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Recent and emerging technologies for the rapid diagnosis of infection and antimicrobial resistance

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The rise in antimicrobial resistance (AMR) is predicted to cause 10 million deaths per year by 2050 unless steps are taken to prevent this looming crisis. Microbiological culture is the gold standard for the diagnosis of bacterial/fungal pathogens and antimicrobial resistance and takes 48 hours or longer. Hence, antibiotic prescriptions are rarely based on a definitive diagnosis and patients often receive inappropriate treatment. Rapid diagnostic tools are urgently required to guide appropriate antimicrobial therapy, thereby improving patient outcomes and slowing AMR development. We discuss new technologies for rapid infection diagnosis including: sample-in-answer-out PCR-based tests, BioFire FilmArray and Curetis Unyvero; rapid susceptibility tests, Accelerate Pheno and microfluidic tests; and sequencing-based approaches, focusing on targeted and clinical metagenomic nanopore sequencing.

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Introduction

More than 700 000 people die per year globally due to antimicrobial resistance (AMR) according to an estimate from the UK government-commissioned review on AMR (O'Neill report) [1]. At the current rate, by 2050, AMR is predicted to cause 10 million deaths annually and cost the world economy $100 trillion in total. It is widely recognised that rapid diagnostics are crucial in the fight against AMR, to improve the management of life threatening infections such as sepsis and pneumonia and to enable earlier and more precise targeting of pathogens with appropriate antibiotics (i.e. improved antibiotic stewardship) [2]. The final O'Neill report states that by 2020 all antibiotic prescriptions should be supported by a rapid diagnostic test where available [1].

Current standard methods for diagnosing bacterial infection are based on microbiological culture and have long turn-around times, offer poor clinical sensitivity and are not fit-for-purpose for acute serious infection such as sepsis, pneumonia and meningitis. Acute infections force clinicians into early broad-spectrum treatment, before culture results become available, highlighting the need for rapid diagnostics [3–6]. A paradigm shift in diagnostic microbiology is urgently required, with the ultimate goal of providing pathogen identification and resistance/susceptibility information to clinicians before antibiotics are administered.

In this review, we highlight recent and emerging tests for the rapid diagnosis of pathogens, antimicrobial resistance and antimicrobial susceptibility and their current/future clinical applications. We describe some of the current tests that utilise genotypic methods such as PCR for pathogen identification and antibiotic resistance testing. We also describe technologies and techniques that combine pathogen identification with rapid phenotypic antibiotic susceptibility testing (AST). Finally, we outline key advances in the application of DNA sequencing for the rapid diagnosis of infection and AMR that could be implemented clinically in the near future.

Rapid PCR-based pathogen and antimicrobial resistance detection

Discerning bacterial from viral infections is the simplest level of diagnosis that can be clinically useful to guide antimicrobial therapy, reducing unnecessary antibiotic prescriptions. FebriDx® is a dipstick test measuring c-reactive protein and Myxovirus resistance protein A levels in blood, differentiating bacterial from viral infections using an inflammation biomarker [7]. Polymerase chain reaction (PCR)-based systems such as ID Now and cobas® Liat® have specific tests for specific targets such as influenza A&B [8]. However, an ideal diagnostic test will identify the specific pathogen and provide guidance on appropriate antimicrobial therapy. This is particularly important in clinical syndromes such as urinary tract infections (UTIs), pneumonia and sepsis, which can be caused by many different pathogens (bacteria, fungi or

viruses in the case of pneumonia and sepsis), many of which may carry acquired antimicrobial resistance.

PCR has evolved since its introduction in the late 80s from manual single target assays [9] to automated multiplex systems such as Unyvero (Curetis) and BioFire® FilmArray® (BioMerieux), which are starting to make an impact in the clinic using tailored cartridges for specific infections [10]. These tests have rapid turnaround times and require very little hands-on time to operate. Studies on the Unyvero Pneumonia cartridges have shown low sensitivity, ranging from 67 to 73%, but with much higher specificity, 94.9–97.9% [10–12]. One study reported average sensitivity for organisms on the panel to be 88.8%, highlighting the contribution of pathogens absent from the panel to the reported low sensitivity. Other issues include Gram-positive detection rates as low as 46.2% [11] and machine failure rates over 10% [12]. The cartridge for blood culture confirmation has demonstrated better results, with a sensitivity of 96.8% and specificity of 99.8% in one study [13]. The test could accurately detect *mecA*, diagnosing MRSA in sepsis within 5 hours of flagging positive on the blood culture machine [13]. However, the high sensitivity is in part related to the prior culturing of the sample, which negates the time advantage of using a PCR tests. For this technology to be widely implemented, the low sensitivity of the cartridges used for direct sample testing needs to be improved. Additionally, panels should include a greater range of targets and machine failure rates must be reduced [10,11,14–16].

The BioFire® FilmArray® (Figure 1) is another multiplex PCR test, capable of simultaneously testing for bacteria, viruses, yeasts and parasites, along with resistance genes [17–21]. Evaluation of the FilmArray® meningitis/encephalitis panel (M/E), which tests for 14 of the most common causes of ME within 1 hour, report categorical agreement with comparator testing of 90.4–99.8% [20,22–25]. A clinical study of the FilmArray® rapid respiratory panel reported significant reductions in antibiotic treatment duration and hospital stay, by 36 and 29.5 hours respectively [26]. While correlation with routine testing is high, FilmArray® is limited in the information it provides as only the pneumonia and blood panels target resistance genes while the remaining panels only identify pathogens.

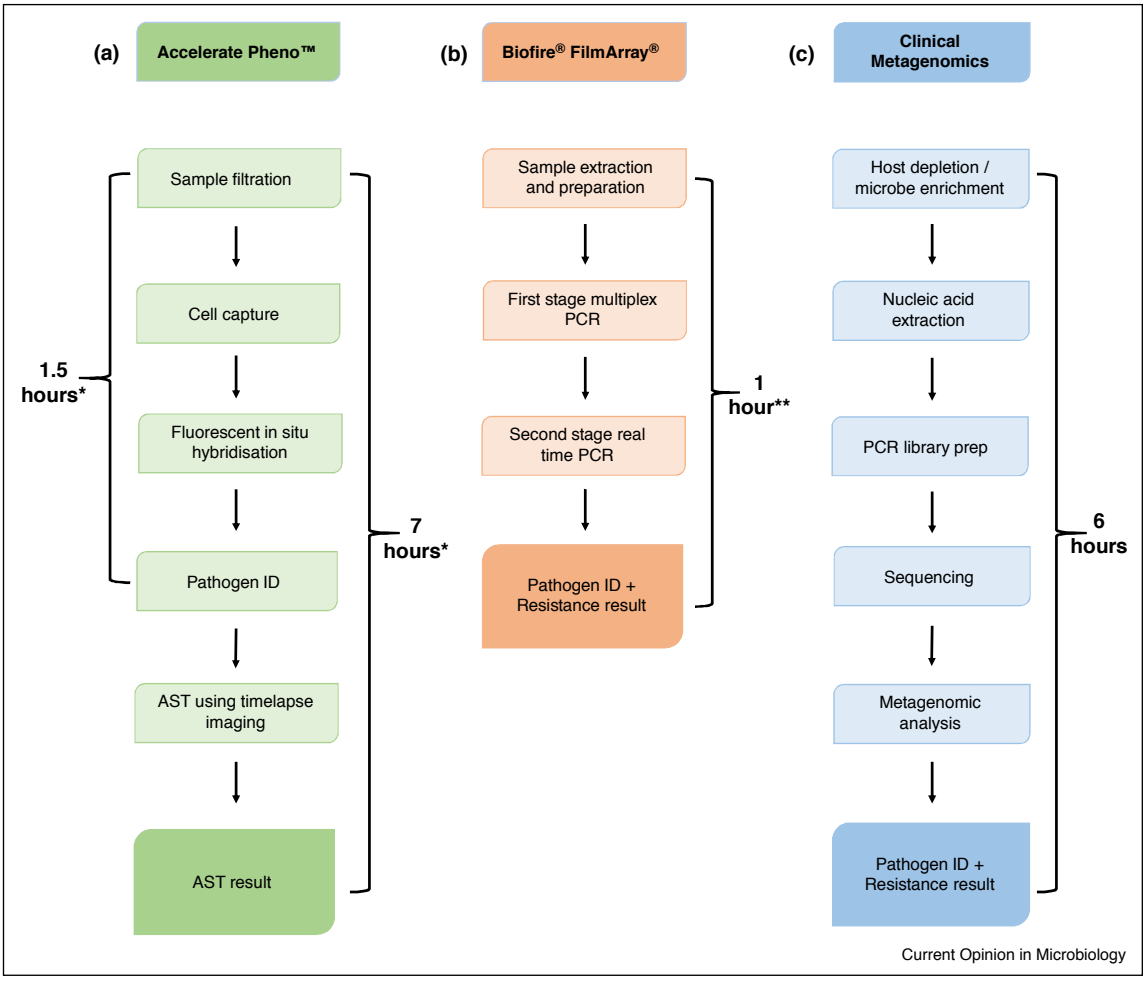
Antibiotic resistance profiling is clinically useful, but antimicrobial susceptibility testing is preferable, something culture can provide but rapid molecular tests cannot. It is better to inform the clinician what treatment they can use, rather than what treatments won't work. Also, although resistance determinants can be accurately identified, there can be poor correlation between genotype and phenotype and it is difficult to tell the source of resistance determinants in samples from non-sterile sites (e.g. lung samples).

Rapid antimicrobial susceptibility testing

Accelerate Pheno™ is a fully automated system that combines total organism count, pathogen ID and AST directly from positive blood cultures (Figure 1). Cells are stained with a limited panel of fluorescence *in situ* hybridisation (FISH) probes to detect species most commonly implicated in septicemia before immobilising them in agarose layers permeated with the antimicrobial being tested. Immobilised cell growth is monitored by automated microscopy with multiple antibiotic concentrations to calculate a minimum inhibitory concentration (MIC) and total organism behaviour is mapped against a predictive database for susceptible, intermediate and resistant phenotypes [27•]. Some early studies on clinical and spiked blood cultures using the Accelerate PhenoTest™ BC panel yielded sensitivities ranging from 94.7 to 97.5% with specificities of 98.9–99.5% against included targets. AST agreement with routine methods were reported as 94.1–96.4% [27•,28–30] Accelerate Pheno™ has been shown to reduce time-to-result down to 1.4 hours for pathogen identification and 6.6 hours for AST result from a positive blood culture, a reduction of 40 hours compared to the current standard-of-care [28]. However, this test still requires a blood culture; AST results should ideally be available within 8 hours of the sample being taken to allow for timely transition to targeted treatment after a first dose of broad-spectrum antibiotics. While studies have shown that Accelerate Pheno™ can identify all 16 organisms on its panel [28,29], studies showing 58.1–88.7% of samples receiving pathogen ID highlights the need for a more comprehensive panel [27•,28–30]. At present, only a cartridge for blood cultures is available.

Microfludic technology can be utilised as an alternative approach to predicting antimicrobial susceptibility. A group at Stanford University have developed a microfludic array for UTI diagnosis using 16S rDNA probes. Pathogens are lysed in urine, releasing rRNA which binds to probes on the sensor surface by electrokinetic hybridisation. Rapid AST and minimum inhibitory concentration (MIC) testing is performed by adding the antibiotic to be tested to the urine at different concentrations and measuring differential rRNA levels after brief culture [31]. The biosensor identified pathogens in 107 urine samples with a 98.5% sensitivity, 96.6% specificity, and categorical agreement of 97.6% for ciprofloxacin MIC within 5 hours [31]. Recent proof-of-concept studies have demonstrated microfluidic techniques achieving AST results within 30 min for uropathogenic *Escherichia coli*. One group used time-lapse phase contrast microscopy of UTI *E. coli* isolates to compare cell growth rates in antibiotic-containing and antibiotic-free media [32••]. They successfully identified 49 clinical isolates as ciprofloxacin resistant or susceptible with 100% agreement with disk-diffusion culture. Another group used digital loop-mediated isothermal amplification (LAMP) targeting the Enterobacteriaceae 23S rRNA gene to

Figure 1



Workflows of highlighted technologies and techniques.
(a) Accelerate PhenoTest Blood Culture kit (*The turnaround time is from machine loading. This kit requires a positive blood culture so time-to-result from primary sample will depend on varying blood culture times). **(b)** BioFire FilmArray (all panels) (**The turnaround time is from machine loading. While most FilmArray panels work directly on primary samples, the Blood Culture Identification (BCID) panel requires a positive blood culture and time-to-result from primary sample will depend on varying blood culture times). **(c)** A typical rapid nanopore metagenomics pipeline not requiring a culture (turnaround time based on Greninger *et al.* [58] and Charalampous *et al.* [59]).

quantitatively detect *E. coli* in microfluid compartments directly from clinical urine samples [33••]. The ratio of signal between the antibiotic-treated and untreated controls was used to determine susceptibility after 15 minutes of incubation. Digital LAMP matched the gold-standard broth microdilution AST call with 96.3% agreement on 51 clinical urine samples tested. It should be noted that rapid phenotypic AST methods require sufficient pathogen biomass to reliably detect differences in growth following the addition of antibiotics.

Sequencing-based technologies for pathogen and AMR detection

Advances in sequencing technologies, often categorised into various generations such as Next Generation Sequencing (NGS) and second, third and fourth generation sequencing (described here as 'sequencing'), have paved the way for whole genome, targeted and metagenomic sequencing approaches to characterise infection. Whole genome sequencing (WGS) of pathogens is now a widely used tool in clinical and public health laboratories for AMR detection, epidemiology and infection control studies. Numerous studies on healthcare associated methicillin-resistant *Staphylococcus aureus* (MRSA) have shown that whole genome sequencing can aid in tracking outbreaks and guide infection control measures where other methods such as multi locus sequence typing (MLST) fail to provide sufficient resolution [34,35]. WGS has also proven particularly useful in TB diagnostics and can be used to confirm *Mycobacterium tuberculosis* (Mtb) infection and provide information about resistance or susceptibility within 1–3 days from a liquid

culture [36]. One of the largest studies conducted on Mtb AMR/susceptibility prediction using WGS showed very high specificities and sensitivities compared to culture (93.6–99.0% and 91.3–97.5% respectively) and it is likely that WGS will be widely implemented for this purpose in the near future [37].

While WGS is useful for predicting AMR in certain pathogens (particularly Mtb), it requires cultured microbes, hence is not applicable for the rapid diagnosis of infection and AMR. Sequencing approaches applied directly to clinical samples to diagnose infection need to be fast enough to guide antimicrobial therapy — <8 hours from sample-to-result. Most sequencing companies now boast run times of 4 hours or less as an option, however, lengthy library preparation as well the requirement for end-point analysis result in turnaround times significantly longer than 8 hours. According to the manufacturers' websites, Illumina sequencers tend to have the longest run times, while Ion Torrent and Pacific BioScience (PacBio) devices are faster. Oxford Nanopore Technologies (ONT) devices offer the most flexible times, with analysable data becoming available in real-time, once sequencing begins. There have been some studies investigating the application of Ion Torrent in infection diagnosis with theoretical turnaround times of less than a day [38]. A direct comparison of Illumina's MiSeq to ONT's MinION for a viral clinical metagenomics pipeline showed that the MinION workflow could provide results within 6 hours compared to the MiSeq's >20 hours [39].

Sequencing for infection diagnosis can either be targeted or non-targeted. Targeted sequencing is performed by capturing-specific pathogens/pathogen sequences or specifically amplifying whole (usually viruses) or portions of genomes using PCR. Non-targeted or agnostic sequencing of microbes in clinical samples is known as 'clinical metagenomics'.

Targeted sequencing

The most common approach for sequencing-based infection diagnostics utilises PCR amplification of housekeeping genes to differentiate microbes [40], particularly the 16S rRNA gene in bacteria due to highly conserved and hypervariable regions in its sequence [41]. Historically, 16S gene sequencing was performed by Sanger sequencing; however, in recent years many have progressed to NGS as it has numerous advantages, particularly when there are multiple microbes present in a sample. This approach is sometimes referred to as 16S metagenomics, but as it targets a fragment of one bacterial gene (not the metagenome), it is more accurately referred to as 16S sequencing. Because of slow turnaround time, 16S sequencing has, until recently, mainly been used in reference laboratories or as a last-line tool for identifying fastidious or uncultivable bacteria when other methods such as MALDI-TOF fail [42]. However, recent studies have demonstrated rapid full-length 16S rRNA gene nanopore sequencing (Oxford Nanopore Technologies) can be used to identify pathogens in clinical samples within hours [43,44].

16S sequencing, however, is not reliable at the species level and is limited to bacterial identification. While there are also universal markers for fungi (e.g. the nuclear ribosomal internal transcribed region), viruses lack a similar universal marker. Furthermore, such markers do not give AMR information. Hence, researchers are beginning to design amplicon sequencing panels targeting a large number of pathogens and resistance genes. These can be organism-specific, for example, specifically targeting TB resistance SNPs [45], or they can be general panels that target multiple bacteria [46••] viruses [47] and resistance determinants. They are particularly useful where high sensitivity tests are required (e.g. for sepsis diagnosis); however, the currently published methods are not sufficiently rapid to be clinically useful currently, taking approximately three days. Switching from Illumina to nanopore sequencing could significantly improve speeds allowing the methods to be utilised in the near future.

An alternative targeted approach is to enrich the target species directly from clinical specimens using RNA baits. One study applying this approach for Mtb diagnosis could detect Mtb in all 43 sequenced sputa that had matched positive mycobacterial growth indicator tubes (MGIT) [48•]. Breadth of coverage was >85% in 32/43 (74%) samples and resistance detection from sputum was concordant across all methods including phenotypic resistance testing in 21/23 resistance mutations identified. This method gives results in 4–5 days, 31 days earlier than phenotypic testing; however, it is currently prohibitively expensive for routine use.

Clinical metagenomics

Metagenomics is the culture-free characterisation of all the genetic material from a sample. It sidesteps limitations of culture and, as a non-targeted method, is more comprehensive than targeted tests. However, human DNA largely dominates primary samples, limiting pathogen and AMR determinant identification [49]. Therefore, enrichment of microbial DNA or depletion of human DNA is an important step in clinical metagenomics [50].

Proof-of-concept studies using Illumina technology have demonstrated the potential of clinical metagenomics in bone and joint infections [51] and in ventilator associated pneumonia (VAP) [52]. Clinical metagenomics have also been applied for the diagnosis of viruses despite lower DNA/RNA yields [53,54]. The advantages of the non-targeted clinical metagenomics approach were demonstrated in a study of a patient with suspected encephalitis with negative bacterial culture and viral PCR results.

Metagenomics revealed an astrovirus infection, an unusual encephalitis pathogen not routinely tested in this context [55]. It is important to note, however, that care must be taken when interpreting metagenomics results due to the risk of contamination and because the presence of a pathogen does not necessarily mean it is the cause of infection.

Illumina sequencing has several disadvantages for implementation in clinical metagenomics including cost, if multiplexing a high number of samples is not possible, turnaround time (long sequencing run times) and short reads. Single-molecule sequencing devices by Pacific Biosciences and Oxford Nanopore Technologies (ONT) have been transformative in sequencing due to their ability to produce long reads [56]. PacBio SMRT technology can generate reads over 300 kb [57] and nanopore sequencing can generate reads >1 Mb in length with the current record at over 2.2 Mb [58]. Long reads aid in genome assembly and matching AMR determinants to their hosts [59]. Nanopore sequencing is particularly applicable to clinical metagenomics due to MinION's low capital cost, portability and real-time data availability. Clinical metagenomics using MinION sequencing has been employed for a wide range of samples including urine [60], feces [61], blood [39] and respiratory samples [62••]. Charalampous *et al.* used a clinical metagenomics pipeline (Figure 1), including human DNA depletion, for the diagnosis of respiratory infection, with a turnaround of 6 hours [62••]. They also demonstrated it was possible to predict some resistance phenotypes using sequence data, including methicillin resistance in *S. aureus* and β-lactamase resistance in *E. coli*. Greninger *et al.* used MinION clinical metagenomics for the detection of Ebola, chikungunya and hepatitis C viruses, also with a turnaround of 6 hours [39]. Resistance variant calling has also been demonstrated using Nanopore sequencing [63] and will improve using metagenomic data as sequencing accuracy and bioinformatics techniques evolve.

Predicting phenotypic resistance using DNA sequence is complex. Resistance can be due to multiple mutations and depend on gene expression levels; additionally, in non-sterile sample sites such as the lung, resistance genes may come from commensal organisms not causing infection, hence linking genes to organisms is important. A potential solution to these complexities is to determine resistance or susceptibility by lineage association [64••]. Using a reference database of *Streptococcus pneumoniae* isolates with known resistances, Brinda *et al.* were able to use metagenomic data to deduce resistance and susceptibility in unknown *S. pneumoniae* culture positive sputum samples by determining their lineage [64••]. A limitation is that the association between the lineage markers and AMR determinants must be strong for the method to accurately predict phenotype; this is not the case with all lineage/AMR determinant combinations.

Conclusion

The current culture gold standard of diagnosing infections is not fit for purpose as it is too slow, not sufficiently comprehensive and is prone to subjective interpretation. This means that most antibiotic treatments are started on empirical grounds, often leading to inappropriate prescribing and contributing to the rise of antimicrobial resistance. While some PCR-based rapid diagnostic tests are being successfully implemented alongside culture for the diagnosis of clinical syndromes such as pneumonia and meningitis, these tests are not comprehensive enough for pathogen and AMR determinant detection to *replace* culture. Clinical metagenomics, on the other hand, can detect bacteria, fungi, viruses, parasites and AMR determinants in a single test within the eight-hour window before second dose of broad-spectrum antibiotics, enabling targeted therapy and reducing unnecessary and inappropriate use of antimicrobials. While predicting phenotype from genotype can be challenging, culture must be replaced with rapid and comprehensive diagnostic methods if we are to avert the looming global AMR crisis.

Conflict of interest statement

J.O.G. has received free MinION flow cells and library preparation reagents as a member of the ONT MinION Access Programme. J.O.G. has received travel and accommodation expenses for speaking at ONT and other conferences, an honorarium for speaking at ONT headquarters and funding towards PhD studentships from ONT. A.A. studentship is part-funded by ONT.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. O'Neill J: **Tackling drug-resistant infections globally: final report and recommendations**. *Review on Antimicrobial Resistance*. 2016.
2. O'Neill J: **Rapid diagnostics: stopping unnecessary use of antibiotics**. *Review on Antimicrobial Resistance*. 2015.
3. Angus DC, Linde-Zwirble WT, Lidicker J, Clermont G, Carcillo J, Pinsky MR: **Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care**. *Crit Care Med* 2001, **29**:1303-1310.
4. Goto M, Al-Hasan MN: **Overall burden of bloodstream infection and nosocomial bloodstream infection in North America and Europe**. *Clin Microbiol Infect* 2013, **19**:501-509.
5. Hall MJ, Williams SN, DeFrances CJ, Golosinskiy A: **Inpatient care for septicemia or sepsis: a challenge for patients and hospitals**. *NCHS Data Brief* 2011:1-8.
6. Nadel S: **Treatment of meningococcal disease**. *J Adolesc Health* 2016, **59**:S21-28.

7. Self WH, Rosen J, Sharp SC, Filbin MR, Hou PC, Parekh AD, Kurz MC, Shapiro NI: **Diagnostic accuracy of FebriDx: a rapid test to detect immune responses to viral and bacterial upper respiratory infections**. *J Clin Med* 2017, **6**.

8. Nolte FS, Gauld L, Barrett SB: **Direct comparison of Alere i and cobas Liat Influenza A and B tests for rapid detection of influenza virus infection**. *J Clin Microbiol* 2016, **54**:2763-2766.

9. Garibyan L, Avashia N: **Polymerase chain reaction**. *J Invest Dermatol* 2013, **133**:1-4.

10. Jamal W, Al Roomi E, AbdulAziz LR, Rotimi VO: **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**:2487-2492.

11. Papan C, Meyer-Buehn M, Laniado G, Nicolai T, Griese M, Huebner J: **Assessment of the multiplex PCR-based assay Unyvero pneumonia application for detection of bacterial pathogens and antibiotic resistance genes in children and neonates**. *Infection* 2018, **46**:189-196.

12. Ozongwu C, Personne Y, Platt G, Jeanes C, Aydin S, Kozato N, Gant V, O'Grady J, Enne VI: **The Unyvero P55 'sample-in, answer-out' pneumonia assay: a performance evaluation**. *Biomol Detect Quantif* 2017, **13**:1-6.

13. Burrack-Lange SC, Personne Y, Huber M, Winkler E, Weile J, Knabbe C, Gorig J, Rohde H: **Multicenter assessment of the rapid Unyvero blood culture molecular assay**. *J Med Microbiol* 2018, **67**:1294-1301.

14. Kunze N, Moerer O, Steinmetz N, Schulze MH, Quintel M, Perl T: **Point-of-care multiplex PCR promises short turnaround times for microbial testing in hospital-acquired pneumonia—an observational pilot study in critical ill patients**. *Ann Clin Microbiol Antimicrob* 2015, **14**:33.

15. Personne Y, Ozongwu C, Platt G, Basurto-Lozada P, Shamin M, Gant VA, Zumla A, Enne VI: **'Sample-in, answer-out'? Evaluation and comprehensive analysis of the Unyvero P50 pneumonia assay**. *Diagn Microbiol Infect Dis* 2016, **86**:5-10.

16. Schulte B, Eickmeyer H, Heininger A, Juretzek S, Karrasch M, Denis O, Roisin S, Pletz MW, Klein M, Barth S *et al.*: **Detection of pneumonia associated pathogens using a prototype multiplexed pneumonia test in hospitalized patients with severe pneumonia**. *PLoS One* 2014, **9**:e110566.

17. Blaschke AJ, Heyrend C, Byington CL, Fisher MA, Barker E, Garrone NF, Thatcher SA, Pavia AT, Barney T, Alger GD *et al.*: **Rapid identification of pathogens from positive blood cultures by multiplex polymerase chain reaction using the FilmArray system**. *Diagn Microbiol Infect Dis* 2012, **74**:349-355.

18. Buss SN, Leber A, Chapin K, Fey PD, Bankowski MJ, Jones MK, Rogatcheva M, Kanack KJ, Bourzac KM: **Multicenter evaluation of the BioFire FilmArray gastrointestinal panel for etiologic diagnosis of infectious gastroenteritis**. *J Clin Microbiol* 2015, **53**:915-925.

19. Gay-Andrieu F, Magassouba N, Picot V, Phillips CL, Peyrefitte CN, Dacosta B, Dore A, Kourouma F, Ligeon-Ligeonnet V, Gauby C *et al.*: **Clinical evaluation of the BioFire FilmArray BioThreat-E test for the diagnosis of Ebola virus disease in Guinea**. *J Clin Virol* 2017, **92**:20-24.

20. Leber AL, Everhart K, Balada-Llasat JM, Cullison J, Daly J, Holt S, Lephart P, Salimnia H, Schreckenberger PC, DesJarlais S *et al.*: **Multicenter evaluation of BioFire FilmArray meningitis/encephalitis panel for detection of bacteria, viruses, and yeast in cerebrospinal fluid specimens**. *J Clin Microbiol* 2016, **54**:2251-2261.

21. Poritz MA, Blaschke AJ, Byington CL, Meyers L, Nilsson K, Jones DE, Thatcher SA, Robbins T, Lingenfelter B, Amiott E *et al.*: **FilmArray, an automated nested multiplex PCR system for multi-pathogen detection: development and application to respiratory tract infection**. *PLoS One* 2011, **6**:e26047.

22. Graf EH, Farquharson MV, Cardenas AM: **Comparative evaluation of the FilmArray meningitis/encephalitis molecular panel in a pediatric population**. *Diagn Microbiol Infect Dis* 2017, **87**:92-94.

23. Hanson KE, Slechta ES, Killpack JA, Heyrend C, Lunt T, Daly JA, Hemmert AC, Blaschke AJ: **Preclinical assessment of a fully automated multiplex PCR panel for detection of central nervous system pathogens**. *J Clin Microbiol* 2016, **54**:785-787.

24. Liesman RM, Strasburg AP, Heitman AK, Theel ES, Patel R, Binnicker MJ: **Evaluation of a commercial multiplex molecular panel for diagnosis of infectious meningitis and encephalitis**. *J Clin Microbiol* 2018, **56**.

25. Messacar K, Breazeale G, Robinson CC, Dominguez SR: **Potential clinical impact of the film array meningitis encephalitis panel in children with suspected central nervous system infections**. *Diagn Microbiol Infect Dis* 2016, **86**:118-120.

26. Rogers BB, Shankar P, Jerris RC, Kotzbauer D, Anderson EJ, Watson JR, O'Brien LA, Uwindatwa F, McNamara K, Bost JE: **Impact of a rapid respiratory panel test on patient outcomes**. *Arch Pathol Lab Med* 2015, **139**:636-641.

27. • Marschal M, Bachmaier J, Autenrieth I, Oberhettinger P, Willmann M, Peter S: **Evaluation of the Accelerate Pheno system for fast identification and antimicrobial susceptibility testing from positive blood cultures in bloodstream infections caused by gram-negative pathogens**. *J Clin Microbiol* 2017, **55**:2116-2126.

Using the Accelerate Pheno BC test, the authors generated antimicrobial susceptibility data directly from positive blood cultures within 7 hours, reducing time to result by 40.39 hours compared to culture-based methods.

28. Lutgring JD, Bittencourt C, McElvania TeKippe E, Cavuoti D, Hollaway R, Burd EM: **Evaluation of the Accelerate Pheno system: results from two academic medical centers**. *J Clin Microbiol* 2018, **56**.

29. Pancholi P, Carroll KC, Buchan BW, Chan RC, Dhiman N, Ford B, Granato PA, Harrington AT, Hernandez DR, Humphries RM *et al.*: **Multicenter evaluation of the Accelerate PhenoTest BC kit for rapid identification and phenotypic antimicrobial susceptibility testing using morphokinetic cellular analysis**. *J Clin Microbiol* 2018, **56**.

30. Charnot-Katsikas A, Tesic V, Love N, Hill B, Bethel C, Boonlayangoor S, Beavis KG: **Use of the Accelerate Pheno system for identification and antimicrobial susceptibility testing of pathogens in positive blood cultures and impact on time to results and workflow**. *J Clin Microbiol* 2018, **56**.

31. Altobelli E, Mohan R, Mach KE, Sin MLY, Anikst V, Buscarini M, Wong PK, Gau V, Banaei N, Liao JC: **Integrated biosensor assay for rapid uropathogen identification and phenotypic antimicrobial susceptibility testing**. *Eur Urol Focus* 2017, **3**:293-299.

32. •• Baltekin O, Boucharin A, Tano E, Andersson DI, Elf J: **Antibiotic susceptibility testing in less than 30 min using direct single-cell imaging**. *Proc Natl Acad Sci U S A* 2017, **114**:9170-9175.

Through time-lapse microscopy, the authors managed to measure ciprofloxacin susceptibility in *E. coli* isolates in under 30 min.

33. •• Schoepp NG, Schlappi TS, Curtis MS, Butkovich SS, Miller S, Humphries RM, Ismagilov RF: **Rapid pathogen-specific phenotypic antibiotic susceptibility testing using digital LAMP quantification in clinical samples**. *Sci Transl Med* 2017, **9**.

Utilizing isothermal nucleic acid amplification, the authors were able to detect ciprofloxacin and nitrofurantoin susceptibility of *E. coli* directly from urine samples in under 30 min.

34. Harris SR, Cartwright EJ, Torok ME, Holden MT, Brown NM, Ogilvy-Stuart AL, Ellington MJ, Quail MA, Bentley SD, Parkhill J *et al.*: **Whole-genome sequencing for analysis of an outbreak of meticillin-resistant *Staphylococcus aureus*: a descriptive study**. *Lancet Infect Dis* 2013, **13**:130-136.

35. Madigan T, Cunningham SA, Patel R, Greenwood-Quaintance KE, Barth JE, Sampathkumar P, Cole NC, Kohner PC, Colby CE, Asay GF *et al.*: **Whole-genome sequencing for methicillin-resistant *Staphylococcus aureus* (MRSA) outbreak investigation in a neonatal intensive care unit**. *Infect Control Hosp Epidemiol* 2018, **39**:1412-1418.

36. Haas CT, Roe JK, Pollara G, Mehta M, Noursadeghi M: **Diagnostic' omics' for active tuberculosis**. *BMC Med* 2016, **14**:37.

37. The CRyPTIC Consortium, Genomes Project, Allix-Beguec C, Arandjelovic I, Bi L, Beckert P, Bonnet M, Bradley P, Cabibbe AM, Cancino-Munoz I *et al.*: **Prediction of susceptibility to first-line tuberculosis drugs by DNA sequencing**. *N Engl J Med* 2018, **379**:1403-1415.

38. Salipante SJ, Sengupta DJ, Rosenthal C, Costa G, Spangler J, Sims EH, Jacobs MA, Miller SI, Hoogestraat DR, Cookson BT *et al.*: **Rapid 16S rRNA next-generation sequencing of polymicrobial clinical samples for diagnosis of complex bacterial infections**. *PLoS One* 2013, **8**:e65226.

39. Greninger AL, Naccache SN, Federman S, Yu G, Mbala P, Bres V, Stryke D, Bouquet J, Somasekar S, Linnen JM *et al.*: **Rapid metagenomic identification of viral pathogens in clinical samples by real-time nanopore sequencing analysis**. *Genome Med* 2015, **7**:99.

40. Bertelli C, Greub G: **Rapid bacterial genome sequencing: methods and applications in clinical microbiology**. *Clin Microbiol Infect* 2013, **19**:803-813.

41. Clarridge JE 3rd: **Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases**. *Clin Microbiol Rev* 2004, **17**:840-862 table of contents.

42. Mongkolrattanothai K, Dien Bard J: **The utility of direct specimen detection by Sanger sequencing in hospitalized pediatric patients**. *Diagn Microbiol Infect Dis* 2017, **87**:100-102.

43. Moon J, Kim N, Lee HS, Shin HR, Lee ST, Jung KH, Park KI, Lee SK, Chu K: **Campylobacter fetus meningitis confirmed by a 16S rRNA gene analysis using the MinION nanopore sequencer, South Korea, 2016**. *Emerg Microbes Infect* 2017, **6**:e94.

44. Mitsuhashi S, Kryukov K, Nakagawa S, Takeuchi JS, Shiraishi Y, Asano K, Imanishi T: **A portable system for rapid bacterial composition analysis using a nanopore-based sequencer and laptop computer**. *Sci Rep* 2017, **7**:5657.

45. Colman RE, Anderson J, Lemmer D, Lehmkuhl E, Georghiou SB, Heaton H, Wiggins K, Gillece JD, Schupp JM, Catanzaro DG *et al.*: **Rapid drug susceptibility testing of drug-resistant *Mycobacterium tuberculosis* isolates directly from clinical samples by use of amplicon sequencing: a proof-of-concept study**. *J Clin Microbiol* 2016, **54**:2058-2067.

46. •• Allicock OM, Guo C, Uhlemann AC, Whittier S, Chauhan LV, Garcia J, Price A, Morse SS, Mishra N, Briese T *et al.*: **BacCapSeq: a platform for diagnosis and characterization of bacterial infections**. *MBio* 2018, **9**.

This study demonstrates the use of high-throughput targeted sequencing of a panel of human pathogens and resistant determinants using probes to capture the target sequence. With improved speeds, this method could be a powerful diagnostic tool.

47. Briese T, Kapoor A, Mishra N, Jain K, Kumar A, Jabado OJ, Lipkin WI: **Virome capture sequencing enables sensitive viral diagnosis and comprehensive virome analysis**. *MBio* 2015, **6**: e01491-01415.

48. • Doyle RM, Burgess C, Williams R, Gorton R, Booth H, Brown J, Bryant JM, Chan J, Creer D, Holdstock J *et al.*: **Direct whole-genome sequencing of sputum accurately identifies drug-resistant *Mycobacterium tuberculosis* faster than MGIT culture sequencing**. *J Clin Microbiol* 2018, **56**.

This study presents the potential to significantly speed up TB diagnosis by sequencing the whole genome of Mtb directly from sputum samples using RNA baits. With this method it is possible to predict Mtb resistance phenotype within days rather than weeks.

49. Doughty EL, Sergeant MJ, Adetifa I, Antonio M, Pallen MJ: **Culture-independent detection and characterisation of *Mycobacterium tuberculosis* and *M. africanum* in sputum samples using shotgun metagenomics on a benchtop sequencer**. *PeerJ* 2014, **2**:e585.

50. Couto N, Schuele L, Raangs EC, Machado MP, Mendes CI, Jesus TF, Chlebowicz M, Rosema S, Ramirez M, Carrico JA *et al.*: **Critical steps in clinical shotgun metagenomics for the concomitant detection and typing of microbial pathogens**. *Sci Rep* 2018, **8**:13767.

51. Ruppe E, Lazarevic V, Girard M, Mouton W, Ferry T, Laurent F, Schrenzel J: **Clinical metagenomics of bone and joint infections: a proof of concept study**. *Sci Rep* 2017, **7**:7718.

52. Yan Q, Cui S, Chen C, Li S, Sha S, Wan X, Yang R, Xin Y, Ma Y: **Metagenomic analysis of sputum microbiome as a tool toward culture-independent pathogen detection of patients with ventilator-associated pneumonia**. *Am J Respir Crit Care Med* 2016, **194**:636-639.

53. Greninger AL, Zerr DM, Qin X, Adler AL, Sampoleo R, Kuypers JM, Englund JA, Jerome KR: **Rapid metagenomic next-generation sequencing during an investigation of hospital-acquired human parainfluenza virus 3 infections**. *J Clin Microbiol* 2017, **55**:177-182.

54. Lewandowska DW, Zagordi O, Zbinden A, Schuurmans MM, Schreiber P, Geissberger F-D, Huder JB, Böni J, Benden C, Mueller NJ *et al.*: **Unbiased metagenomic sequencing complements specific routine diagnostic methods and increases chances to detect rare viral strains**. *Diagn Microbiol Infect Dis* 2015, **83**:133-138.

55. Naccache SN, Peggs KS, Mattes FM, Phadke R, Garson JA, Grant P, Samayoa E, Federman S, Miller S, Lunn MP *et al.*: **Diagnosis of neuroinvasive astrovirus infection in an immunocompromised adult with encephalitis by unbiased next-generation sequencing**. *Clin Infect Dis* 2015, **60**:919-923.

56. Heather JM, Chain B: **The sequence of sequencers: the history of sequencing DNA**. *Genomics* 2016, **107**:1-8.

57. PacBio: *SMRT Sequencing: Read Lengths*. 2017 . Accessed: 2018 *https://www.pacb.com/smrt-science/smrt-sequencing/read-lengths/*.

58. Oxford Nanopore Technologies: *Longer and Longer: DNA Sequence of More than Two Million Bases Now Achieved with Nanopore Sequencing*. 2018 . Accessed: 2018 *https://nanoporetech.com/about-us/news/longer-and-longer-dna-sequence-more-two-million-bases-now-achieved-nanopore*.

59. Jain M, Koren S, Miga KH, Quick J, Rand AC, Sasani TA, Tyson JR, Beggs AD, Dilthey AT, Fiddes IT *et al.*: **Nanopore sequencing and assembly of a human genome with ultra-long reads**. *Nat Biotechnol* 2018, **36**:338.

60. Schmidt K, Mwaigwisya S, Crossman LC, Doumith M, Munroe D, Pires C, Khan AM, Woodford N, Saunders NJ, Wain J *et al.*: **Identification of bacterial pathogens and antimicrobial resistance directly from clinical urines by nanopore-based metagenomic sequencing**. *J Antimicrob Chemother* 2017, **72**:104-114.

61. Leggett RM, Alcon-Giner C, Heavens D, Caim S, Brook TC, Kujawska M, Hoyles L, Clarke P, Hall L, Clark MD: **Rapid MinION metagenomic profiling of the preterm infant gut microbiota to aid in pathogen diagnostics**. *bioRxiv* 2017 https://doi.org/10.1101/180406.

62. •• Charalampous T, Richardson H, Kay GL, Baldan R, Jeanes C, Rae D, Grundy S, Turner DJ, Wain J, Leggett RM *et al.*: **Rapid diagnosis of lower respiratory infection using nanopore-based clinical metagenomics**. *bioRxiv* 2018 https://doi.org/10.1101/387548.

This study describes a newly developed clinical metagenomics pipeline that incorporates host DNA depletion and MinION sequencing to characterise lower respiratory infections in as little as 6 hours, giving both pathogen ID and information about resistance determinants in a clinically useful timeframe.

63. Quick J, Loman NJ, Duraffour S, Simpson JT, Severi E, Cowley L, Bore JA, Koundouno R, Dudas G, Mikhail A *et al.*: **Real-time, portable genome sequencing for Ebola surveillance**. *Nature* 2016, **530**:228-232.

64. •• Břinda K, Callendrello A, Cowley L, Charalampous T, Lee RS, MacFadden DR, Kucherov G, Grady J, Baym M, Hanage WP: **Lineage calling can identify antibiotic resistant clones within minutes**. *bioRxiv* 2018 https://doi.org/10.1101/403204.

The authors in this study design a database to rapidly identify pathogen lineage from clinical samples to predict resistance/susceptibility phenotype in as little as five minutes from nanopore metagenomics data.