is associated with outbreaks from devices that aerosolize water. There were 532 cases in the UK in 2018\textsuperscript{27}, earlier sensitive detection of these would allow outbreak investigation to occur sooner and potentially stop further cases occurring.

**Syndromic molecular tests for pneumonia**

At the current time there are 2 FDA approved, CE marked syndromic molecular panels for pneumonia which are commercially available: the Filmarray (Biofire diagnostics LLC, Salt Lake City,
Utah, US) Pneumonia panel and the Unyvero (Curetis GmbH, Holzgerlingen, Germany) Hospitalised Pneumonia (HPN) panel. Fast Track Diagnostics respiratory panel 33 (Fast Track Diagnostics SARL, Luxembourg) is another available platform with a large number of targets, but insufficient bacterial targets for it to be considered a true pneumonia panel so this will only be considered in brief. The commercially available platforms are summarised in table 1.

The authors are aware of further panels in development from Mobidiag, Bruker, Accelerate and Axo Science but published data is only available for the latter. There are also several research groups who have developed their own syndromic molecular pneumonia tests, most notably Gadsby et al.

There are a multitude of other ‘respiratory pathogen’ multiplex panels which have targets only for respiratory viruses, atypical bacterial targets or a very small range of typical bacteria. These are beyond the scope of this review article. We have only included assays with targets for a wide range of typical pathogens for pneumonia.

**BioFire FilmArray Pneumonia panel**

This is an FDA approved and CE marked platform that uses nested real-time PCR to detect 34 clinically important respiratory targets (15 semi-quantitative bacterial targets, 3 qualitative atypical bacterial targets, 8 resistance genes and 8 viral targets). The semi-quantitative bacterial targets are *S. pneumoniae*, *H. influenzae*, *M. catarrhalis*, *S. aureus*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, *P. aeruginosa*, *E. coli*, *Enterobacter cloacae complex*, *Acinetobacter calcoaceticus-baumanii complex*, *Klebsiella aerogenes*, *K. oxytoca*, *K. pneumoniae* group, *Proteus* species and *Serratia marcescens*. The qualitative bacterial targets are *Chlamydia pneumoniae*, *L. pneumophila* and *M. pneumoniae*. Resistance gene targets are 5 carbapenemases (*bla*<sub>KPC</sub>, *bla*<sub>VIM</sub>, *bla*<sub>NDM</sub>, *bla*<sub>OXA-48</sub>, *bla*<sub>VIM</sub>, *bla*<sub>IMP</sub>), one ESBL (*bla*<sub>CTX-M</sub>) and two MRSA genes (*mecA/C* and *MREJ*). The viral targets are *Influenza A*, *Influenza B*, *Rhinovirus/Enterovirus*, *Adenovirus*, *RSV*, *Coronavirus*, human *Metapneumovirus*, and *Parainfluenza* viruses (types 1, 2, 3 and 4). The assay is validated on several sample types; sputum (including expressed sputum), bronchoalveolar lavage fluid and endotracheal aspirates. Sample
preparation takes 5 minutes and the test has a run time of around an hour and 15 minutes. A Pneumonia plus panel is also available which has an additional Middle-Eastern Respiratory Syndrome Coronavirus (MERS CoV) target.

The negative percent agreement (NPA) is the specificity of a test when compared to a non-reference standard. Some authors use this when reporting results in lieu of specificity as a result of the imperfect nature of current diagnostics. The NPA of bacterial detection between culture-based methods and the FA pneumonia panel varies between different organisms but is consistently very high. In the manufacturers dataset only two organisms on the panel have an NPA below 95%: H. influenzae (91.4% [95% CI 89.3-93.1%]) and S. aureus (91.2% [95% CI 89.1-93.0%]). Furthermore, the pneumonia panel detects pathogens in a much higher proportion of samples than culture. Buchan et al\textsuperscript{31} reported that the Filmarray detected a bacterial target in 71% more specimens than routine culture, equating to over 100% increase in total bacterial detections.

The relative abundance of organism for the 15 bacterial targets is estimated based on real-time PCR relative to a material of known quantity and is grouped for reporting into bins. These represent approximately $10^4$, $10^5$, $10^6$ and $>10^7$ genomic copies of bacterial nucleic acid per millilitre of specimen respectively. Concordance with reference molecular testing is very high\textsuperscript{33} but as expected the overall concordance between bin and reference sputum culture (CFU/ml) concentration was lower at around 40\%\textsuperscript{29} and was highly variable between organisms. As such the manufacturer advises clinical correlation in interpretation of semi-quantitative results.

To date there have been no published prospective interventional studies evaluating the clinical impacts of using the pneumonia panel in patients with pneumonia. Observational data based on lower respiratory tract assays which preceded the final, FDA approved pneumonia panel suggested change of antibiotics could be supported in >50% of cases\textsuperscript{31,34}.

\textbf{Curetis Unyvero Hospitalised Pneumonia (HPN) panel (formerly P55: LRT panel in USA)}\textsuperscript{35}
The HPN panel is CE marked and runs on the Unyvero platform which includes the Unyvero Lysator, the Unyvero Cockpit and the Unyvero Analyzer. Amplicons generated by 8 parallel multiplex PCR reactions are qualitatively detected by hybridisation on arrays in a single use cartridge. It has a wide range of bacterial and resistance gene targets including 29 pathogens and 19 resistance genes. The bacterial targets are *S. pneumoniae*, *S. aureus*, *Citrobacter freundii*, *E. coli*, *E. cloacae complex*, *E. aerogenes*, *Proteus species*, *K. pneumoniae*, *K. oxytoca*, *K. variicola*, *S. marcescens*, *Morganella morganii*, *M. catarrhalis*, *P. aeruginosa*, *A. baumanii complex*, *Stenotrophomonas maltophilia*, *L. pneumophila*, *H. influenzae*, *C. pneumoniae*, and *M. pneumoniae*. Resistance gene targets are: *bla*KPC, *bla*NDM, *bla*OXA-23, *bla*OXA-24, *bla*OXA-48, *bla*VIM, *bla*IMP, *bla*CTX-M, *bla*SHV, *bla*TEM, sul1, *ermB*, GyrA83 and GyrA87 for *E. coli* and *P. aeruginosa*, mecA/C. There is one fungal target (*Pneumocystis jirovecii*).

The assay is validated for use on sputum (including expectorated sputum, BAL and ET aspirate). Like the FilmArray, the Unyvero is a platform designed as a ‘sample-to-answer’ solution taking 5 minutes of low skill hands-on time with a total turnaround time of 4-5 hours. An equivalent test, the lower respiratory tract panel (LRT) has FDA approval in the US but is only validated for use on tracheal aspirates.

Manufacturer reported diagnostic sensitivity for bacterial detection (when compared to reference culture and molecular detection in cases of discrepancy) is between 80-100% with the majority of targets >90%; the exceptions are *A. baumanii complex* (88.9%), *K. pneumoniae* (80%) and *S. marcescens* (90%). Reported specificity is 98.3%-100%. Enne et al tested 608 surplus ICU samples and reported sensitivity of bacterial targets of between 50-100%; with the majority of targets >90%. The most notable exceptions were *E. aerogenes* (50% [95% CI, 12-88%]) and *S. marcescens* (77.8% [95% CI, 40-97%]). Peiffer-Smadja et al evaluated the HPN cartridge on VAP and severe HAP samples and reported a pooled sensitivity of 80% whilst only detecting 3/6 and 7/13 Gram positive isolates.
In the diagnostic performance data presented by the manufacturer, resistance marker detection aligned poorly with organism antibiogram: for example, matching in only 4/11 mecA detections or 9/13 quinolone resistance markers in *E. coli*. This issue was noted by Gadbsy et al\(^{39}\) for the P55 assay where the sensitivity for antibiotic resistance detection was 18%.

Two predecessors to the HPN cartridge have been developed and CE marked: the earlier P50, and the later P55. The former of these was evaluated most extensively by Personne et al who found the test to be sensitive for bacterial detection but with a run failure rate of 12.6% and extensive discrepancies with regards to sensitivity testing\(^ {40}\). Furthermore, the test was unable to differentiate *S. pneumoniae* from the *S. mitis* group. Papan et al reported that the P50 had a low sensitivity for Gram positive organisms (when evaluated on paediatric samples)\(^ {41}\).

The resistance panel on the P50 was broad but lacked several key emerging carbapenemase gene targets. The P55 panel rebalanced this by removing less clinically relevant resistance genes. It added targets for *S. pneumoniae* and *M. pneumoniae*. Again, the sensitivity for bacterial detection remained high when assessed by Ozongwu et al albeit with a high overall run failure rate of 10%\(^ {17}\).

The targets on the panel for the HPN are the same as the P55, but the manufacturer claims it has a higher sensitivity and specificity.

To date there are no published randomised controlled trials evaluating the clinical impact of the Unyvero HPN system in patients with pneumonia. Jamal et al\(^ {42}\) performed a non-randomised interventional study using the P50 assay where antibiotics were adjusted based on the results and pathogens detected were compared to culture. The turnaround time for result was very quick (~4 hours) compared to culture (48-96 hours) and a large proportion of patients had antibiotics changed based on the P50 results, however the small number of patients studied and the lack of a comparator group make definitive conclusions impossible. Gadsby et al retrospectively tested BAL samples with the P55 and reviewed patient notes. They reported that 53.6% of patients who had positive standard of care microbiology could potentially have had a change in antibiotics earlier.
based on P55 results. Conversely, they reported a false negative P55 result in ~20% of those with a positive culture which could have caused harm if acted upon.

**Fast Track Diagnostics (FTD) Respiratory pathogens 33**

The Respiratory pathogens 33 panel differs from the first two tests discussed in that it is exclusively a laboratory centred assay. The CE marked Respiratory pathogens 33 kit can be used on several standard laboratory cyclers. As such there is no reported standard turnaround time although it is greater than 6 hours. Positive signals are detected from eight multiplex real-time PCR reactions. It is not an automated process so will have a considerably longer hands-on time requiring skilled extraction and setup. The panel has 12 bacterial targets, 20 viral targets and 1 fungal target (*P. jiroveci*). The bacterial targets are: *H. influenzae* (with additional specific HiB target), *Bordatella* species (excluding *B. parapertussis*), *M. catarrhalis*, *Salmonella* species, *L. pneumophila*/*longbeachiae*, *K. pneumoniae*, *S. aureus*, *S. pneumoniae*, *C. pneumoniae* and *M. pneumoniae*. The viral targets are: *Influenza* (A, A(H1N1), B, C), *Rhinovirus*, *Coronaviruses* (NL63, 229E, OC43, HKU1), *Parainfluenza* (1-4), *Metapneumoviruses* A/B, *Bocavirus*, *RSV* A/B, *Adenovirus*, *Enterovirus* and *Parechovirus*.

**Comparing systems**

There is very little published data comparing different syndromic molecular pneumonia tests. Enne et al and the INHALE group presented data at ECCMID 2019 where they compared the Unyvero and the Filmarray on 654 surplus intensive care respiratory tract samples. The Filmarray had slightly greater sensitivity for common pathogens, fewer major discordances (defined as routine culture finding 1 or more undetected organisms) and fewer machine failures. The Unyvero had slightly higher specificity and overall concordance with reference culture.

*Table 1: commercially available pneumonia syndromic tests*
| Panel                              | Turn-around time (Hands on) | Targets                                                                 | Comments                                                                                       | Refs |
|-----------------------------------|-----------------------------|-------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------|------|
| BioFire FilmArray Pneumonia panel | 75 minutes (5 minutes)      | • 15 Bacterial (Semi-quantitative)                                      | • CE marked, FDA approved                                                                   |      |
|                                   |                             | • 3 Atypical bacteria                                                  | • Potentially deployable as point-of-care test¹                                               |      |
|                                   |                             | • 8 Resistance genes                                                   | • Semi-quantification (Genome copies)                                                         |      |
|                                   |                             | • 8 Viruses                                                            |                                                                                               |      |
| Curetis Unyvero Hospitalised      | 5 hours (5 minutes)         | • 17 Bacterial                                                         | • CE marked, FDA approved equivalent LRT panel (latter only validated on ET aspirates)       |      |
| Pneumonia panel (HPN)⁵ (formerly  |                             | • 3 Atypical bacterial                                                 | • Very extensive range of resistance genes                                                   |      |
| P55 and P50)                      |                             | • 1 Fungal (Pneumocystis jirovecii)                                    | • No viral targets                                                                              |      |
|                                   |                             | • 19 Resistance genes                                                  |                                                                                               |      |
| FTD Respiratory Pathogens ³³     | Platform dependent² (>6 hours) | • 8 Bacterial                                                         | • CE marked                                                                                    |      |
|                                   |                             | • 4 Atypical bacterial                                                 | • Laboratory based                                                                            |      |
|                                   |                             | • 20 Viruses                                                           | • Insufficient bacterial targets for true pneumonia panel: lacking critical Gram negative     |      |
|                                   |                             | • 1 Fungal (Pneumocystis jirovecii)                                    | targets                                                                                       |      |
|                                   |                             |                                                                       | • Not automated                                                                                |      |
|                                   |                             |                                                                       | • No resistance targets                                                                        |      |
|                                   |                             |                                                                       | • Qualitative only                                                                             |      |

**Discussion**

Whilst the data presented for syndromic molecular test for pneumonia clearly demonstrates high accuracy and the detection of many more pathogens than culture, no data has yet been published showing that this translates into improved antibiotic use or clinical benefit. Other molecular diagnostics studies for blood stream infection⁴⁵ have shown improved diagnostic performance, but

---

¹ Not CLIA waivered in the US
² Validated on Applied Biosystems® 7500 and NucliSENS® easyMag®, other platforms are compatible.
negligible impact on clinical outcomes when results were not provided to clinicians along with infection specialist advice. It seems likely that such a wealth of information generated will require careful interpretation by an infection specialist in consultation with the clinicians directly caring for the patient, for these benefits to be maximised.

Rapid syndromic molecular platforms have the potential to significantly improve the use of antibiotics and clinical outcomes in patient with pneumonia, but high quality randomised controlled trials are urgently required to evaluate their clinical impact. We are aware of 5 trials that are currently underway or in set up that may address this evidence gap: the SARIPOC study is a single centre randomised controlled trial (RCT) recruiting critically unwell patients with pneumonia in Southampton, UK. The INHALE study is a UK multicentre, RCT recruiting critically unwell patients with HAP and VAP. PIBCAP is a UK multicentre RCT recruiting patients with CAP. The NORCAP trial, in Norway is a single centre RCT in set up, also aiming to recruit patients with CAP. A further single centre RCT in Edinburgh is using molecular testing for broader community acquired LRTI microbial diagnosis. The first of these two studies are testing patients at the point-of-care, whereas the others use rapid laboratory-based testing.

Translating quicker tests into antibiotic savings: is antibiotic de-escalation safe?

Antibiotic de-escalation based on results is a key component of antibiotic stewardship and is widely accepted as good practice. Trials looking at the efficacy and safety of antimicrobial de-isolation based on culture results are sparse. The vast majority of published studies are observational and comparison between studies for so many variables (HAP, CAP, VAP, ICU/ non-ICU, severe sepsis etc) are fraught with difficulties. Furthermore, due to the geographic variability in causative organisms and prescribing practices, they are often poorly transferrable between regions.

To our knowledge no interventional studies have looked at the safety or efficacy of antimicrobial de-escalation based on multiplexed PCR for pathogens of pneumonia. Studies to date have made their de-escalation intervention after at least 48 hours when the patient has stabilised, and culture results
are available. Both the IDSA and the National Institute for Clinical Excellence (NICE) cite an urgent need for well-run RCTs on the impact of de-escalating antimicrobial therapy\textsuperscript{46,47}. The IDSA and the American Thoracic Society advise antibiotic de-escalation in HAP/VAP according to culture results on the basis of expert opinion, citing a high level of confidence that it ‘reduces costs, burdens, and side effects, and that it is very likely that de-escalation also reduces antimicrobial resistance’\textsuperscript{47}. There a small number of interventional studies looking at antibiotic de-escalation based upon microbiological culture results in HAP/VAP which have suggested this practice is safe\textsuperscript{48,49}. High quality data for outcomes, including length of intensive care stay and antibiotic savings, are lacking and conflicting. A meta-analysis by Khan et al\textsuperscript{50} of observational studies reviewing antibiotic de-escalation in pneumonia in ICU (HAP and VAP only) found no difference in mortality between those who were de-escalated according to culture result and those that weren’t.

In the context of CAP, both the IDSA\textsuperscript{51} and NICE/BTS\textsuperscript{46} guidelines recommend organism directed therapy when a pathogen has been identified by culture. High quality data is lacking but observational data and limited interventional data suggests this is safe\textsuperscript{52–54}. A systematic review by Paul et al\textsuperscript{55} included studies with CAP, HAP, VAP and Blood stream infection. The reviewers found no association between de-escaltion and survival with pneumonia (OR 0.97, 95% CI 0.45–2.12).

**Detection of colonising flora**

Concern has been raised that the high sensitivity of molecular tests will lead to excessive detection of colonising flora which may paradoxically increase unnecessary antibiotic use. This is particularly pertinent in expectorated sputa where small numbers of potentially pathogenic bacteria can be present in the absence of disease. A potential solution to this is the development of semi-quantitative molecular methods such as with the BioFire\textsuperscript{®} FilmArray\textsuperscript{®} Pneumonia panel. This provides a representation of the amount of bacterial DNA present which is highly concordant with reference molecular techniques.
Interpreting genotypic resistance results

As highlighted by studies using the Unyvero\textsuperscript{17,39}, molecular detection of resistance genes may correlate poorly with phenotypic sensitivity in its current form. Detection of genes from ‘off panel’ organisms, for example mecA genes in colonising coagulase negative staphylococci, may be incorrectly attributed to those organisms which are on the panel. As such, clinicians will need to be cautious in interpreting these results.

Practical Issues: where to test

As well as having relatively quicker run times, syndromic multiplex molecular tests could potentially be deployed at the point-of-care. The RespPOC trial by Brendish et al, demonstrated with a respiratory viral panel that this was logistically feasible and associated with a number of clinical benefits compared to routine clinical care\textsuperscript{24}. A post hoc analysis\textsuperscript{56} of patients who tested positive for respiratory viruses in the trial highlighted an association between rapid turn-around time (defined as <1.6 hours), shorter hospital admission and shorter durations of antibiotic therapy. It is our belief that point-of-care testing represents the ideal strategy for new, rapid diagnostic test platforms allowing clinicians to maximise the benefit from such accurate tests early in the decision-making process. Clearly, rigorous quality assurance is essential for any diagnostic test irrespective of the site of testing. It should also be noted that the tests described in this article are not currently CLIA waived - a requirement for use at the point-of-care in the US.

Conclusion

Rapid syndromic molecular tests for pneumonia have improved diagnostic accuracy compared to the current gold standard of culture and can provide results in real time. In the era of widespread AMR their use has the potential to dramatically improve the rational use of antibiotics and to improve clinical outcomes in patient with pneumonia. High quality data from well conducted randomised controlled trials are now urgently needed to assess the impact of these platforms on antibiotic use and patient outcome.
Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of Interest

Poole, S. - Declarations of interest: none.

Clark, T.W. has received speaker fees, reimbursement for travel and honoraria from Biofire LLC and BioMerieux and has also received equipment and consumables from these companies for the purposes of independent research. No commercial entities had any input into this manuscript.

Acknowledgements

The authors would like to thank Paula Sands, Research engagement Librarian at the Southampton General Hospital Healthcare library for her help and expertise in constructing search terms for literature review.

References

1. Wang Haidong, Naghavi Mohsen, Allen Christine, Barber Ryan M, Bhutta Zulfiqar A, Carter Austin, et al. Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980–2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet* 2016;388(10053):1459–544. Doi: 10.1016/S0140-6736(16)31012-1.

2. Mortality from pneumonia (ICD-10 J12 - J18 equivalent to ICD-9 480 - 486): directly standardised rate, all ages, annual trend: NHS Digital. Available at https://digital.nhs.uk/data-and-information/publications/clinical-indicators/compendium-of-population-health-indicators/compendium-mortality/current/mortality-from-respiratory-diseases. Accessed February 25, 2019, 2018.
3 Torres Antoni, Peetermans Willy E, Viegi Giovanni, Blasi Francesco. Risk factors for community-acquired pneumonia in adults in Europe: a literature review. *Thorax* 2013;**68**(11):1057–65. Doi: 10.1136/thoraxjnl-2013-204282.

4 Jain Seema, Self Wesley H., Wunderink Richard G., Fakhran Sherene, Balk Robert, Bramley Anna M., et al. Community-Acquired Pneumonia Requiring Hospitalization among U.S. Adults. *N Engl J Med* 2015;**373**(5):415–27.

5 Magill Shelley S., O'Leary Erin, Janelle Sarah J., Thompson Deborah L., Dumyati Ghinwa, Nadle Joelle, et al. Changes in Prevalence of Health Care–Associated Infections in U.S. Hospitals. *N Engl J Med* 2018;**379**(18):1732–44. Doi: 10.1056/NEJMoa1801550.

6 Giuliano Karen K., Baker Dian, Quinn Barbara. The epidemiology of nonventilator hospital-acquired pneumonia in the United States. *Am J Infect Control* 2018;**46**(3):322–7. Doi: 10.1016/j.ajic.2017.09.005.

7 Wang Yun, Eldridge Noel, Metersky Mark L., Verzier Nancy R., Meehan Thomas P., Pandolfi Michelle M., et al. National Trends in Patient Safety for Four Common Conditions, 2005–2011. *N Engl J Med* 2014;**370**(4):341–51. Doi: 10.1056/NEJMsai300991.

8 Nguile-Makao Molière, Zahar Jean-Ralph, Français Adrien, Tabah Alexis, Garrousie-Orgeas Maité, Allouchiche Bernard, et al. Attributable mortality of ventilator-associated pneumonia: respective impact of main characteristics at ICU admission and VAP onset using conditional logistic regression and multi-state models. *Intensive Care Med* 2010;**36**(5):781–9. Doi: 10.1007/s00134-010-1824-6.

9 Kollef Marin H., Hamilton Cindy W., Ernst Frank R. Economic Impact of Ventilator-Associated Pneumonia in a Large Matched Cohort. *Infect Control Hosp Epidemiol* 2012;**33**(03):250–6. Doi: 10.1086/664049.

10 Tackling drug-resistant infections globally: final report and recommendations chaired by Jim
O’Neill. Available at https://amr-review.org/sites/default/files/160525_Final paper_with cover.pdf. Accessed February 12, 2019, 2016.

11 UK 5-year action plan for antimicrobial resistance 2019 to 2024 - GOV.UK. Available at https://www.gov.uk/government/publications/uk-5-year-action-plan-for-antimicrobial-resistance-2019-to-2024. Accessed November 14, 2019, n.d.

12 Dellinger R Phillip, Carlet Jean M, Masur Henry, Gerlach Herwig, Calandra Thierry, Cohen Jonathan, et al. Surviving Sepsis Campaign guidelines for management of severe sepsis and septic shock. *Crit Care Med* 2004;32(3):858–73.

13 Daniel Priya, Rodrigo Chamira, Mckeever Tricia M, Woodhead Mark, Welham Sally, Lim Wei Shen, et al. Time to first antibiotic and mortality in adults hospitalised with community-acquired pneumonia: a matched-propensity analysis. *Thorax* 2016;71(6):568–70. Doi: 10.1136/thoraxjnl-2015-207513.

14 Chalmers J. D., Taylor J. K., Singanayagam A., Fleming G. B., Akram A. R., Mandal P., et al. Epidemiology, Antibiotic Therapy, and Clinical Outcomes in Health Care-Associated Pneumonia: A UK Cohort Study. *Clin Infect Dis* 2011;53(2):107–13. Doi: 10.1093/cid/cir274.

15 Musher Daniel M., Reig Ingrid L., Cazares Guillermo, Stager Charles E., Logan Nancy, Safar Hossam. Can an etiologic agent be identified in adults who are hospitalized for community-acquired pneumonia: Results of a one-year study. *J Infect* 2013;67(1):11–8. Doi: 10.1016/j.jinf.2013.03.003.

16 Gadsby Naomi J., Russell Clark D., McHugh Martin P., Mark Harriet, Conway Morris Andrew, Laurenson Ian F., et al. Comprehensive Molecular Testing for Respiratory Pathogens in Community-Acquired Pneumonia. *Clin Infect Dis* 2016;62(7):817–23. Doi: 10.1093/cid/civ1214.

17 Ozongwu C., Personne Y., Platt G., Jeanes C., Aydin S., Kozato N., et al. The Unyvero P55
'sample-in, answer-out' pneumonia assay: A performance evaluation. *Biomol Detect Quantif* 2017;13:1–6. Doi: 10.1016/J.BDQ.2017.06.001.

18 Kaur Amarjeet, Kumar Navin, Sengupta Sharmila, Mehta Yatin. Respiratory Multiplex Polymerase Chain Reaction: An Important Diagnostic Tool in Immunocompromised Patients. *Indian J Crit Care Med* 2017;21(4):192–8. Doi: 10.4103/ijccm.IJCCM_2_17.

19 Baudel Jean-Luc, Tankovic Jacques, Dahoumane Redouane, Carrat Fabrice, Galbois Arnaud, Ait-Oufella Hafid, et al. Multiplex PCR performed of bronchoalveolar lavage fluid increases pathogen identification rate in critically ill patients with pneumonia: a pilot study. *Ann Intensive Care* 2014;4(1):35. Doi: 10.1186/s13613-014-0035-7.

20 Jones Ronald N. Microbial Etiologies of Hospital-Acquired Bacterial Pneumonia and Ventilator-Associated Bacterial Pneumonia. *Clin Infect Dis* 2010;51(S1):81–7. Doi: 10.1086/653053.

21 Sader Helio S, Castanheira Mariana, Arends S J Ryan, Goossens Herman, Flamm Robert K. Geographical and temporal variation in the frequency and antimicrobial susceptibility of bacteria isolated from patients hospitalized with bacterial pneumonia: results from 20 years of the SENTRY Antimicrobial Surveillance Program (1997–2016). *J Antimicrob Chemother* 2019. Doi: 10.1093/jac/dkz074.

22 Ruuskanen Olli, Lahti Elina, Jennings Lance C, Murdoch David R. Viral pneumonia. *Lancet* 2011;377(9773):1264–75. Doi: 10.1016/S0140-6736(10)61459-6.

23 Choi Sang-Ho, Hong Sang-Bum, Ko Gwang-Beom, Lee Yumi, Park Hyun Jung, Park So-Youn, et al. Viral Infection in Patients with Severe Pneumonia Requiring Intensive Care Unit Admission. *Am J Respir Crit Care Med* 2012;186(4):325–32. Doi: 10.1164/rccm.201112-2240OC.

24 Brendish Nathan J, Malachira Ahalya K, Armstrong Lawrence, Houghton Rebecca, Aitken Sandra, Nyimbili Esther, et al. Routine molecular point-of-care testing for respiratory viruses
in adults presenting to hospital with acute respiratory illness (ResPOC): a pragmatic, open-label, randomised controlled trial. *Lancet Respir Med* 2017;5(5):401–11. Doi: 10.1016/S2213-2600(17)30120-0.

25 Muthuri Stella G, Venkatesan Sudhir, Myles Puja R, Leonardi-Bee Jo, Al Khuwaitir Tarig S A, Al Mamun Abdullah, et al. Effectiveness of neuraminidase inhibitors in reducing mortality in patients admitted to hospital with influenza A H1N1pdm09 virus infection: a meta-analysis of individual participant data. *Lancet Respir Med* 2014;2(5):395–404. Doi: 10.1016/S2213-2600(14)70041-4.

26 Katzen Jeremy, Kohn Rachel, Houk Jessica L, Ison Michael G. Early Oseltamivir After Hospital Admission Is Associated With Shortened Hospitalization: A 5-Year Analysis of Oseltamivir Timing and Clinical Outcomes. *Clin Infect Dis* 2019;69(1):52–8. Doi: 10.1093/cid/ciy860.

27 Public Health England. Monthly Legionella Report December 2018 National Surveillance Scheme for Legionnaires’ disease in Residents of England and Wales. 2019.

28 Villiers L., Caspar Y., Marche H., Boccoz S., Maurin M., Marche P.N., et al. ReSynPlex: Respiratory Syndrome Linked Pathogens Multiplex Detection and Characterization. *IRBM* 2018;39(5):368–75. Doi: 10.1016/J.IRBM.2018.10.002.

29 Biofire Diagnostics - FilmArray Pneumonia Panel. Available at https://www.biofiredx.com/products/the-filmarray-panels/filmarray-pneumonia/. Accessed February 25, 2019, n.d.

30 Iannello A, Dubost C, Weber C, Alberti-Segui C, Mousset C, Ginocchio C, et al. Evaluation of the BioFire® FilmArray® Pneumonia Panel in ICU Patients with Suspected Ventilator-Associated Pneumonia n.d. Doi: 10.1016/j.cmi.2016.06.013.

31 Buchan B, Windham S, Faron M, Balada-Llasat J, Relich R, Humphries R, et al. Clinical Evaluation and Potential Impact of a Semi-Quantitative Multiplex Molecular Assay for the
Identification of Pathogenic Bacteria and Viruses in Lower Respiratory Specimens. ATS. 2018.

32 Kerr S;, Graue C;, Broadbent K;, Balada-Llasat JM, Carroll A, Stone H, et al. Clinical Evaluation of the Biofire FilmArray Pneumonia Panel plus. ECCMID. 2018.

33 M Buccambuso, L O’Connor, M Brooks, J Manwaring, T Edwards, M Hockin, J Arce, R Lems, J Larsen, A Fratto, D Abbott, J Southwick A Judd. Precision of the FilmArray® Pneumonia Panel Considerations for Interpreting Relative Abundance of Bacterial Nucleic Acids in Lower Respiratory Specimens. ECCMID. 2018.

34 A.M. Huang, S.L. Windham, D. Mahmutoglu, J.M. Balada-Llasat; R.F. Relich, R. Humphries, S. Miller, A. Harrington, C. Murphy, A. Leber7, J. Dien Bard8, C. Zimmerman, S. Kerr, C. Graue, N.A. Ledeboer and B.W. Buc. Potential Clinical Impact of a Semi-Quantitative Multiplex Molecular Assay for the Identification of Bacteria, Viruses, and Fungi in Lower Respiratory Specimens. CVS. 2017.

35 UNYVERO HPN HOSPITALIZED PNEUMONIA APPLICATION MANUAL. Available at https://curetis.com/wp-content/uploads/00255_HPN_ApplicationManual_Rev6-EN.pdf. Accessed February 27, 2019, n.d.

36 Enne, Virve I; Aydin, Alp; Richardson, Hollian; Owen, Dewi; Baldan, Rossella; Russell, Charlotte; Nonamiukor, Brenda; Swart, Ann Marie; High, Juliet; Barber, Anthony; Gant, Vanya; Livermore, David M.; O’Grady Justin. INHALE WP1: An observational study comparing the performance of two multiplex PCR platforms against routine microbiology for the detection of potential pathogens in patients with suspected hospital acquired/ventilator associated pneumonia (HAP/VAP) across. ECCMID. 2019.

37 Peiffer-Smadja N., Bouadma L., Mourvillier B., Reboul M., Dilly M.-P., Montravers P., et al. Performance du test Unyvero HPN chez des patients de réanimation avec une pneumopathie acquise sous ventilation mécanique ou une pneumopathie nosocomiale sévère. Médecine
Peiffer-Smadja, N.; Bouadma, L.; Allouche, K.; Reboul, M.; Montravers, P.; Timsit, J.-F.; Armand-Lefevre L. Impact of the Unyvero HPN test in ICU patients with ventilator-associated pneumonia (VAP) or severe hospital-acquired pneumonia (HAP). *ECCMID*. 2019.

Gadsby Naomi J, Mchugh Martin P, Forbes Callum, Mackenzie Laura, Hamilton Stephen K D, Griffith David M, et al. Comparison of Unyvero P55 Pneumonia Cartridge, in-house PCR and culture for the identification of respiratory pathogens and antibiotic resistance in bronchoalveolar lavage fluids in the critical care setting n.d. Doi: 10.1007/s10096-019-03526-x.

Personne Y., Ozongwu C., Platt G., Basurto-Lozada P., Shamin M., Gant V. A., et al. ‘Sample-in, answer-out’? Evaluation and comprehensive analysis of the Unyvero P50 pneumonia assay. *Diagn Microbiol Infect Dis* 2016;86(1):5–10. Doi: 10.1016/j.diagmicrobio.2016.06.010.

Papan Cihan, Meyer-Buehn Melanie, Laniado Gudrun, Nicolai · Thomas, Griese Matthias, Huebner Johannes. Assessment of the multiplex PCR-based assay Unyvero pneumonia application for detection of bacterial pathogens and antibiotic resistance genes in children and neonates 2018;46:189–96. Doi: 10.1007/s15010-017-1088-y.

Jamal Wafaa, Al Roomi Ebtehal, AbdulAziz Lubna R., Rotimi Vincent O. Evaluation of curetis Unyvero, a multiplex PCR-based testing system, for rapid detection of bacteria and antibiotic resistance and impact of the assay on management of severe nosocomial pneumonia. *J Clin Microbiol* 2014;52(7):2487–92. Doi: 10.1128/JCM.00325-14.

Manual FTD Respiratory pathogens 33. Available at http://www.fast-trackdiagnostics.com/media/897519/ftd-2p3-32_64-manual-v7-2018_02-en.pdf. Accessed February 27, 2019, n.d.

Personne Y., Ozongwu C., Platt G., Basurto-Lozada P., Shamin M., Gant V. A., et al. ‘Sample-in,
answer-out’? Evaluation and comprehensive analysis of the Unyvero P50 pneumonia assay. 
Diagn Microbiol Infect Dis 2016;86(1):5–10. Doi: 10.1016/j.diagmicrobio.2016.06.010.

45 Poole Stephen, Kidd Stephen P, Saeed Kordo. A review of novel technologies and techniques associated with identification of bloodstream infection etiologies and rapid antimicrobial genotypic and quantitative phenotypic determination. Expert Rev Mol Diagn 2018;18(6):543–55. Doi: 10.1080/14737159.2018.1480369.

46 National Clinical Guidelines Centre. Diagnosis and management of community- and hospital-acquired pneumonia in adults. NICE clinical guideline 191. NICE; 2014.

47 Kalil Andre C., Metersky Mark L., Klompas Michael, Muscedere John, Sweeney Daniel A., Palmer Lucy B., et al. Management of Adults With Hospital-acquired and Ventilator-associated Pneumonia: 2016 Clinical Practice Guidelines by the Infectious Diseases Society of America and the American Thoracic Society. Clin Infect Dis 2016;63(5):e61–111. Doi: 10.1093/cid/ciw353.

48 Kim Jong Wook, Chung Joowon, Choi Sang-Ho, Jang Hang Jea, Hong Sang-Bum, Lim Chae-Man, et al. Early use of imipenem/cilastatin and vancomycin followed by de-escalation versus conventional antimicrobials without de-escalation for patients with hospital-acquired pneumonia in a medical ICU: a randomized clinical trial. Crit Care 2012;16(1):R28. Doi: 10.1186/cc11197.

49 Leone Marc, Bechis Carole, Baumstarck Karine, Lefrant Jean-Yves, Albanèse Jacques, Jaber Samir, et al. De-escalation versus continuation of empirical antimicrobial treatment in severe sepsis: a multicenter non-blinded randomized noninferiority trial. Intensive Care Med 2014;40(10):1399–408. Doi: 10.1007/s00134-014-3411-8.

50 Ambaras Khan Rahela, Aziz Zoriah. Antibiotic de-escalation in patients with pneumonia in the intensive care unit: A systematic review and meta-analysis. Int J Clin Pract
Musher Daniel M., Mandell Lionel A., Niederman Michael S., File Thomas M., Dowell Scott F., Torres Antonio, et al. Infectious Diseases Society of America/American Thoracic Society Consensus Guidelines on the Management of Community-Acquired Pneumonia in Adults. *Clin Infect Dis* 2007;44(Supplement_2):S27–72. Doi: 10.1086/511159.

Yamana Hayato, Matsui Hiroki, Tagami Takashi, Hirashima Junko, Fushimi Kiyohide, Yasunaga Hideo. De-escalation versus continuation of empirical antimicrobial therapy in community-acquired pneumonia. *J Infect* 2016;73(4):314–25. Doi: 10.1016/J.JINF.2016.07.001.

Falguera M, Ruiz-González A, Schoenenberger J A, Touzón C, Gázquez I, Galindo C, et al. Prospective, randomised study to compare empirical treatment versus targeted treatment on the basis of the urine antigen results in hospitalised patients with community-acquired pneumonia. *Thorax* 2010;65(2):101–6. Doi: 10.1136/thx.2009.118588.

Van Der Eerden M M, Vlaspolder F, De Graaff C S, Groot T, Bronsveld W, Jansen H M, et al. Comparison between pathogen directed antibiotic treatment and empirical broad spectrum antibiotic treatment in patients with community acquired pneumonia: a prospective randomised study. *Thorax* 2005;60:672–8. Doi: 10.1136/thx.2004.030411.

Paul M., Dickstein Y., Raz-Pasteur A. Antibiotic de-escalation for bloodstream infections and pneumonia: systematic review and meta-analysis. *Clin Microbiol Infect* 2016;22(12):960–7. Doi: 10.1016/J.CMI.2016.05.023.

Brendish Nathan J, Malachira Ahalya K, Beard Kate R, Ewings Sean, Clark Tristan W. Impact of turnaround time on outcome with point-of-care testing for respiratory viruses: a post hoc analysis from a randomised controlled trial. *Eur Respir J* 2018;52(2):1800555. Doi: 10.1183/13993003.00555-2018.