Introduction

The ongoing global health risk of sepsis has been widely reported in recent years. Despite being one of the leading causes of death in patients admitted to hospital emergency departments, its diagnosis, treatment and, therefore, clinical outcomes, are relatively poor. Between 27 and 30 million people worldwide are estimated to develop sepsis per year [1], and a recent global assessment of the mortality rate of patients with sepsis treated in an intensive care unit (ICU) found that more than one third died [2]. These high mortality rates, combined with the global prevalence of sepsis, makes rapid diagnosis and treatment a top priority for public health organisations worldwide.

In addition to the devastating impact on patient health, the cost of sepsis to healthcare systems is of great concern. It costs NHS England approximately £2 billion per year to treat sepsis [3], and it is estimated that if the US achieved earlier sepsis identification and evidence-based treatment, there would be an annual reduction in hospital expenditures of over $1.5 billion [4]. But what actually is sepsis?

Although commonly referred to as blood poisoning or septicaemia, sepsis is the result of the body’s immune response damaging tissues and organs when attempting to fight an infection, and does not necessarily involve bloodstream infection. The seriousness of sepsis can vary, from mild cases, to severe sepsis and septic shock. Vital organs become damaged as the condition progresses and the whole body can be affected. Sepsis can be caused by viral, fungal or bacterial infections, with the latter being the more common cause.

Anyone can develop sepsis, but higher risk groups include those with weaker immune systems, such as infants and the elderly, people with chronic diseases (e.g. diabetes, AIDS, cancer, liver disease or kidney disease), and patients with invasive devices, such as intravenous catheters or breathing tubes.
A number of factors also influence the speed and severity of sepsis progression, such as genetics, other co-morbidities, and the nature of the infecting microorganism. Some patients may not deteriorate until the sepsis has progressed significantly, whereas other cases can rapidly decline and become fatal within a few hours.

Early recognition of sepsis is therefore imperative for good clinical outcomes. There is a widespread need for a better understanding of sepsis diagnosis as a final common pathway of illness due to infection, across healthcare professionals, governments and the general public. Rapid and appropriate antimicrobial therapy as a result of timely diagnosis can save lives – for every hour that correct treatment is delayed, survival decreases by 7.6% [5].

Improving sepsis therapy

The standard procedure for treating sepsis is to begin with broad-spectrum antimicrobials that target a range of microorganisms. While this may help to bring the infection under control, without knowing the identity of the microorganism, the effectiveness of broad-spectrum treatment is limited, and potentially exposes the patient to adverse health effects. Following correct identification of the microorganism, patients can be switched to targeted treatment, which often includes a combination of two or three drugs. However, an incorrect assessment of the source of infection often hinders the administration of appropriate antimicrobial treatment, and the time taken from hospital admission to correct microbial identification is often slow with traditional biochemical identification methods.

In addition to minimising prolonged exposure to potentially aggressive treatment, another advantage of de-escalating from broad-spectrum antimicrobials sooner is reducing the risk of microorganisms developing multi-drug resistance (MDR). Changing to a more targeted therapy also aligns with numerous guidelines worldwide, such as the UK’s National Institute for Health and Clinical Excellence (NICE) Guidelines on Antimicrobial Stewardship [6], which aims to change antimicrobial prescribing practice to help slow the emergence of resistance and ensure that antimicrobials remain an effective treatment for infection, and the World Health Organization (WHO) Global Action Plan on Antimicrobial Resistance [7].

Until recently, the majority of clinical laboratories have used biochemical methods to identify microorganisms detected in patient samples, which take up to 48 hours after the blood culture turns positive. These phenotypic methods depend on the microorganism growth rate, and many require specialised culture methods and experienced technicians to obtain accurate identification. On top of this, many microbes are difficult to cultivate, or are non-cultivatable. Molecular methods go some way to remedy this, but there remains a lack of standardisation for identification of microorganisms in a streamlined, cost-efficient workflow.
Speeding up time-to-result

Mass spectrometry (MS) is fast emerging as a rapid, robust technology for clinical microbiology laboratories. The high resolving power, analytical sensitivity and ease-of-use of modern mass spectrometers make it an important tool for identifying microorganisms causing sepsis. Matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) MS is a highly reliable technique for identifying a broad range of microbes, by generating a proteomic ‘fingerprint’ that is then matched at the genus or species level with mass spectra from a reference library. Samples used by MALDI-TOF MS are typically isolates grown on culture media.

In cases of a potential blood stream infection, 2-3 blood culture bottles are drawn from the patient and placed into blood culture systems where it takes approximately 18-24 hours to turn positive.

Traditional biochemical methods can take up to 48 hours following positive blood culture to obtain an identification, whereas in a MALDI-TOF MS workflow bacteria are isolated from positive blood culture media, using either a dedicated sample preparation procedure or subculture methods. The most sophisticated systems, such as the Bruker CE-IVD MALDI Biotyper® (MBT) system, are capable of identification up to 24 hours after positive blood culture using isolates from subculture methods. However, when the MALDI Biotyper is combined with Bruker’s MBT Sepsityper® IVD Kit, laboratories can obtain direct pathogen identifications in 15-20 minutes after blood cultures turn positive. This enables the clinical laboratory to provide microbial identification 18-24 hours from the patient sample draw and up to 48 hours faster than traditional methods.

Gram-negative bacteria are the most common cause of sepsis, and the large Enterobacterales order are emerging as a class that are showing increasing resistance to first-line antibiotics, such as carbapenems and 3rd generation cephalosporins. Isolates that produce carbapenem-hydrolysing β-lactamases (carbapenemases), or enzymes that hydrolyse the β-lactam ring of cephalosporins (cephalosporinases) are thought to significantly contribute to the MDR threat. Bacterial cell isolates from positively flagged blood cultures – isolated by the rapid Sepsityper workflow – can undergo β-lactamase activity detection with Selective Testing of Antibiotic Resistance (STAR) using the MBT STAR-Carba and STAR-Cepha IVD Kits. This enables rapid detection of carbapenemase/cephalosporinase activity and identification of bacteria in one workflow. Combining the MALDI Biotyper with the MBT Sepsityper and MBT STAR assays allows microorganism identification and carbapenemase and/or cephalosporinase phenotypical resistance testing 60-90 minutes from a blood culture turning positive. This would allow physicians to manage patient treatment more effectively compared to classical biochemical methods.

**Rapid MBT Sepsityper® IVD Workflow**

1. 1 ml Positive Blood Culture liquid
2. Add lysis buffer and mix
3. Centrifuge 2 min and discard supernatant
4. Add washing buffer and mix
5. Centrifuge 1 min and discard supernatant
6. DT and eDT of the pellet, add 1 µl matrix
7. ID on MALDI Biotyper® "Rapid Sepsityper Workflow"
Minimising hospital costs

There are several ways in which incorporating a system such as the MALDI Biotyper combined with the MBT Sepsityper Kit can save hospitals money. Length of bed stays represents a significant cost to any health organisation, so reducing the number of days a patient spends in hospital is therefore a primary goal. Fast identification of causative microbial species and subsequent timely therapeutic treatment enables patients to leave hospital sooner, minimising bed stay costs. For example, a study carried out by the Royal Bolton Hospital (RBH), UK, calculated that by incorporating the MALDI Biotyper with Sepsityper into its protocol, length of bed stay for patients with bacteraemia was reduced by an average of 1.6 days for gram-negative bacilli, and 1.8 days for gram-positive cocci, compared to the standard laboratory protocol (SLP – traditional biochemical methods). Based on an estimated cost per bed day in England of £500, hospitals could save between £800-900 per patient by using MALDI-TOF MS technology.

Reducing the cost of antibiotics is another source of considerable savings for hospitals. By moving patients onto a targeted, more effective course of treatment sooner, costs of both oral and intravenous (IV) antimicrobials can be reduced. The same study at RBH found that implementing the MALDI Biotyper with the MBT Sepsityper solution reduced the number of IV and oral antibiotic doses by 33% and 35% respectively, compared to the SLP. This finding also translates to cost savings associated with nursing time, which is reduced particularly as a result of less IV drug doses.

In addition to costs to the hospital, poor patient outcomes as a result of delayed sepsis treatment have cost implications for the wider economy, although these are harder to quantify. Of those who survive severe sepsis, many suffer long-term, life altering conditions which may see them re-hospitalised, or unable to work for long periods of time or requiring rehabilitation. Known as post-sepsis syndrome (PSS), these conditions can include impaired physical and neurocognitive functioning, mood disorders, and a low quality of life [8], as well as a compromised immune system, increasing susceptibility to future infections [9].

Improving patient outcomes

Implementing the MALDI Biotyper with the MBT Sepsityper Kit shortens the time to identification after positive blood culture by up to 48 hours, by eliminating the time consuming step of culturing the microorganisms, and utilising the fast MALDI-TOF MS identification technology. Clinicians are able to act upon these earlier results and optimise antibiotic therapy in sepsis patients faster than conventional workflows. This is particularly important with critical organisms, such as *Staphylococcus aureus*, which frequently cause sepsis and have a high mortality rate if appropriate treatment is not rapidly given. Methicillin-resistant *S. aureus* (MRSA) is particularly serious in healthcare environments, where a large number of immunocompromised patients are exposed.
As well as improving patient outcomes, fast identification saves the laboratory critical time to focus on important samples. For example, the MBT Sepsityper Kit frees more time for biomedical scientists to focus on moving clinically significant samples to antimicrobial susceptibility testing (AST), which is crucial for establishing the resistance profile of important antimicrobials and ensuring effective treatment is provided, and recognising resistant microorganisms quickly to effectively manage hospital hygiene. The laboratory users can also eliminate potential contaminants earlier in the workflow, for example *Staphylococcus epidermidis*, saving time on running follow up AST tests for non-relevant samples.

**Clinical impact: Case examples**

As well as the positive laboratory and hospital impacts of running the MALDI Biotyper with the MBT Sepsityper Kit, there are also specific cases where achieving a faster result provided strong clinical benefits to the individuals affected.

A 40-year-old female diabetic patient with renal dialysis and a permanent catheter *in situ* was presented to the University Hospital Crosshouse, Scotland, with an infection. Rigors affected the patient when treated with vancomycin and gentamicin, and the catheter was identified as the presumed source of infection. Gram staining showed gram-positive cocci (GPC), and the infecting organism was identified as *Streptococcus agalactiae* using the MBT Sepsityper solution. The results indicated that the catheter was not the source of infection. Further investigation revealed a large vulval abscess, and treatment with co-amoxiclav commenced while vancomycin and gentamicin were stopped. The abscess was drained and the permanent catheter line salvaged without surgery, and additional blood cultures taken through catheter and peripherally showed no infection. The MBT Sepsityper solution allowed identification 24 hours earlier than traditional methods, enabling targeted therapy and reducing the time using vancomycin and gentamycin, therefore minimising associated negative side effects. The real cause of infection was promptly identified and treated quicker than with their previous methods, and the need for surgery was prevented.

Another example from Crosshouse demonstrates how the MBT Sepsityper solution prevented a patient being incorrectly discharged. The patient was admitted with a fever and headache, but with no photophobia or neck rigidity. The patient had a history of sleep apnoea, and presented with no skin or soft tissue infection, and no pain in joints or sign of osteomyelitis in the long bones. The patient was difficult to examine on ward rounds due to sleep apnoea, but the transthoracic echocardiogram showed no vegetations on the heart valves, which are indicative of bacterial or fungal infection. Gram-positive cocci were found in the blood, and the advice was to discharge following one or two doses of ceftriaxone (which was started on admission to cover for meningitis). The MBT Sepsityper identification result was *S. aureus*, and the patient developed tenderness of the lower spine. The orthopaedic team advised an MRI scan, which detected diskitis of the thoracic (T12) and lumbar (L1) vertebrae. The patient was started on flucloxacinil but developed cholestatic hepatitis, which is a very rare side effect of this treatment but can be life threatening, so the therapy was switched to IV daptomycin.
patient made a full recovery. If the MBT Sepsityper solution had not been used for quick identification, the patient would have been sent home without an additional diagnostics investigation. In this case, the MBT Sepsityper solution prevented early discharge and the need for re-admission, which is not a straightforward process and would have significantly delayed further investigations and therapy.

**Continuing advances in sepsis treatment**

Given that the incidence of sepsis is increasing by approximately 1.5 percent per year [10] – a trend that is compounded by the aging population – the need for rapid microbial identification methods is growing. The ability to identify microorganisms based on their protein profiles, rather than physical or biochemical characteristics, makes MALDI-TOF MS a highly versatile method, while also being accurate and specific, for identifying causative pathogens from positive blood culture bottles. A growing body of evidence highlights the patient- and hospital-wide benefits of fast species identification made possible by implementing the MBT Sepsityper workflow, for immediate direct analysis from positive blood cultures.

Governments and public health organisations have announced a range of actions to tackle the rise in sepsis cases. For example, in 2015 the Department of Health and Social Care, England, announced measures to address sepsis across the NHS, government and national health bodies. This included a General Practitioner (GP) performance audit to help improve practice in recognising sepsis, new NICE clinical guidelines on the diagnosis and treatment of sepsis in adults in 2016 [11], and working with Public Health England and Health Education England to improve public awareness. Despite these actions, the need for a rapid identification system in clinical laboratories remains an important goal for hospitals and health trusts worldwide.

For more information on the study conducted at the Royal Bolton Hospital, a case study is available [https://www.bruker.com/fileadmin/user_upload/8-PDF-Docs/Separations_MassSpectrometry/Literature/Brochures/1864704_Case_Study_Sepsityper_Rushana_Hussain_ebook.pdf](https://www.bruker.com/fileadmin/user_upload/8-PDF-Docs/Separations_MassSpectrometry/Literature/Brochures/1864704_Case_Study_Sepsityper_Rushana_Hussain_ebook.pdf)

References

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Bruker Daltonik GmbH
Bremen - Germany
Phone +49 (0) 421-2205-0
ms.sales.bdal@bruker.com - www.bruker.com/microbiology