

## Expert Insights

- Improving patient outcomes with rapid, reliable microbial identification and resistance testing

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# Improving Patient Outcomes with Rapid, Reliable Microbial Identification and Resistance Testing

Scientists are pioneering the *Bologna Workflow*, for microorganism identification and antimicrobial susceptibility testing after positive blood culture, with state-of-the-art MALDI-TOF MS technology.



Working with Bruker

Miriam Cordovana, Biomedical Laboratory Technician at the Sant'Orsola-Malpighi University Hospital of Bologna, has introduced the MALDI Biotyper positive blood culture (PBC) workflow into her laboratory, for fast and accurate microorganism identification and resistance detection.

*“We can now identify microorganisms within 15-20 minutes after a PBC alert using the Bruker MALDI Biotyper® with the Rapid MBT Sepsityper®, enabling a dramatically shorter reporting time compared to classical routine methods.”*

## Bacteriology, Mycology and Mycobacteriology Section, Sant'Orsola-Malpighi University Hospital

The Sant'Orsola-Malpighi University Hospital in Bologna, Italy, is a polyclinic which develops and delivers multi-specialist research, education and training, promoting innovation and delivering high quality patient care and medical training. Founded in 1592, the S.Orsola-Malpighi University Hospital is one of the largest and oldest hospitals in Italy, with more than 1,500 beds and over 5,100 employees. The hospital has approximately 70,000 admitted cases, 3 million outpatient visits and 33,000 surgeries per year, and contains 67 Operative Units.

The hospital comprises all medical and surgical specialties, except for neurosurgery and dentistry, and is among the main Italian centers for transplants as well as an excellence center for oncology and hematology. Italy is currently facing a dramatic epidemiological situation regarding bacterial resistance to antibiotics. So, the Bacteriology,

Mycology and Mycobacteriology section of the hospital's Operative Unit of Microbiology is dedicated to conducting rapid identification and antimicrobial susceptibility testing (AST) to combat this issue. This section has a staff number of 18, with seven medical doctors and microbiologists, and five students specializing in microbiology.

Miriam Cordovana is a Biomedical Laboratory Technician in the Bacteriology, Mycology and Mycobacteriology section at the hospital, and oversees the processing of biological samples, from their arrival at the laboratory to the execution of AST. She has played an integral role in the department's aim to optimize the whole workflow for blood culture microbial testing. Miriam joined the S.Orsola-Malpighi University Hospital in May 2007, having held positions at the University Hospital of Florence Careggi and the Human Genetic Laboratory of the University of Florence.

## Antimicrobial resistance in Italy

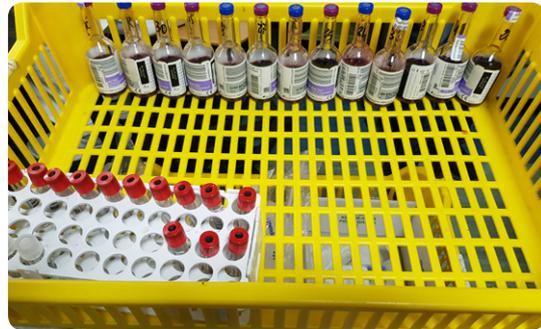
The global rise of antimicrobial resistance has been widely reported over the last decade, par-

ticularly in some regions of Europe, which are battling increasing levels of multi-drug resistant (MDR) microorganisms in the population. The prevalence of antimicrobial resistance shows distinct variations depending on the bacterial species, antimicrobial group and geographical region, and for several years, a clear pattern of increased resistance has become evident in southern and eastern European countries. Carbapenems are the most important last-line antimicrobial family for the treatment of infections with MDR Gram-negative bacteria, so particular attention has been paid to carbapenem-resistant bacteria. In countries with high levels of multi-drug resistance, such as Italy, only a few therapeutic options are now available for highly pathogenic infections, hence research is being directed towards technologies that are capable of rapidly detecting infecting organisms and establishing the degree of antimicrobial susceptibility.

Gram-negative bacteria are the most common cause of sepsis, followed by Gram-positive bacteria and fungi. Fast isolation and species identification are therefore critical to provide a targeted therapeutic strategy and de-escalate from broad spectrum antibiotics as soon as possible. Sepsis is thought to impact approximately 27-30 million people globally every year [1], with one third dying from the condition [2], so clinical microbiology laboratories are in need of an identification method which can allow clinicians to rapidly act upon results and manage blood stream infections.

## Carbapenemase-producing *Enterobacteriaceae*

The *Enterobacteriaceae* are a large family of Gram-negative bacteria, which includes many harmless species such as most strains of *E. coli*, in addition to some well-known pathogens, such as *Klebsiella* and *Salmonella*. *Klebsiella pneumoniae* is a common cause of urinary tract, respiratory tract and blood stream infections, and can spread rapidly between patients in healthcare settings. In Europe, more than one third of the *K. pneumoniae* isolates reported to the European Antimicrobial Resistance Surveillance Network (EARS-Net) in 2015 were resistant to at least one of the antimicrobial groups under surveillance (fluoroquinolones, third-generation cephalosporins, aminoglycosides and



carbapenems) [3]. Carbapenem-resistant *Enterobacteriaceae* (CRE) have emerged relatively recently as a class of bacteria that do not respond to this last-line antimicrobial treatment. Not all mechanisms of resistance have been fully understood or characterized, but isolates that produce carbapenem-hydrolyzing  $\beta$ -lactamases (carbapenemases) are the focus of studies of resistance determination, and are thought to primarily contribute to the rapid dissemination of MDR organisms. Italy has one of the highest instances of carbapenem-resistant *K. pneumoniae* in Europe, with 33.5% of invasive isolates showing resistance [4]. The *Klebsiella pneumoniae* Carbapenemase (KPC)-producing *K. pneumoniae* family is endemic in Italy, and represents a severe public health concern.

The rapid detection of KPC-producing isolates is therefore highly desired, and many techniques have been developed in the past to attempt this, such as the modified Hodge test, the disk diffusion synergy test with inhibitors, and Carba NP (Nordmann Poirel) test. However, until recently these techniques have shown to be both slow (up to 24 hours) and time-consuming, lacking sensitivity (phenotypic methods), or having a limited number of antibiotic targets included. Other assays that are based on molecular methods can be expensive or have not been optimized for routine use. The power of matrix-assisted laser desorption/ionization (MALDI) - time-of-flight (TOF) mass spectrometry (MS) technology is now being harnessed for the rapid detection of carbapenemase-producing bacterial strains. The hydrolytic activity of bacterial carbapenemases can now be detected by a functional assay – the Bruker MALDI Biotyper Selective Testing of Antibiotic Resistance- $\beta$ -lactamase (MBT STAR-BL) assay – which is based on the distinct mass changes of the carbapenem molecule after enzymatic cleavage. An important advantage of this worldwide first MS-based phenotypic CE-IVD

labeled assay is that it can be applied directly from positive blood cultures (PBC) after MBT Sepsityper isolation.

## Implementation of the *Bologna Workflow*

The S.Orsola-Malpighi University Hospital recently introduced the integrated *Bologna Workflow* to the Bacteriology, Mycology and Mycobacteriology section, for rapid and cost-effective microbial identification and AST from PBCs. The Bologna laboratory has validated all non-IVD methods, software, kits and workflows described in the current article in house for clinical use in the Bologna laboratory. The subtyping module is currently available for research use only, but will soon be available as an IVD-CE product. There is an urgent priority in clinical microbiology, as the spread of resistance against third-generation cephalosporins and carbapenems among Gram-negative bacteria poses a high burden on the Italian healthcare system, and is rapidly rising elsewhere in Europe and worldwide. The complete workflow speeds up time to result, enabling clinicians to make critical treatment decisions sooner. Miriam explains the importance of a new laboratory protocol:

*“Our institution has been trying for many years to optimize the whole workflow for blood culture. We are continuously trying to reinforce the need for rigorous procedures, from the correct disinfection of the skin before sampling, to indications about optimal timing for sampling.”*

*Since the introduction of MALDI-TOF MS as part of our routine microbiology workflow, we are constantly evaluating procedures allowing shorter identification from blood cultures. One of these methods is based on the Rapid Workflow of the MBT Sepsityper Kit.”*

## Bruker’s MALDI Biotyper®

The MALDI Biotyper (MBT), based on state-of-the-art MALDI-TOF mass spectrometry technology, allows for the identification of thousands of different microbial species. This, combined with automated detection of specific resistances in one workflow, makes the MBT a powerful tool in clinical microbiology laboratories.

## MBT Sepsityper® IVD kit

The Sepsityper workflow is performed in conjunction with Bruker’s MBT system, for rapid isolation of microorganisms from positive blood cultures with a time to identification of 15-20 minutes. This enables up to 24 h faster decision-making for clinicians and, therefore, improved patient outcomes in sepsis cases.

## MBT STAR®-BL IVD assays

Bacterial cell isolates from positively flagged blood cultures – using the rapid Sepsityper workflow – can undergo  $\beta$ -lactamase activity detection with the MBT STAR-Carba and STAR-Cepha IVD Kits. This enables rapid detection of carbapenemase/cephalosporinase activity and identification of bacteria in one workflow.

For the past few years, the most concerning infectious diseases have been hospital-acquired infections by carbapenem-resistant bacterial strains and, given the dramatic epidemiological situation in Italy, focus on minimizing infection transmission and reducing morbidity and mortality is key. The *Bologna Workflow* is composed of the Bruker MBT platform, the MBT Subtyping Module, the MBT Sepsityper Kit, the MBT STAR-Carba assay for fast carbapenem-resistance testing, and the new STAR-Cepha kit for functional resistance testing against third-generation cephalosporins.

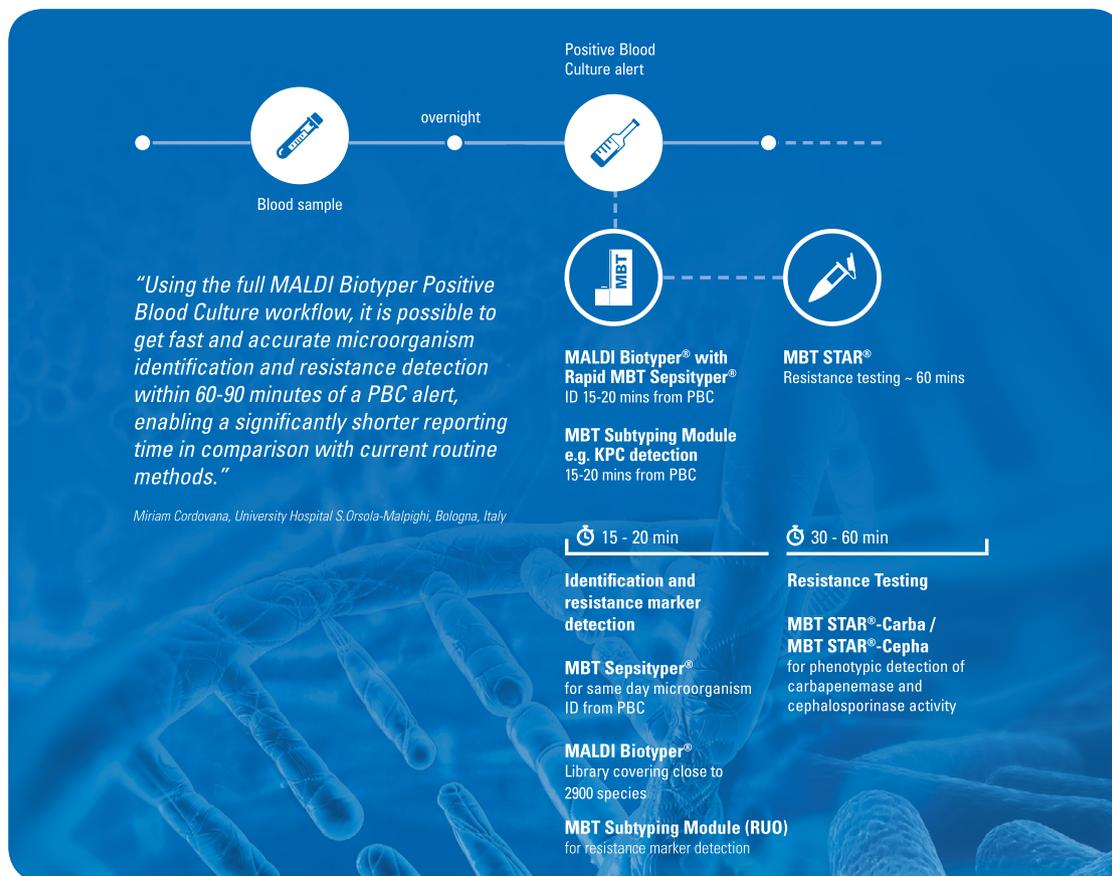


Figure 1: The *Bologna Workflow* implemented at the S.Orsola-Malpighi University Hospital, Bologna.

Miriam describes the workflow in more detail:

“We use the Rapid Sepsityper kit to extract the bacterial pellet, used for species identification performed by the MBT system, equipped with the MBT Subtyping Module. The isolates from a positively flagged blood culture, using the Sepsityper workflow, can then undergo further resistance testing using the same residual bacterial pellet with the MBT STAR-Carba and MBT STAR-Cepha assays to detect  $\beta$ -lactamase activity.

*Compared with traditional methods, all requiring a subculturing step, the Bologna Workflow is shorter by up to 24 hours, saving up to one day, and the methods are much easier for users to handle.*

*In addition they provide higher quality results (correct identifications) and better throughput (number of samples identified). The previous subculture-based methods provided the same number of results per day, but the percentage of correct identifications was significantly lower (around 65%) with a longer time-to-report (approx. 3 h). The implementation of the Rapid Sepsityper workflow has improved this significantly, with over 91% correct identifications (Table 1). It also fills in the gaps of hard-to-identify bacteria that the previous method left behind. This reflects in a higher degree of satisfaction from the operators and clinicians.”*

The *Bologna Workflow* combines rapid identification with the MBT STAR-Carba assay for fast carbapenem-resistance testing. An automated warning for carbapenem-resistant *K. pneumoniae* (KPC), associated with the *bla*<sub>KPC</sub> gene, is performed with the MBT Subtyping Module. For the S.Orsola-Malpighi University Hospital, the KPC cases (around 95%)

Table 1: Results from the S.Orsola-Malpighi University Hospital: comparison between the formerly used 'chocolate' method (subculturing of an enriched bacterial pellet on chocolate agar), and the Bruker MBT Sepsityper kit, both followed by identification using the MALDI Biotyper (MBT).

	Correct identification results	No identification	Total
MBT Sepsityper + MBT identification	587 (91.7%)	53	640
Chocolate method + MBT identification	231 (67.3%)	112	343

make up the majority of *K. pneumoniae* identifications. Continuous expansions and developments of the MBT reference library also enable the Bologna Hospital to detect very rare species in a PBC. A recent MBT library update (2018) added another 239 species across 24 microbial genera, which improves the coverage of biological diversity for the identification workflow. The complete workflow covers identification of over 2,700 microbial species from PBCs, speeding up time to result and enabling clinicians to make critical treatment decisions sooner.

## Detecting Resistant Microbes using the MBT Subtyping Module

KPC-producing *K. pneumoniae* first emerged in Italy in 2010, and, since then, Miriam and the Bologna hospital laboratory have been conducting a research project to phenotypically characterize and store the strains, and evaluate different methods of detection. Lau *et al.* (2014) discovered a specific MALDI-TOF mass peak at 11,109 m/z appearing in KPC-producing isolates, which is coded at the pKpQIL plasmid carrying the *bla*<sub>KPC</sub>

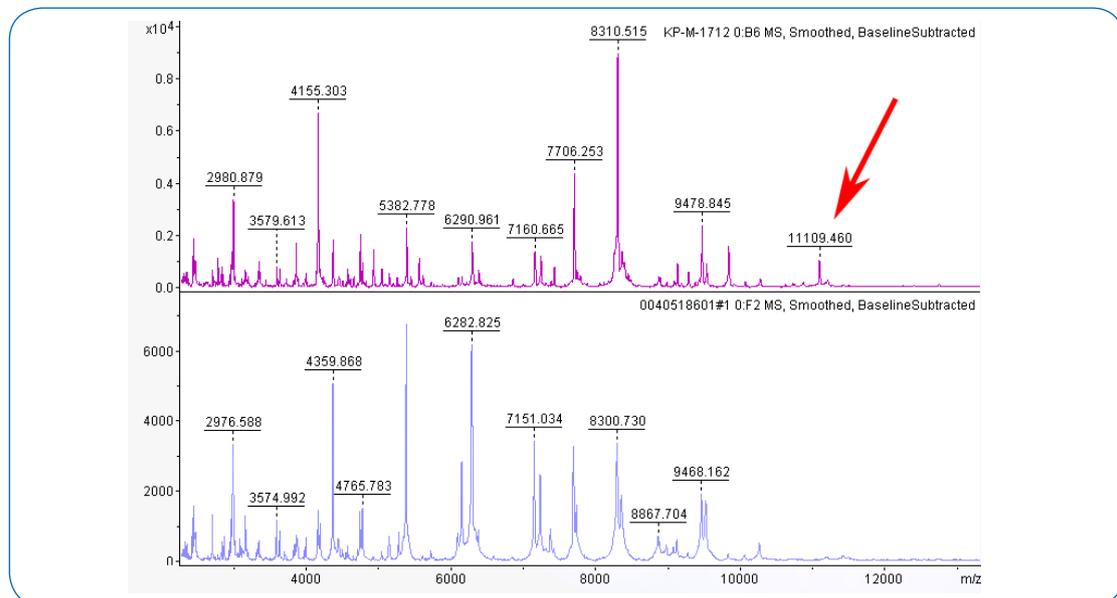


Figure 2: The pKpQIL plasmid-related peak in the MALDI mass spectra of *K. pneumoniae*. The upper spectrum shows a KPC-producing strain exhibiting the specific 11,109 m/z peak. The lower spectrum shows a negative control, without the specific peak. Reproduced from reference [6] in accordance with the Creative Commons License (<https://creativecommons.org/licenses/by/4.0/>).

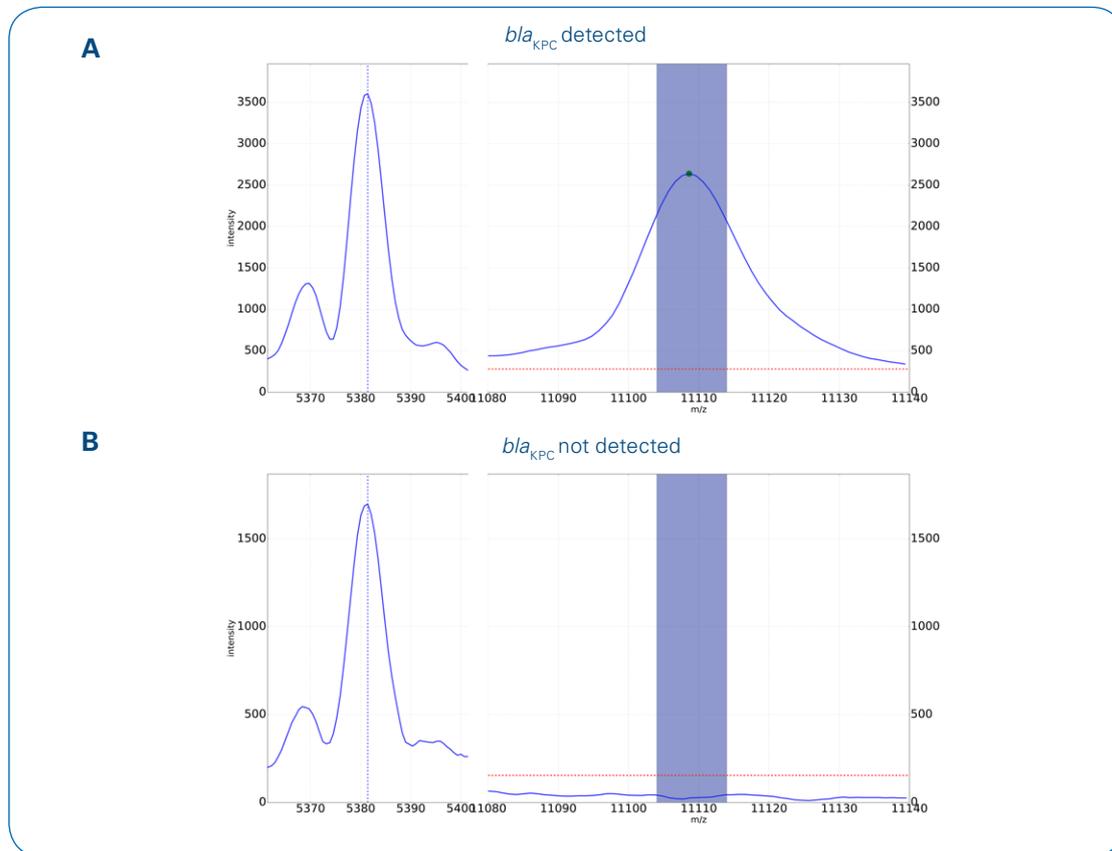


Figure 3: Detection in the window of  $m/z$  11,109  $\pm$ 5. The dotted red line corresponds to a multiple of the average noise in the spectrum. It is used as a threshold for the intensity in peak detection. (A) KPC-positive *K. pneumoniae* strain with a peak for the pKpQIL plasmid. (B) KPC-negative strain. In the detection window only noise below the detection threshold can be observed. Reproduced from reference [6] in accordance with the Creative Commons License (<https://creativecommons.org/licenses/by/4.0/>).

gene [5]. The automated identification of these KPC-producing *K. pneumoniae*, by detecting this specific KPC-related peak in the MALDI-TOF MS spectra, is now implemented in the MBT Subtyping Module, allowing a “presumptive KPC” result (Figure 2 and Figure 3). This innovative approach was applied first to bacterial plate cultures, then directly to PBC, using the bacterial pellet obtained using the MBT Sepsityper Kit, which enabled reliable and rapid identification of KPC-producing *K. pneumoniae* strains. The automated peak detection allowed for 100% specificity and 85.1% sensitivity. In addition to the MBT Subtyping Module, the MBT STAR-Carba test for detection of carbapenemase activity showed 100% specificity and sensitivity [6]. The Bologna group is achieving a turnaround time for detection of KPC-producing isolates from 10 minutes to 1.5 hours, and showed that this novel MALDI-based

approach uniquely provides real-time detection of antibiotic resistance with simultaneous species identification. By incorporating this technology as part of the *Bologna Workflow*, the laboratory at the S.Orsola-Malpighi University Hospital can perceive early warnings for KPC-producing strains, facilitating the rapid initiation or change of therapeutic action and future infection control measures.

This complete approach is ideally suited to any kind of clinical laboratory, from high-throughput routine laboratories to smaller “spoke” rapid response laboratories, due to the minimal handling time, the high level of automation, and ease of use that makes it also suitable for operators who may not have much experience with spectra processing and analysis.

“We also work with other carbapenemase gene families beyond KPC-producing *K. pneumoniae*, as soon as they emerge in the region” adds Miriam, continuing: “For example, we worked on the first Italian outbreak of New Delhi metallo-β-lactamase (NDM) in 2011, as well as the Verona integron-mediated metallo-β-lactamase (VIM)-producing *Citrobacter freundii* hospital outbreak in 2012.

*I have extensively worked with the MBT STAR-Carba assay, testing it first on hundreds of strains from plate cultures, and then applying it to blood cultures, and to the extension of the KPC subtyping method to other species of bacteria, in collaboration with Bruker scientists.”*

## Patient Impact

The reduced confirmation and identification times achieved by the MBT *Bologna Workflow* enable prompt optimization of antimicrobial therapy, providing patients the best possible chance to recover from infectious disease. Timely alterations of therapeutic treatment are particularly crucial in the case of KPC-producing bacterial strains, since the report of KPC-producing *K. pneumoniae* immediately leads to the escalation of therapy (addition of ceftazidime/avibactam and colistin), and can therefore save patient lives. Every half hour saved to provide a narrowed treatment can be crucial, as this is the time frame required for *Enterobacteriaceae* to duplicate. In the case of sepsis, it has been shown that, for every hour that the appropriate treatment is delayed, the chance of survival decreases by 7.6% [7].

## Working with Bruker

The time savings the MALDI Biotyper provides during microbial identification as part of the *Bologna Workflow* allows technicians to set up AST earlier, conferring a direct effect on treatment. Miriam comments on how the MALDI Biotyper has benefitted the laboratory further:

*“Investing in technology is fundamental, not only to meet the needs of the hospital, but most importantly to meet patient needs. An improvement in patient outcomes should be the driving force for the implementation of any new technology.*

*The introduction of the MALDI Biotyper has enabled us to provide species identification in minutes, between 24-72 hours faster than with previous methods. I couldn't hope to find better collaborative partners than Bruker scientists, from the scientific assistance point of view, but also on a personal level. The excellent instrument quality was the key deciding factor when assessing how to meet our needs, but the expertise, integrity and collaborative mindset of the scientists helped our decision in choosing Bruker.”*

By integrating the MALDI Biotyper into the *Bologna Workflow*, the laboratory can now perform faster and more reliable diagnostics in cases of sepsis, which poses a huge public

health risk across the globe, as well as in the field of epidemiological surveillance of carbapenemase-producing *K. pneumoniae*. Miriam comments on the impact of the MBT on the hospital:

*“The MBT Subtyping Module and MBT Sepsityper workflow have simplified and improved our workflow. Consultants continuously ask us for a MALDI Biotyper result, but we only have one instrument at the moment.*”

*Ever since the introduction of the MBT Subtyping Module and MBT Sepsityper, we have had a long queue for measurements. We are very proud of our work, as we are able to provide more than 91% of correct identifications with the Sepsityper (Table 1). We are also able to identify uncommon species, or a KPC-producing isolate from a single colony in a mixed culture, which would previously require overnight subculturing and a further two tests.”*

## Future Developments

There is wide scope for the extension of KPC-subtyping in the future, as well as for the addition of new resistance markers and resistance detection methods. Miriam comments on where she sees the future of the field heading:

*“I see the need for an increasingly more “customized” susceptibility testing, tailored by the results of species identification, combined with the epidemiological data of each setting. I do, however, see the ever-increasing spread of uncommon opportunistic pathogens, given the growing proportion of immunocompromised patients for specific pathologies or medical treatments. This could present a challenge to AST, so the faster we can identify these organisms, the better.”*

In the near future, Miriam plans to carry out a quantitative evaluation of the impact of the *Bologna Workflow* on patients and the hospital, including length of hospital stay, amount/type of antibiotics used, and cost savings.

For more information on the S.Orsola-Malpighi University Hospital, please visit

<https://www.aosp.bo.it/content/home>

For more information on Bruker’s MALDI Biotyper systems, please visit

<https://www.bruker.com/products/mass-spectrometry-and-separations/maldi-biotyper-systems.html>

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S.Orsola-Malpighi University Hospital



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## About the S.Orsola-Malpighi University Hospital

With more than 400 years of history, the Bologna University Hospital Authority Sant'Orsola-Malpighi Polyclinic was the first hospital in Bologna, and is today home to the School of Medicine and Surgery. The Polyclinic is an internationally acclaimed institution for the study and treatment of pathologies, and, each year, organizes medical conferences and conventions attended by professionals of international fame. The hospital develops and delivers multi-specialist research, education and training in an integrative manner, promotes innovation and pursues the highest degree of patient/customer care and the training of medical students, as well as expanding the full potential of its skilled staff through goal sharing and assuming responsibility for any results.

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Bruker is enabling scientists to make breakthrough discoveries and develop new applications that improve the quality of human life. Bruker's high-performance scientific instruments and high-value analytical and diagnostic solutions enable scientists to explore life and materials at molecular, cellular and microscopic levels. In close cooperation with our customers, Bruker is enabling innovation, improved productivity and customer success in life science molecular research, in applied and pharma applications, in microscopy and nanoanalysis, and in industrial applications, as well as in cell biology, preclinical imaging, clinical phenomics and proteomics research and clinical microbiology.

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● **Bruker Daltonik GmbH**

Bremen • Germany  
Phone +49 (0) 421-2205-0

[ms.sales.bdal@bruker.com](mailto:ms.sales.bdal@bruker.com) - [www.bruker.com/microbiology](http://www.bruker.com/microbiology)