




New Microbiological Techniques for the Diagnosis of Bacterial Infections and Sepsis in ICU Including Point of Care

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Abstract

Purpose of Review The aim of this article is to review current and emerging microbiological techniques that support the rapid diagnosis of bacterial infections in critically ill patients, including their performance, strengths and pitfalls, as well as available data evaluating their clinical impact.

Recent Findings Bacterial infections and sepsis are responsible for significant morbidity and mortality in patients admitted to the intensive care unit and their management is further complicated by the increase in the global burden of antimicrobial resistance. In this setting, new diagnostic methods able to overcome the limits of traditional microbiology in terms of turn-around time and accuracy are highly warranted. We discuss the following broad themes: optimisation of existing culture-based methodologies, rapid antigen detection, nucleic acid detection (including multiplex PCR assays and microarrays), sepsis biomarkers, novel methods of pathogen detection (e.g. T2 magnetic resonance) and susceptibility testing (e.g. morphokinetic cellular analysis) and the application of direct metagenomics on clinical samples. The assessment of the host response through new “omics” technologies might also aid in early diagnosis of infections, as well as define non-infectious inflammatory states.

Summary Despite being a promising field, there is still scarce evidence about the real-life impact of these assays on patient management. A common finding of available studies is that the performance of rapid diagnostic strategies highly depends on whether they are integrated within active antimicrobial stewardship programs. Assessing the impact of these emerging diagnostic methods through patient-centred clinical outcomes is a complex challenge for which large and well-designed studies are awaited.

Keywords Rapid diagnostics · Bloodstream infection · Sepsis · Antimicrobial resistance · Critical care

Introduction

Bacterial infections are common in adults and children admitted to the intensive care unit (ICU). In a cohort of 198 ICUs in 24 European countries, including 3147 patients, 37.4% had sepsis, with 24.7% presenting with sepsis on admission [1]. Infections in these patients are associated with significant

morbidity, mortality and cost [2]. The risks associated with infection also result in high usage of antibiotics; in a global point-prevalence study, 70% of all ICU patients were receiving at least one antibiotic on any given day [3]. Being able to rapidly, and accurately, determine the causative pathogen in bacterial infections is a critical step in clinical management. Furthermore, with the growing global burden of antimicrobial resistance, rapid antimicrobial susceptibility testing (AST) is increasingly important to guide therapy. Given the necessity of reducing excessive antibiotic use, we also urgently need diagnostic strategies that can help exclude the presence of infection and define non-infectious inflammatory states for which antibiotics are not required [4].

Current diagnostic methods in patients presenting with sepsis largely rely on the culture of micro-organisms from blood to detect bacteraemia. However, not only is this approach relatively slow and laborious, culture-based systems suffer from a number of pre-analytical limitations that may affect performance, such as inadequate blood volume collection,

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prior antibiotic exposure and delays in laboratory processing or transportation, especially if laboratory facilities are off-site. Furthermore, even when an organism is cultured, definitive identification and susceptibility testing may be delayed for few days. Contamination is a frequent problem that may occur at blood culture (BC) collection and may drive inappropriate antibiotic use, misdirect clinical diagnosis and expose patients to unnecessary toxicities [5]. There are also many fastidious pathogens, which can be challenging to culture in standard automated systems [6].

While, in some respects, clinical microbiology laboratories have relied upon techniques that have evolved little for many decades, there are a number of emerging or newly established technologies that are set to revolutionise how microbial diagnostics may be performed in the near future. Mass-spectrometry methods were not part of routine laboratory practice a decade ago, yet matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF) has now rapidly replaced conventional bacterial identification methods in many laboratories, with step-wise improvements in turn-around times (TATs), accuracy and reduced costs [7]. This article aims to review the current state of the art, as well as new and emerging technologies, that may improve our capacity for rapid and accurate microbiological diagnosis in patients with significant bacterial infections and sepsis.

Established Rapid Diagnostic Methods

In recent years, implementation of existing rapid diagnostic technologies and improved automated workflow systems into clinical laboratories has enabled better delivery of healthcare [8, 9]. Currently, automated BC systems are the gold standard for bloodstream infection detection [10]. Many automated BC systems exist (e.g. BACTEC™ FX, BacT/ALERT®) which apply different methods to detect organism growth (i.e. different nutrients and antimicrobial binding agents) and their performance has been compared [11–15]. In one study, a shorter time to detection and bacterial recovery rate was observed in the BacT/ALERT® VIRTUO system when compared to others [11]. Bottles containing antibiotic binding agents typically have better bacterial recovery rates [16, 17]. Most automated BC systems have an internal sensor that detects carbon dioxide or pH as an indicator of microbial growth [18]. Microscopy and Gram stain on sterile fluids such as blood is a crucial step in providing critical information to inform diagnosis and management of severe infection. Despite automated Gram staining systems, interpretation remains laborious and time intensive, and is still operator dependent [19]. Automated image acquisition and machine learning-based approaches for automated Gram stain classifications have been explored, showing promising accuracy although still far from being able to perform as fully automated systems [20]. Early

identification of important organisms such as *Staphylococcus aureus* directly from BC by coagulase testing has improved TAT and is inexpensive and easily incorporated into standard workflows [21, 22]. Rapid antimicrobial susceptibility methods have been developed to reduce the time to results. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) has developed a standardised rapid method based on disc diffusion that offers antimicrobial susceptibility results within 4–8 h from BC positivity [23–25]. Detection of common mediators of resistance, such as β -lactamases, direct from clinical samples, is also possible. Rapid detection of clinically relevant carbapenemase- and extended-spectrum β -lactamase (ESBL)-producing organisms can be achieved through a variety of commercial assays (e.g. RAPIDEC® CARBA NP, ESBL-NP) [26, 27]. Using a pH indicator to detect carbapenem hydrolysis, accurate and rapid (under 2 h) detection of carbapenemases from clinical isolates using a short incubation culture time can be demonstrated [28]. Similar reductions in TAT have been seen with rapid ESBL detection assays when compared with conventional susceptibility testing [29].

Direct antigen testing of clinical samples has aided in the rapid species identification. Urine antigen testing has been widely used for pathogen detection in respiratory infections caused by *Legionella pneumophila* and *Streptococcus pneumoniae* [30]. Antigens shed from these organisms and excreted in the urinary tract are usually detected by enzyme immunoassay (EIA) or lateral flow assay (LFA) [31]. Despite a shortened TAT, antigen testing suffers from poor sensitivity and specificity (for example, in children colonised with *S. pneumoniae*) and is unable to provide antibiotic susceptibility profiles or other epidemiological data [32]. Rapid antigen detection from other clinical samples such as blood, throat swab, synovial fluid, pleural fluid and cerebrospinal fluid (CSF) has been examined previously but is not as commonly used in practice [33–35]. A large retrospective multicentre study assessed the clinical utility of rapid bacterial antigen detection using latex agglutination and concluded that they were costly and of no detectable clinical benefit [36]. Nucleic acid amplification testing (NAAT) or polymerase chain reaction (PCR) tests are a reliable non-culture microbial detection method, frequently used in laboratories around the globe for the diagnosis of a wide array of microbial pathogens. In addition, multiplex PCR incorporates several primers and probes within one reaction tube to amplify gene targets from multiple pathogens [37]. This highly sensitive approach increases the diagnostic yield and can be used on many clinical specimens including respiratory secretions, CSF, sterile fluids and diarrhoeal faeces. Limitations of PCR testing include reporting of incidental results, a lack of distinction between colonisation versus infection, requirement for experienced operators and a dedicated laboratory environment, and the absence of antibiotic susceptibility data [38]. Furthermore, PCR

will only detect pathogens specifically targeted by the assay design. Rare and unexpected organisms, or strains with variants in target sequences, may be missed. The accuracy and favourable positive predictive value rests upon the correct clinical setting, e.g. *Clostridoides difficile* PCR testing in diarrhoea and multiplex bacterial PCR testing on CSF with pleocytosis.

The immune response to severe infection and sepsis is complex with a wide variety of inflammatory and anti-inflammatory mediators released [39]. Numerous biomarkers have been explored to assist in the rapid diagnosis of serious infections in ICU. Along with the leucocyte count, the most established of these is C-reactive protein (CRP). CRP is an acute phase protein that increases following interleukin-6 secretion by macrophages and T-cells, and has been shown to be a sensitive but not specific marker of sepsis [40]. Procalcitonin is a peptide secreted by many cells in the body in response to a pro-inflammatory stimulus, and may be more specific as a marker of bacterial infection than CRP [41]. The complexity of the host response is reflected in the range of biomarkers under investigation as potential markers of serious infection, including acute phase reactants, cytokines (in particular interleukin-6 and interleukin-8), soluble receptors and cell surface and endothelial markers [42]. Used individually or in combination, the role of biomarkers is to stratify the risk of serious infection, or crudely predict the likely aetiology and guide decisions on initiating or stopping antibiotics. Despite the limited nature of their predictions, evidence from randomised trials appears to support a role in ICU. While of limited value in guiding treatment initiation, the use of procalcitonin supported decisions to stop antibiotics, and reduced the duration of antimicrobial therapy in both adult and neonatal ICU [43, 44].

New and Emerging Methods

In recent years, new rapid diagnostic tests (RDTs) have emerged that are able to provide pathogen identification and resistance profile within a short TAT. Their potential in improving patient management is promising although studies on their clinical impact remain scarce [45].

Emerging Diagnostics for Meningitis and Severe Respiratory Infections

Multiplex PCR are increasingly used in clinical practice for the diagnosis of central nervous system infections and pneumonia in the ICU setting. The BioFire FilmArray Meningitis/Encephalitis panel (bioMérieux) is an FDA-cleared, multiplex PCR detecting 14 pathogens from CSF in 1 h. Estimated sensitivity and specificity are 90 and 97% respectively [46]

although evidence is scarce about its impact on patients' outcomes [47, 48].

Similarly, the BioFire FilmArray Pneumonia plus Panel can detect 27 microorganisms and 7 resistance markers on respiratory specimens, including nosocomial pathogens associated to hospital-acquired or ventilator-acquired pneumonia, and its role in improving antimicrobial stewardship (AMS) in critically ill patients with coronavirus disease 2019 has been suggested [49]. Other multiplex PCR (e.g. Seegene Allplex Respiratory panel) have narrower panels, more useful for community-acquired respiratory infections [50].

Nucleic Acid Detection from Blood Cultures

Several techniques are emerging for pathogen identification from positive BCs. The BioFire FilmArray BC identification panel (BCID, bioMérieux) is a multiplex PCR which detects 24 pathogens and 3 resistance genes from positive cultures with good analytical performance [51, 52]. In a study on ICU patients with culture-confirmed sepsis, this test reduced the time to optimal treatment compared to standard BC [53] and a role in diagnosing ventilator-associated pneumonia has also been suggested [54]. A new version of this test has been recently released (BioFire BCID2) with a broader panel including 43 targets, although clinical evaluation studies are awaited.

In the specific setting of *S. aureus* bacteraemia, the Xpert MRSA/SA BC Assay (Cepheid) can detect through a real-time PCR methicillin-susceptible and methicillin-resistant *S. aureus* (MRSA) from positive BC. This test is associated with high sensitivity and specificity [55] and its automation easily fits the laboratory routine. Similarly, the Cepheid Xpert Carba-R assay is designed for the detection of genes encoding for carbapenemases from cultured bacterial isolates; however, it has been assessed as a method of direct detection from BC in settings with high carbapenem resistance prevalence [56]. The Verigene system (Luminex) uses multiplex PCRs and subsequent microarray hybridisation for detection of 22 bacteria and their resistance determinants from positive BC [57, 58], and comprises two different panels, for Gram positives and Gram negatives, whose choice can be driven by the Gram stain results. Verigene has proved able to identify susceptibility to new β -lactam/ β -lactamase inhibitors [59], and when implemented within an AMS program, reduced the time to optimal therapy in bacteraemic patients [60, 61].

Other technologies applied to positive BC include fluorescent in situ hybridisation (FISH) using peptide nucleic acid (PNA) probes targeting 16S or 18S rRNA of bacteria and fungi respectively. PNA-FISH (AdvanDx) comprises 4 different panels and improvement of early treatment appropriateness has been demonstrated when integrated with AMS [62, 63].

Pathogen Detection Direct from Blood

To skip the time-consuming step of BC growth, new technologies are emerging that may be used directly on whole blood. Among NAAT-based methods, Lightcycler SeptiFast Test (Roche) and Magicplex Sepsis Real-Time test (Seegene) are real-time PCR assays detecting several microorganisms and some markers of resistance from whole blood. Despite having broad panels, their low sensitivity [64–68] makes recommendations about their clinical use difficult. Indeed, SeptiFast was recently discontinued.

The combination of pathogen-specific PCR with miniaturised magnetic resonance has been realised in the T2 magnetic resonance (T2MR), able to identify microorganisms from whole blood with a brand-new methodology; specifically, the DNA amplified by PCR binds by complementary probes to paramagnetic nanoparticles, whose signal is identified by T2MR [69]. The T2Candida test (T2 Biosystems) is an automated system which identifies the most common *Candida* species with high negative predictive values across a wide range of pre-test probabilities [70, 71]. T2Candida has shown to shorten the time to effective antifungal therapy and reduce inappropriate empirical treatments, as well as to predict poor clinical outcomes in suspected and proven candidemia [71]. Similarly, the T2Bacteria detects the ESKAPE bacteria (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Escherichia coli*) [72–74]. However, due to the limited panel of pathogens and resistance genes detected, its clinical benefit remains uncertain [75]. MALDI-TOF has been used directly on clinical samples including blood, urine [76] and CSF [77]. PCR/ESI-MS combines pathogen-specific PCR with mass spectrometry based on electrospray ionisation (ESI-MS): the IRIDICA system (Abbott) could detect 780 microorganisms and 4 resistance genes from various samples. However, despite promising performances, this assay has been discontinued [78] illustrating that a clear benefit of implementing these expensive tests is not yet apparent.

Direct Metagenomics

Metagenomics-based assays are among the most promising emerging tools in clinical microbiology as they can potentially identify any microorganisms in a given sample.

16S metagenomics is based on amplification through universal primers of the bacterial 16S rRNA gene, followed by amplicon sequencing, which leads to bacterial identification and taxonomic profiling [79]. Sepsitest (Molzym) is a semiautomated assay based on such technology used for pathogen detection from blood: despite being able to detect polymicrobial infections and fastidious organisms, its role in informing clinical decisions is limited as it suffers of low sensitivity and does not provide AST [64]. Compared to 16S metagenomics, shotgun metagenomics is based on untargeted

next-generation sequencing (NGS), which reads complete bacterial genomes by massive parallel sequencing, providing a precise taxonomic resolution of all pathogens in a sample and potentially detects markers of antimicrobial resistance [80]. iDTECT Dx Blood (PathoQuest) is based on untargeted NGS and has been demonstrated to detect more clinically relevant microorganisms than conventional microbiology in immunocompromised patients, with a high negative predictive value [81]. Similarly, Karius NGS Plasma Test (Karius), which can identify microbial cell-free DNA from over 1200 microorganisms, showed 93.7% sensitivity compared to BCs in patients with suspected sepsis [82] and may be able to identify clinically relevant pathogens in blood in the days before the onset of bloodstream infections [83].

The ability of shotgun-metagenomics to perform a comprehensive analysis of the microbial genetic material in a biological sample holds great promise. However, limitations exist that make the implementation of these assays complex and a limited real-life clinical impact for diagnosis of infection has been reported by some studies [84, 85]. The frequent detection of contaminants and colonisers affects NGS specificity and complicates the interpretation of results in diagnosing bloodstream infections; to address this limitation, a recent study on patients with septic shock showed the utility of the sepsis indicating quantifier (SIQ) score as a means of discriminating clinically relevant pathogens from the others [86]. Moreover, NGS sensitivity is decreased in samples with high nucleic acid background such as blood, thus requiring human DNA depletion. Such techniques also lack standardisation of analysis methods. Frequently, bioinformatics skills needed to analyse NGS data are unavailable in a standard diagnostic laboratory and may require external expertise or data transfer to other facilities. This introduces delays, as well as additional costs, challenges in computational and data storage capacity, data privacy issues and complexities for accreditation with regulatory authorities.

New Rapid AST Methods

The detection of resistance genes is not always reliable to reflect the actual susceptibility pattern of the identified pathogen. The FDA-approved Accelerate Pheno system (Accelerate Diagnostics) can detect 16 microorganisms from positive BC based on FISH technology as well as perform phenotypic AST by morphokinetic cellular analysis [87–89], with over 96% categorical agreement in comparison to standard methods [89, 90] (Table 1). Studies showed this test improves achievement of and time to optimal therapy in patients with bacteraemia [92, 93].

Advances in microfluidics, electronics, optic and biosensor techniques are promising approaches for next-generation rapid AST and at the early stages of translation into practice. Evidences on their role to address point of care testing

Table 1 Commercially available rapid diagnostic tests for the diagnosis of bloodstream infections

Technology	Assay (manufacturer)	TAT (h)	Organisms detected	Resistance genes detected	Sensitivity/specificity (%)	Ref
From positive blood cultures						
Multiplex PCR	The BioFire FilmArray blood culture identification panel 2 (BCID2) (bioMérieux)	1	11 Gram positives <i>Staphylococcus</i> spp., <i>Staphylococcus aureus</i> , <i>S. epidermidis</i> , <i>S. lugdunensis</i> , <i>Streptococcus</i> spp., <i>S. agalactiae</i> , <i>S. pyogenes</i> , <i>S. pneumoniae</i> , <i>E. faecalis</i> , <i>E. faecium</i> , <i>L. monocytogenes</i> 15 Gram negatives <i>A. calcoaceticus-baumannii</i> complex, <i>B. fragilis</i> , <i>H. influenzae</i> , <i>N. meningitidis</i> , <i>P. aeruginosa</i> , <i>S. maltophilia</i> , <i>Enterobacteriales</i> : <i>E. coli</i> , <i>E. cloacae</i> complex, <i>K. aerogenes</i> , <i>K. oxytoca</i> , <i>K. pneumoniae</i> group, <i>Proteus</i> spp., <i>Salmonella</i> , <i>S. marcescens</i> 7 fungal species <i>C. albicans</i> , <i>C. auris</i> , <i>C. glabrata</i> , <i>C. krusei</i> , <i>C. parapsilosis</i> , <i>C. tropicalis</i> , <i>C. neoformans/gattii</i>	<i>mecA/C</i> , <i>mecA/C</i> and <i>MREJ</i> (MRSA), <i>van A/B</i> , <i>blaKPC</i> , <i>blaIMP</i> , <i>blaOXA-48</i> , <i>blaNDM</i> , <i>blaVIM</i> , <i>mcr-1</i> , <i>CTX-M</i>	91–96/98–100	[51–53]
Real-time multiplex PCR	Xpert MRSA/SA Blood Culture Assay (Cepheid)	1–2	<i>Staphylococcus aureus</i> , MRSA	<i>mecA</i>	98–100/99.5	[55]
DNA microarray	Verigene Gram Positive Blood Culture Test (Luminex)	2.5	13 Gram positives <i>Staphylococcus</i> spp., <i>Staphylococcus aureus</i> , <i>S. epidermidis</i> , <i>S. lugdunensis</i> , <i>Streptococcus</i> spp., <i>S. agalactiae</i> , <i>S. pneumoniae</i> , <i>S. pyogenes</i> , <i>S. anginosus</i> , <i>E. faecalis</i> , <i>E. faecium</i> , <i>Micrococcus</i> spp., <i>Listeria</i> spp.	<i>mecA</i> , <i>van A/B</i>	93–100/94.5–100	[58]
	Verigene Gram Negative Blood Culture Test (Luminex)	2.5	9 Gram negatives <i>E. coli</i> , <i>K. pneumoniae</i> , <i>K. oxytoca</i> , <i>S. marcescens</i> , <i>Citrobacter</i> spp., <i>Enterobacter</i> spp., <i>Proteus</i> spp., <i>Acinetobacter</i> spp., <i>P. aeruginosa</i>	<i>mecA</i> , <i>van A/B</i> , <i>bla_{CTX-M}</i> , <i>bla_{KPC}</i> , <i>bla_{OXA-48}</i> , <i>bla_{IMP}</i> , <i>bla_{VIM}</i> , <i>bla_{NDM}</i>	98/100	[57]
In situ hybridization	- <i>Staphylococcus aureus</i> /CNS PNA FISH (AdvanDx)	1.5–3	<i>S. aureus</i> , CoNS	-	88–98/>98	[91]
	- <i>E. faecalis</i> /OE PNA FISH (AdvanDx)		<i>E. faecalis</i> , <i>E. faecium</i> , <i>Enterococcus</i> spp.	-	97/100	[91]
	-Gram-Negative PNA FISH (AdvanDx)		<i>E. coli</i> , <i>K. pneumoniae</i> , <i>P. aeruginosa</i>	-	99/98	[91]
	- <i>Candida</i> PNA FISH (AdvanDx)		<i>C. albicans</i> / <i>C. parapsilosis</i> , <i>C. tropicalis</i> , <i>C. glabrata</i> / <i>C. krusei</i>	-	99/100	[91]
In situ hybridization + morphokinetic cellular analysis for AST	Quick-FISH Accelerate PhenoTest BC (Accelerate Diagnostics)	0.5 1 (7 for A-S-T)	(same 4 panels of PNA-FISH) 6 Gram positives <i>CoNS</i> spp., <i>E. faecalis</i> , <i>E. faecium</i> , <i>S. aureus</i> , <i>S. lugdunensis</i> , <i>Streptococcus</i> spp. 8 Gram negatives <i>A. baumannii</i> , <i>Citrobacter</i> spp., <i>Enterobacter</i> spp., <i>E. coli</i> , <i>Klebsiella</i> spp., <i>Proteus</i> spp., <i>P. aeruginosa</i> , <i>S. marcescens</i> 2 <i>Candida</i> species <i>C. albicans</i> , <i>C. glabrata</i>	- AST results as MIC	98–100/98–100 95–97.5/99–99.5 (for ID)	[91] [87, 88, 90]
From whole blood						
Multiplex real-time PCR	Magicplex Sepsis Real-Time test (Seegene)	3–5	73 Gram positives (40 <i>Streptococcus</i> spp., 30 <i>Staphylococcus</i> spp., 3 <i>Enterococcus</i> spp.)	<i>mecA</i> , <i>van A/B</i>	29–65/66–95	[67, 68]

Table 1 (continued)

Technology	Assay (manufacturer)	TAT (h)	Organisms detected	Resistance genes detected	Sensitivity/specificity (%)	Ref
			12 Gram negatives <i>E. coli</i> , <i>K. pneumoniae</i> , <i>K. oxytoca</i> , <i>S. marcescens</i> , <i>B. fragilis</i> , <i>S. thyphi</i> , <i>E. cloacae</i> , <i>E. aerogenes</i> , <i>P. mirabilis</i> , <i>P. aeruginosa</i> , <i>A. baumannii</i> , <i>S. maltophilia</i>			
			6 fungi <i>C. albicans</i> , <i>C. tropicalis</i> , <i>C. parapsilosis</i> , <i>C. krusei</i> , <i>C. glabrata</i> , <i>A. fumigatus</i>			
PCR + miniaturised magnetic resonance	T2Candida panel (T2 Biosystems)	3–5	5 <i>Candida</i> species <i>C. albicans</i> / <i>C. tropicalis</i> , <i>C. glabrata</i> / <i>C. krusei</i> and <i>C. parapsilosis</i>	-	89–91/98–100	[70, 71]
	T2Bacteria panel (T2 Biosystems)	4–7	<i>E. faecium</i> , <i>S. aureus</i> , <i>K. pneumoniae</i> , <i>A. baumannii</i> , <i>P. aeruginosa</i> , <i>E. coli</i>	-	83–90/90–98	[72–74]
Broad range PCR + sequencing	SepsiTest (Molzzy)	8–18	Over 345 bacteria and 8 fungi	-	48/86	[64]
Untargeted NGS	iDTECT Dx Blood (PathoQuest)	60 ^a	Over 1200 pathogens (bacteria and viruses)	-	(Negative predictive value: 98.4%)	[81]
Untargeted NGS	Karius NGS plasma Test (Karius)	53 ^a	Over 1200 pathogens (bacteria, fungi, viruses and parasites)	-	93/63	[82]

TAT, turn-around time; PCR, polymerase chain reaction; MRSA, methicillin-resistant *S. aureus*; CoNS, coagulase-negative staphylococci; PNA, peptide nucleic acid; FISH, fluorescent in situ hybridisation; AST, antimicrobial susceptibility testing; MIC, minimum inhibitory concentration; ID, identification

^a Including sample shipment

(POCT) needs, however, are still scarce, and studies are still in progress to achieve FDA approval and CE mark [94].

Table 1 summarises performance of the main commercially available RDTs for bloodstream infections.

Host Response and Transcriptomics

Existing biomarkers provide a limited insight into the complex host response to infection, and consequently offer limited discrimination between infectious aetiologies. Indeed, commonly used infection biomarkers offer only a binary outcome of severity of infection, or probability of bacterial infection to guide antibacterial use. There is growing interest in the role of omics technologies to interrogate the proteome, metabolome, epigenome or transcriptome to more comprehensively characterise infection phenotypes. Such biological classifiers are established in the fields of oncology [95] and cardiovascular disease [96], though the time-critical nature of infections in ICU adds a further challenge. Diagnostics based on differential gene expression in acute infections are of substantial interest. Classifiers such as the ‘Integrated Antibiotics Decision Model’ [97] and a ‘Disease Risk Score’ in febrile children [98] have undergone external validation to suggest they have potential value in guiding treatment decisions. Septicyte was the first such transcript-based infectious disease diagnostic to receive FDA approval in 2017 and continues to

undergo external validation to demonstrate its value in different clinical contexts [99]. Transcript-based classifiers have the potential to characterise patients not only by pathogen (bacterial, viral or fungal) but by inflammatory phenotype thereby offering the possibility of successful personalised immunomodulation in sepsis [100]. Establishing the role of transcript-based disease classifiers in infections on ICU will require an understanding of how such assays can be performed in a timely way, and a demonstration of their impact, including cost-effectiveness, in clinical trials.

How Should the Clinical Utility of Novel Rapid Diagnostics Be Evaluated?

Reduced turn-around time (TAT) in either identification or susceptibility information is not sufficient to indicate the improved utility of a test, though it is an important component [101]. Other parameters include the sensitivity, specificity, type of result yielded and the confidence of the relevant clinician acting upon the result [102]. Arguably, a full assessment of the impact and value of rapid diagnostic microbiology technologies evaluates more than TATs and AMS outcomes. We need controlled trials or interrupted time series analyses over extended periods, evaluating multiple key clinical and process outcomes such as mortality, acute kidney injury, length of stay

and readmission. This would ideally be combined with a comprehensive cost-effectiveness analysis, assessing not only hospital admission costs, but value of quality-adjusted-life-year (QALY) saved, costs of laboratory implementation of RDT programs and adjunct AMS programs.

There is a paucity of high-quality evidence in this field, though there are numerous quasi-studies that have evaluated AMS outcomes with several incorporating a selection of clinical or process outcomes [45, 103, 104]. The most consistent, though not universal finding, has been that rapid technologies alone do not translate even to better AMS outcomes, let alone improved clinical outcomes, without also embedding customised AMS support strategies and this is reflected in the Infectious Diseases Society of America (IDSA) guidelines [45, 52, 101, 105, 106].

Targeted AMS strategies that have been evaluated to support implementation of RDT range from extended hours of the service, notification of critical results to a member of the AMS team who provides targeted direct advice and other activities that increase clinical interaction [105, 107]. When these strategies are coupled with RDTs, improvements in optimal antimicrobial use and de-escalation are the most consistent findings, with cost saving the least represented [45, 108, 109]. Impact on clinical outcomes has been highly variable in studies assessing length of stay, mortality and re-admission [110–112]. The reasons for this have not been rigorously studied but based on other stewardship research likely pertain to prescribing behaviour, lack of familiarity, and experience or expert knowledge in the actionability of RDT results [113, 114]. The likelihood of a clinical de-escalation of antimicrobials overnight is low, even if microbiology and AMS teams extend their hours of operation for a 24/7 model, reflecting most likely a combination of either junior clinical staff or caretaker culture overnight [105].

The most actionable results include (i) identification of likely contaminants and (ii) detection of a molecular target correlated with resistance not covered by the empiric regimen. One of the molecular RDTs most rigorously assessed for AMS and clinical impact has been the Verigene system. The results for Gram-positive BC organisms were demonstrably more actionable than for Gram negatives, reflecting both the complexity of genetic markers of resistance and the morbidity and mortality associated with early suboptimal treatment of Gram-negative sepsis [105]. Multiplexed PCR assays are limited in the number of target genes that can be identified and do not comprehensively cover all relevant resistance mechanisms. Despite this, Verigene has outperformed clinical risk tools for predicting third-generation cephalosporin resistance, though the applicability of this result will be variable depending on the community prevalence of ESBL, in particular [107]. Another significant limitation of most molecular RDT systems is the suboptimal sensitivity in detecting polymicrobial infection limiting confidence in de-escalation [105].

Implementation of molecular RDTs is a relatively resource-intensive measure and current technologies are not stand-alone tests. Conventional phenotypic testing would still need to be performed, particularly for AST [115]. As discussed above, molecular RDT does have some significant limitations including decreased sensitivity in detecting polymicrobial bacteraemia, the potential for cross-contamination or genetic similarity (e.g. *Shigella* and *E. coli*), the restricted range of resistance mechanisms and the lack of clinically validated correlation of genetic makers with minimum inhibitory concentrations (MICs). The latter can be critical to therapeutic drug monitoring and wild-type surveillance or to determine suitability of use of an agent such as meropenem even in the presence of a carbapenemase. Phenotypic RDTs and biomarkers are also usually utilised in conjunction with conventional testing. Improved cost-efficiencies can be associated with targeted use for critically unwell patients, specifically within intensive care, haematology and oncology [113].

Demonstrating benefits from introduction of such services will depend on the institution including local antibiograms, patient complexity and strength of current AMS, and interactions with microbiology and infectious disease teams [109]. Low- and middle-income nations with high rates of community multidrug-resistant organisms may find the costs of molecular methods prohibitive but rapid phenotypic tests or optical sensor portable low footprint techniques may have a significant role [116].

Point of Care Diagnostics

Most of the methods discussed thus far require a well-functioning laboratory, with at least a basic requirement for scientific skills and training. Only assays which are simple to use and have a low risk of incorrect results are generally approved for POCT, with higher complexity tests reserved for suitably equipped laboratories. However, using a laboratory-based test introduces some delay and a degree of distance from the patient and treating clinicians. In geographically dispersed countries with remote locations, this can result in major delays for critical tests such as BCs or molecular diagnostics. In Australia, as in most other jurisdictions, organisations offering POCT must adhere to certain standards that define appropriate governance, maintenance of test integrity, minimisation of pre-analytical, analytical and post-analytical errors, provision of suitable training and competency assessment, with all such processes embedded within a robust quality management system [117]. Such processes have evolved over time to ensure that clinicians have confidence in test results they receive. Currently, no POCT exists for the accurate diagnosis of bloodstream infections or most other critical infections. The future holds some hope for technological advances such as microfluidic devices that can integrate

sampling handling and signal generation within a POCT setting, maybe using testing platforms such as “on-chip” immunoassays or nucleic acid analysis. Such technology can, in theory, incorporate all the key steps of molecular detection: cell lysis and extraction, nucleic acid purification, amplification and detection of reaction products. Such miniaturisation may also allow multiplexing to enable high-throughput testing within a single portable device [118]. There is substantial pre-clinical research into the design, construction materials and detection technologies for such devices, but as yet no commercial products are ready for clinical evaluation.

Conclusions

It is likely that a number of new microbiological methods will enhance our capacity to rapidly and accurately identify pathogens in critically unwell patients. However, well-designed studies assessing key clinical outcomes are needed to define their role in improving the management of severe infections.

Availability of Data and Material Not applicable

Code Availability Not applicable

Declarations

Conflict of Interest PNAH reports grants from Shionogi, MSD and Sandoz, as well as personal fees from Sandoz and Pfizer, outside the submitted work. All the other authors declare no conflicts of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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