INSTANT DETECTION OF ANTIBIOTIC RESISTANCE MARKERS BY MALDI-TOF MS

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INTRODUCTION

The increasing spread of antibiotic resistances at global level demands always more urgently for rapid methods for their identification.
The novel MALDI-TOF MS subtyping applications fit well this purpose, and allow an innovative and extremely fast approach.
The search of specific peaks in the bacterial mass spectrum, corresponding to specific markers of antibiotic resistance, enables the instant detection of the respective resistance mechanisms.

In this study, the usage of the MALDI Biotyper (Bruker Daltonik) for the automatic detection of

- ✓ **KPC-producing *K. pneumoniae* (Kp-KPC)**
- ✓ **Methicillin resistant *S. aureus* (MRSA)**
- ✓ **Carbapenemase producing *B. fragilis***

in "real time" during the routine species identification process was investigated.

MATERIALS AND METHODS

The specific resistance markers were detected processing the same mass spectra recorded for the species identification during the routine processes (all spots were prepared by direct transfer).
The detection was performed by using an automatic and integrated specific algorithm for *K. pneumoniae, B. fragilis* and *S. aureus* in the MALDI Biotyper software.

Klebsiella pneumoniae KPC

The presence of a KPC carbapenemase was detected searching the peak at m/z 11109 (Lau, 2014), specifically associated to the pKpQIL plasmid carrying bla_{KPC} genes. This plasmid is present in the more prevalent KPC-Kp clones among all the circulating ones. **Fig. 1 & 2**

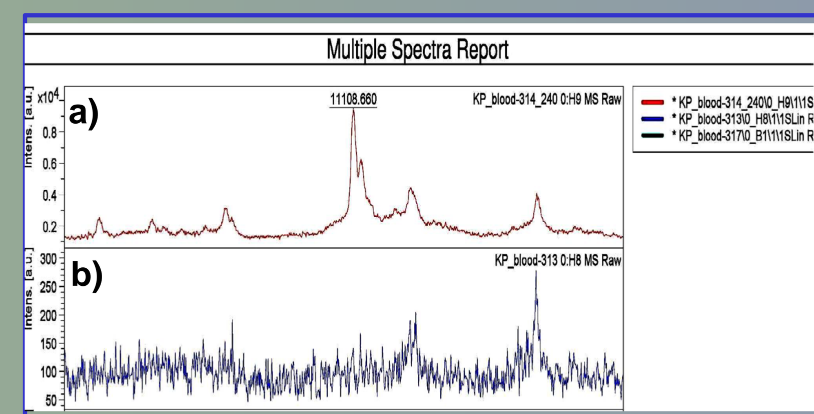

Fig. 1 KPC-specific peak at m/z 11109
a) *Kp*-KPC **b)** *Kp* "non-KPC"

Fig. 2 Final identification result provided by the MALDI Biotyper software

A total of n=4725 non-duplicated *K. pneumoniae* strains were analyzed, among them n=1329 KPC-producers (characterized by PCR or synergy test with boronic acid), and n=3396 carbapenem susceptible (*wild type* or beta-lactamase producers) or other classes carbapenemase producers.

Methicillin resistant *Staphylococcus aureus* (MRSA)

The detection of the methicillin resistance in *S. aureus* was performed by searching the peak at m/z 2411-2419, related to the PSM-mec peptide, present in a subgroup of agr-positive MRSA strains (Josten, 2014). **Fig. 3**

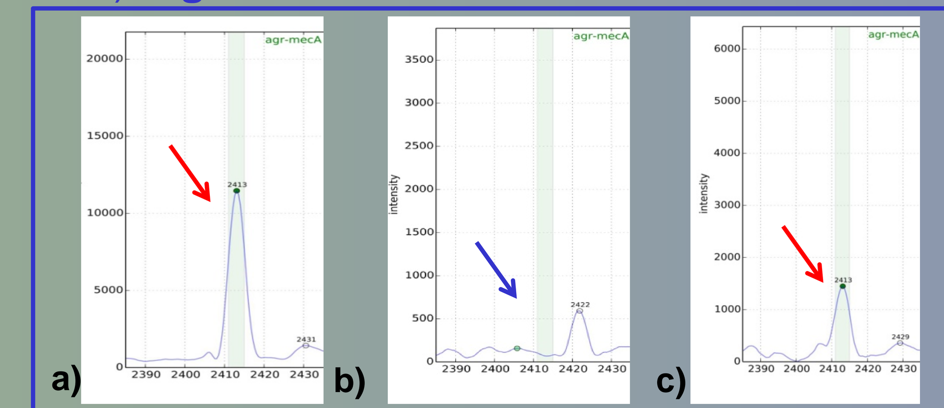

Fig. 3 Automated detection of the peak at m/z 2411-2419
a) *S. aureus* ATCC 33591 (PSM-mec positive)
b) Clinical isolate of MRSA (PSM-mec negative)
c) Clinical isolate of MRSA (PSM-mec positive)

N=1304 non duplicated *S. aureus* clinical isolates were analyzed, among them n=211 MRSA and n=1093 MSSA, phenotypically characterized by their susceptibility to oxacillin.

Carbapenemase producing *Bacteroides fragilis* (*cfiA*+)

The presence of the class B carbapenemase encoded by the *cfiA* gene, constitutively present in a subgroup of *B. fragilis* (division II), was identified detecting the modifications in the mass spectral pattern, that are specific to this subfamily (Nagy, 2011). **Fig. 4 & 5**

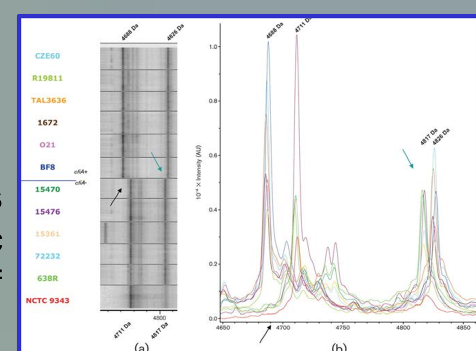

Fig. 4 Mass differences of some peaks specific for both subgroups of *B. fragilis*

Fig. 5 Final identification result provided by the MALDI Biotyper software

N=401 consecutive *B. fragilis* clinical isolates were subtyped into division I/II by matching of their mass spectra with the specific MALDI Biotyper sublibrary for *cfiA*-positive/*cfiA*-negative *Bacteroides fragilis.*

[1] **Lau *et al.* (2014).** A rapid matrix-assisted laser desorption ionization-time of flight mass spectrometry-based method for single-plasmid tracking in an outbreak of carbapenem-resistant Enterobacteriaceae. J Clin Microbiol. 2014 Aug;52(8):2804-12
[2] **Josten *et al.* (2014).** Identification of agr-positive methicillin-resistant *Staphylococcus aureus* harbouring the class A mec complex by MALDI-TOF mass spectrometry. Int J Med Microbiol. 2014 Nov;304(8):1018-23
[3] **Nagy *et al.* (2011).** Differentiation of division I (cfiA-negative) and division II (cfiA-positive) *Bacteroides fragilis* strains by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. J Med Microbiol. 2011 Nov;60(Pt 11):1584-90

RESULTS

Klebsiella pneumoniae KPC

The KPC-specific peak was detected in 1050/1329 of the Kp-KPC strains (sensitivity 79.0%), and in 0/3396 of the carbapenem susceptible / other carbapenemase producing strains (specificity 100%). **Table 1**
Moreover, an increasing trend of the strains that show the specific peak along the years was observed (from 60.2% in 2010 to 92.6% in 2016), likely due to an increase of the prevalence of this/these clone/s among all the circulating KPC clones. **Fig. 6**

	KPC n=1329	MBL n=67	OXA-48 n=20	*Wild type* β-lactamase ESBL AmpC n=3309
Peak at 11109 m/z	1050 (79%)	0	0	0
No peak at 11109 m/z	279	67	20	3309 (100%)

Table 1

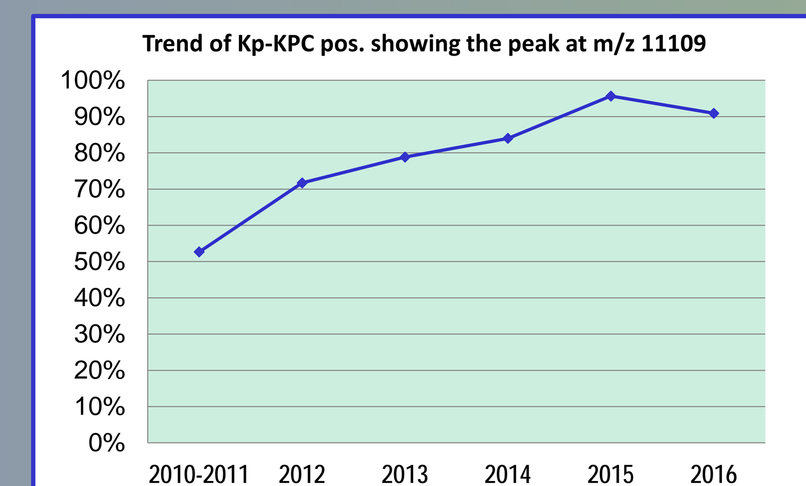

Fig. 6

Methicillin resistant *Staphylococcus aureus*

The specific peak related to the PSM-mec peptide was detected in 49/211 of the MRSA isolates (23.2%), and in none of the MSSA strains (specificity 100%). **Table 2**

	OXA=R n=211	OXA=S n=1093
Peak PSM-mec	49 (23,2%)	0
No peak PSM-mec	162	1093 (100%)

Table 2

Carbapenemase producing *Bacteroides fragilis*

41/401 (10.2%) *B. fragilis* clinical isolates were classified by the MALDI Biotyper software as *cfiA*-positive, all confirmed by PCR.
Although the presence of this carbapenemase causes a reduced susceptibility to carbapenems, no other routine method is able to detect its presence, meaning consequently the risk of possible therapeutic failures.

DISCUSSION

In this study, the detection of bacterial antibiotic resistance markers by MALDI-TOF MS subtyping proved to be a reliable, innovative and extremely easy method, that uses the same mass spectra recorded for the bacterial species identification.
The method showed absolute specificity and positive predictive values, and a sensitivity related to the prevalence of the specific resistance determinant among all the strains that exhibit such resistance, and therefore resulted optimal for KPC-producing *Klebsiella pneumoniae* and carbapenemase producing *Bacteroides fragilis*, and limited, as predictable, for methicillin resistant *Staphylococcus aureus*, given the multiplicity of determinants of such resistance.

The **novel applications of the MALDI Biotyper** allow the **automatic detection** of such **specific resistance markers**, and their instant identification during the routine species identification processes.

Although the three groups of bacteria involved in this study have a very different clinical and epidemiological relevance, our results suggest that the introduction of this approach in routine practice could give a significant contribution to the adoption of always earlier and more effective therapeutic and infection control measures.