A“FULL MALDI-BASED” APPROACH FOR A RAPID DIAGNOSIS OF SEPSIS CAUSED BY CARBAPENEMASE-PRODUCING ENTEROBACTERIA

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INTRODUCTION

The dramatic spread of carbapenem-producing enterobacteria (CPE) in the hospital settings represents a situation of risk for a continuously increasing number of patients in the epidemiological context of Italy, where, despite the surveillance and control measures applied, the infections caused by such (multi)-resistant strains are always more common.

The increase of sepsis by CPE observed in the last years represents the most worrying expression of the spread of these strains in the healthcare facilities.

The identification as prompt as possible of the carbapenemase production has a crucial importance for the management of these infections, and might have an extremely relevant impact for the patients’ clinical outcome.

In this study, an innovative “full MALDI-based” approach for the fast detection of CPE in positive blood cultures, by using a combination of the most recent applications of the MALDI-TOF MS MALDI Biotyper system (Bruker Daltonik) directly on the positive bottles, was evaluated.

The automatic detection of the KPC-specific peak at m/z 11109 was performed by the MALDI Biotyper software, simultaneously with the identification at species level, by processing the mass spectra generated from the bacterial pellet extracted from the positive blood culture bottle. The carbapenemase activity was evaluated by an imipenem hydrolysis assay.

MATERIALS AND METHODS

77 consecutive positive blood cultures with *K. pneumoniae* (n=46 producing different classes of carbapenemases, and n=31 carbapenem-susceptible), collected in June-August 2017, were analyzed with this novel approach. **Fig. 1.**

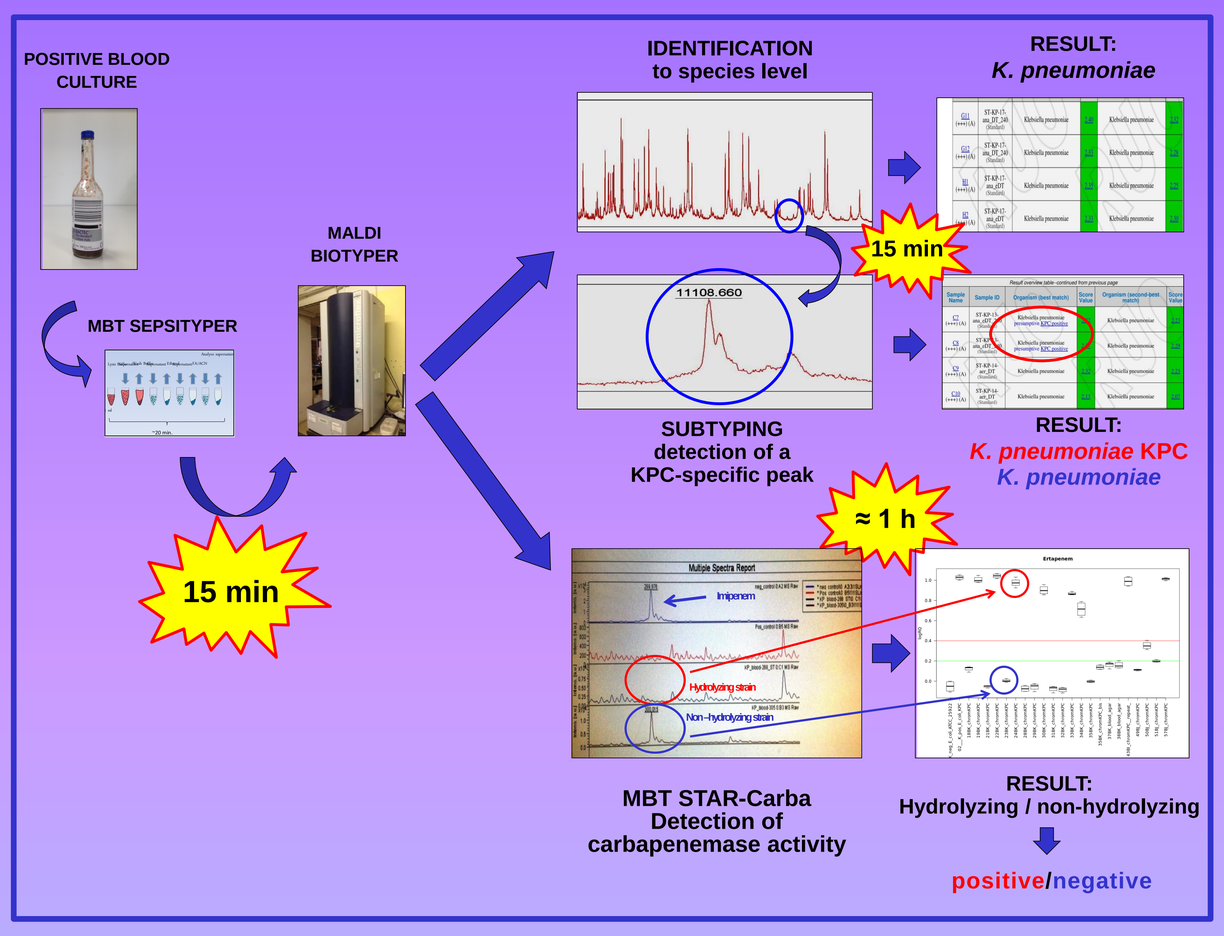

Fig. 1 Schematic representation of the “full-MALDI-based” approach starting from the positive blood culture bottles to the final result.

✓Extraction of the bacterial pellet

From the positive blood culture bottle, the bacterial pellet was extracted following the Sepsityper® Kit protocol (Bruker Daltonik).

✓Acquisition of mass spectrum

The bacterial mass spectra recorded from the pellet were processed by the MALDI Biotyper software, for the **species identification and to search for the KPC-specific peak at 11109 m/z**, using an algorithm which is automated and integrated in the software.

✓Detection of carbapenemase activity - imipenem hydrolysis assay

The same pellet used for the acquisition of the mass spectra was used for the imipenem hydrolysis assay MBT STAR-Carba (Bruker Daltonik), that detects the carbapenemase activity by the analysis of the mass spectra of the antibiotic intact/hydrolyzed molecules, after a short incubation (30 min) with the tested bacterial strain.

This test was performed both to confirm the carbapenemase activity (in the strains positive for the KPC-specific peak), and to detect it (in case of strains producing carbapenemases of other classes or KPC+ strains negative for the specific peak.)

RESULTS

✓Species identification and detection of the peak at m/z 11109

All the samples were identified at species level (ID log score >2.0) .

The MALDI Biotyper software detected the KPC-specific peak in 34/35 (97.1%) of the KPC-producing strains in which such a peak was present, corresponding to 34/37 (91.9%) of the total number of KPC-positive strains, and in none of the other samples. **Table 1**

	KPC n=37	MBL n=8	NDM+OXA-48 n=1	Carbapenem-Susceptible n=31
Peak at 11109 m/z	34/35 (97,1%)	0	0	0
No peak at 11109 m/z	2+1	8 (100%)	1 (100%)	31 (100%)

Table 1. Sensitivity and specificity of automated picking of the KPC-specific peak at m/z 11109.

✓Confirmation of carbapenemase activity by MBT STAR-Carba

The MBT STAR-Carba assay resulted positive for 46/46 of the samples positive with CPE, and negative in the remaining samples (31/31). **Table 2**

	KPC n=37	MBL n=8	NDM+OXA-48 n=1	Carbapenem-Susceptible n=31
STAR-CARBA+	37 (100%)	8 (100%)	1 (100%)	0
STAR-CARBA-	0	0	0	31 (100%)

Table 2. Sensitivity and specificity of the imipenem hydrolysis assay MBT STAR-Carba.

✓Turn-around time

The novel approach provided a conclusive result in a time frame between 30 min [in the cases where the KPC-specific peak was detected – 73.9% (34/46) of the positives] and 2 h (in all the other cases).

DISCUSSION

The “full MALDI-based” approach allowed the detection of enterobacteria producing different classes of carbapenemases directly from the positive blood culture bottle, with optimal sensitivity and specificity, and enabling a significant shortening of the potential reporting time in comparison with the current routine (30 min – 2 h).

Considering also the extreme ease of use of the workflow, these promising results suggest the possibility of an effective implementation of these novel MALDI Biotyper applications in the routine practice.