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SHORT COMMUNICATION

Laboratory validation of a KPC-producing strain identification method based on the detection of a specific 11,109 Da peak via Maldi-Tof-Vitek MS in an endemic area

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SUMMARY

Matrix Assisted Laser Desorption/Ionization-Time of Flight (MALDI-TOF) Vitek MS system is a useful technique to identify bacteria strains isolated in clinical samples. In this paper, we applied this method to KPC-producing *Enterobacteriaceae* detection through the determination of a specific 11,109 (±8) Da peak. We assayed the presence, specificity and reliability of this peak on routine workflow through the analysis of 183 *Enterobacteriaceae* strains isolated from clinical samples and characterized by classical approaches. The peak was detected in 95.5% (129/135) of carbapenemase-producing strains spectra compared with the 48 extended spectrum beta-lactamase producing controls strains, which all lacked this peak. Hence, this 11,109 Da peak determination showed a Positive Predictive Value (PPV) of 100% and a Negative Predictive Value (NPV) of 94.4%. The characterization of this specific peak in a MALDI-TOF Vitek MS system might be considered a valuable tool to reveal KPC-producing *Enterobacteriaceae* especially in KPC endemic region.

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Carbapenemase-producing *Enterobacteriaceae* (CPE) strains represent a pivotal concern in public health because the worldwide onset of antibiotic-resistance spreading is dramatically increasing. This phenomenon requires immediate intervention measures, including rapid detection of antibiotic resistance bacteria strains (Nordmann *et al.*, 2011) to identify the carbapenemase producing strains driven patients, and to provide an appropriate therapeutic regimen for every patient and preventive measures to avoid nosocomial outbreaks.

The Mass-Spectrometry (MS)-based MALDI-TOF method has replaced traditional methods for bacterial identification. Recently, several studies suggested a further application of MALDI-TOF technology in the analysis and determination of susceptible bacteria strains through the pattern of specific peaks related to resistance determinants. In particular, the MALDI-TOF MS technique has been investigated as tool for detecting carbapenemase through the correspondence between KPC enzyme and a specific peak at 11,109 Da in MALDI-TOF MS spectra (Lau *et al*., 2014, Gaibani *et al*., 2016, Centonze *et al*., 2018). Our microbiology laboratory performs 50 to 80 swab samples daily to check the presence of CPE and ESBL producing strains. We performed the screening of Carbapenemase-producing Enterobacteria (CPE) strains on ESBL ChromID agar plate, with ertapenem disk (10 µg) plus a McConkey agar plate with a meropenem disk (10 µg). Strains showing an inhibition zone diameter <22 mm, according to European Committee Antimicrobial Susceptibility Testing (EUCAST) guidelines, and with positive Carba NP test (Nordmann *et al*., 2012) were considered as CPE. All strains were identified with MALDI-TOF Vitek MS system.

The primary goal of our study was to demonstrate the usefulness, reliability and feasibility of the MALDI-TOF Vitek MS system to quickly identify KPC-producing *Enterobacteriaceae* in a clinical diagnostics lab and to validate a routine workflow, by searching for the 11,109Da peak.

From May to July 2016, we examined daily the MALDI-TOF MS spectra obtained during bacterial species identification of CPE detected during screening according to protocols in use for MDR screening at the Microbiology Laboratory of Verona Hospital. Rectal and pharyngeal swabs were processed by different technicians. In parallel, we also investigated control group strains isolated in the same context, showing zone diameter for ertapenem disk >22 mm, with negative Carba NP test and positive ESBL NDP test (Dortet *et al*., 2014).

We analyzed the MALDI-TOF MS spectra of 183 *Enterobacteriaceae,* thus determining the presence of: 124 *Klebsiella pneumoniae*; 8 *Escherichia coli*; 2 *Enterobacter aerogenes*; 1 *Enterobacter cloacae*. All these bacteria strains were carbapenemase-producing. The remaining 48 bacteria isolated strains were ESBL-producing *K. pneumoniae*. All strains were identified withMALDI-TOF Vitek MS RUO *Axima@Saramis* (bioMérieux, France), according to manufacturer indications. Parameter settings to detect the 11,109 Da peak were: Relative Intensity ≥0; Absolute

Key words:
KPC carbapenemase, 11,109 peak, MALDI-TOF carbapenemase detection.

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Table 1 - *Results of MALDI-TOF spectra analysis on strains with Carba NP test or ESBL NDP test positive isolated during routine screening from May to July 2016. On discrepant strains MALDI-TOF, measurement was repeated after re-isolation 11,109 Da peak.*

		First MALDI-TOF spectra analysis			*Spectra analysis after repetition of discrepant strains*		
		Present	*Absent*	*Tot*	*Present*	*Absent*	*Tot*
Carba NP test positive	*K. pneumoniae*	114	10	124	122	2	124
	E. coli	6	2	8	6	2	8
	E. aerogenes	1	1	2	1	1	2
	E. cloacae	0	1	1	0	1	1
ESBL NDP test positive	*K. pneumoniae*	0	48	48	0	48	48

Intensity ≥0; Error % 0.08. In the first MALDI-TOF MS spectra analysis, 121 out of 135 (89.6%) Carba NP positive strains were positive for 11,109 Da peak (*Table 1*), whereas this peak was not detectable in all 48 positive strains for the ESBL NDP test. We repeated MALDI-TOF MS identification on 14 peak negative strains or with a confidence of spectrum analysis less than 90%. After the MALDI-TOF test was performed with a good confidence level, 8 more strains positive for Carba NP test showed the 11,109Da peak in their spectrum. Hence, 129 out of 135 MALDI-TOF MS spectra of Carba NP test positive strains showed the 11,109 Da peak, increasing the method sensitivity to 95.5%. Spectra analysis for detection of the 11,109 Da peak had 95.5% sensitivity (129/135) and 100% specificity (48/48).

We investigated 6 out of 135 Carba NP test positive bacteria strains negative for the 11,109 Da peak using multiplex and single PCRs for *bla*IMP/*bla*VIM/*bla*KPC and *bla*NDM and single *bla*OXA-48 PCR (Dallenne *et al.*, 2010). 3 out of 6 strains showed harbor gene for carbapenemases other than KPC, namely NDM (1 *E. coli*) and VIM (1 *E. aerogenes*, 1 *E. cloacae*). All 3 strains had a metal-beta-lactamase, and, for this reason, they did not show the peak in their spectrum. The other 3 strains were confirmed positive for *bla*KPC and negative for the presence of the 11,109 Da peak, resulting in discrepant strains.

These global analysis spectra results showed that 132 out of 135 carbapenemase producers were KPC producers, and the final sensitivity of MALDI-TOF MS detection was 97.7% (129/132). Only 3 (2.2%) KPC producer strains didn't show the 11,109 Da peak, namely: 87/24U, MDR4547 and MDR4597 (*Table 2*). Method specificity confirmed 100% (51/51).

Analysis of these 3 KPC-producing strains was performed to clarify the absence of the peak.

Additional primers: KPC Fw 5' TGT CAC TGT ATC GCC GTC TAG 3'; KPC Rev 5' TTA CTG CCC GTT GAC GCC CAA TCC were used to amplify and sequence the entire *bla*KPC gene in discrepant strains. By sequencing the entire *bla*KPC gene, as reported in *Table 2*, we concluded that 2 harbored KPC-19 and one KPC-3.

To characterize the plasmid content of discrepant strains, PCR-based replicon typing (PBRT) (Carattoli 2005) other than PCR was performed to detect IncQ, IncR, IncU, OriColE plasmids (Garcia-Fernandez, 2009). The same plasmid analysis was also performed on 7 strains (3 *E. coli* and 4 *K. pneumoniae*) selected among strains harboring *bla*KPC and with the presence of peak.

Through PBRT protocol plasmid characterization we proved a different scaffold on discrepant strains: 1 strain was positive for OriColE and IncR, 1 positive for IncFIA and IncFIB, and 1 positive for IncQ (*Table 2*).

The 7 strains presenting the 11,109 Da peak showed different replicons according to plasmid characterization,

Table 2 - *Results of PCRs for carbapenemase variant, p019 and plasmid profiles performed on 3 discrepant strains (KPC-producing/missing 11,109Da peak) and 7 control strains (KPC- producing/with 11,109Da peak).*

Strains	*KPC variant*	*11,109Da peak*	*p019*	*IncQ*	*IncR*	*OriColE*	*N*	*FIA*	*FIB*	*Y*	*FII*	*FrepB*
87/24 U(ECO)	KPC19	Absent	Neg	Neg	Pos	Pos	Neg	Neg	Neg	Neg	Neg	Neg
MDR4547 (KPN)	KPC3	Absent	Neg	Neg	Neg	Neg	Neg	Pos	Pos	Neg	Neg	Neg
MDR4597 (ECO)	KPC19	Absent	Neg	Pos	Neg	Neg	Neg	Neg	neg	Neg	Neg	Neg
MDR 53(KPN)	KPC3	Present	Pos	Neg	Neg	Pos	Neg	Neg	Neg	Neg	Pos	Neg
MDR368 (ECO)	KPC19	Present	Pos	Neg	Neg	Pos	Pos	Pos	Pos	Neg	Pos	Pos
MDR488 (KPN)	KPC3	Present	Pos	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Pos	Neg
MDR600 (ECO)	KPC3	Present	Pos	Neg	Neg	Pos	Neg	Pos	Pos	Neg	Pos	Pos
MDR624 (KPN)	KPC3	Present	Pos	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Pos	Neg
MDR 1985 (KPN)	KPC3	Present	Pos	Neg	Neg	Pos	Neg	Neg	neg	Neg	Pos	Neg
MDR1696 (ECO)	KPC3	Present	Pos	neg	Neg	Pos	Neg	Neg	Pos	Pos	Pos	Neg

KPN, *K. pneumoniae*; ECO, *E. coli*; Pos, positive; Neg, negative.

but the element that appeared common and yet different from discordant strains was the presence of FIIs replicon, indicating the possibility of a different plasmid harboring *bla*KPC gene in the discrepant strains, confirming the correlation between 11,109 Da peak and plasmid, (Gaibani *et al*., 2016). The FIIs rep is the replicon most frequently present in plasmid pKpQIL, carrying transposon Tn4401, very common in KPC-producing strains (Leavitt 2010).

In order to confirm this, PCRs for *p019* gene correlated to the 11,109 Da peak (Gaibani et al 2016) were performed with the following primers: p019 Fw 5' GCG GTT GAC AAA ACC ATG- 3'; p019 Rev: 5'GCT CAA ACG TCA CTA TGG C-3'. The amplification profiles were: 30 cycles of 94ºC for 1 min, 56ºC for 1 min, 72ºC for 1 min and final elongation at 72ºC for 2 min, obtaining ~300bp of product. For the 3 discrepant strains and the 7 control strains, PCR results showed a direct correlation between the 11,109 Da peak and p019. In fact, all three discrepant strains were also negative for *p019* gene, while the 7 strains presenting the 11,109 Da peak in MALDI-TOF spectra were also positive for *p019* gene.

In our study, we analyzed a large number of bacteria strains isolated from patients to validate the use of 11,109 Da peak in the diagnostic routine. Our results demonstrated the consistency of this marker for KPC production determination without the need for a molecular test in a KPC endemic region (Giani *et al*., 2013; Mazzariol *et al*., 2012, Centonze *et al*., 2018). We suggest the use of MALDI-TOF spectra analysis in combination with Carba NP test, because we find them complementary, and in this way carbapenemase-producing strains without 11,109 Da peak are always detected. These latter strains can be checked at the molecular level to find the carbapenemse enzyme produced.

The correlation between the presence of 11,109 Da peak and positive results by Carba NP test confirmed the reliability of MALDI-TOF MS analysis as a rapid screening method to detect KPC carbapenemase in carbapenemase-producing bacterial strains.

It is noteworthy that our observations confirmed and reinforced those indicated in previous studies (Lau *et al*., 2014, Gaibani *et al*., 2016, Centonze *et al*., 2018) using the Bruker Daltonics Maldi TOF system. On the other hand, to our knowledge, our validation was the first to be carried out on the BioMérieux MALDI TOF platform, which uses the same technology but with a different software (Shimadzu Launchpad) and a different database (SARAMIS™ Premium (Spectral ARchivee And Microbial Identification System) ID-professional (AnagnosTec-bioMérieux)).

Epidemiology of our geographic area, with over 90% of carbapenemase, which are KPC (Giani *et al*., 2013), is favorable to implement this analysis. The utility of the 11,109 Da peak detection, moreover, is directed not only to find KPC-producing strains during the routine identification, but also to find strains producing other types of carbapenemases (for example NDM or VIM, which are increasing in frequency) or new carbapenemases. The presence of the 11,109 Da peak combined with a positive Carba NP test confirms KPC production. On the other hand, the absence of the peak combined with positive Carba NP test can lead to perform PCR for *bla* genes and to search for carbapenemase other than KPC. In fact, the 3 above-mentioned strains were detected and characterized as NDM and VIM producers precisely through spectra analysis, since the Carba NP test provides information only about carbapenemase production, whereas it is unable to reveal the type of carbapenemase involved. This aspect is important not only to provide epidemiologic information, but, mainly, to detect strains with a high level of resistance and transmissibility, such as NDM producers, the new threat emerging worldwide. Moreover, detailed information about the specific type of carbapenemase is noteworthy when an effective treatment against only some specific types of carbapenemase producers is required, as with Ceftazidime-Avibactam, which is not active against MBL producer strains.

The 11,109 Da peak search is a sensitive, specific screening method that provides immediately useful information to clinicians, allowing them to adopt prompt containing measures for carriers. This approach is rapid, simple and does not require high expertise or extra steps in the current identification flow with the MALDI-TOF MS system. Moreover, the costs are much lower compared to other diagnostic tests, such as, for example, molecular methods, which are also time-consuming and more difficult to perform, and their use could be then restricted for discrepant strains only.

In addition, MALDI-TOF MS measurements can be performed directly from colony, and as we have shown are not operator dependent and allow easy screening of a large number of samples. MALDI-TOF spectrum analysis to peak detection can also be performed retrospectively, on spectra already acquired for identification. This is very useful when Enterobacteria strains showed carbapenems resistance in their antibiotic susceptibility test, rapidly identifying KPC producers. The test limitation is the quality of spectra. To ensure reliable results, it is extremely important to acquire high quality spectra (confidence ≥90%). Therefore, it is necessary to guarantee the calibration of the instrument and the standardization of the pre-analytical phase.

In conclusion, our data validated the peak at 11,109 Da as a valuable marker for KPC producers in a cohort of isolated strains in an endemic area for the presence of these antibiotic resistances usingMALDI-TOF Vitek MS (BioMerieux). The reliability and sensitivity of this marker suggest the need to improve the current MALDI-TOF software with an automatic procedure to check the presence of 11,109 Da peak for a useful diagnostic analysis approach for the laboratory. In KPC endemic area, we propose this diagnostic tool to find a KPC producer without additional cost, monitoring the insurgence of outbreaks in a rapid and inexpenseive way.

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