Evaluation of the Vitek MS ® system in the identification of KPC-type carbapenemase-producing *Enterobacterales* carrying the Tn*4401a* transposon

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Abstract:

Identification of carbapenemase-producing clinical isolates is crucial for timely treatment guidance and control of in-hospital dissemination. Objective: To determine the diagnostic validity of the MALDI-TOF Vitek MS technique in the detection of KPC-type carbapenemases through the identification of the characteristic peak 11.109 Da in the mass spectrum, using PCR as a reference method. Methods: We evaluate 210 isolates, including 34 positive controls consisting of carbapenemase-producing *Klebsiella pneumoniae* isolates positive for the *bla*$_{KPC}$ gene, the pKpQIL plasmid and the Tn*4401a* transposon, 30 Enterobacteriaceae *bla*$_{KPC}$ isolates positive by PCR but of unknown plasmid background, and 146 negative controls. Agreement was established between MALDI TOF MS, the RAPIDEC® CARBA NP and modified Carbapenemase Inactivation Method (mCIM) test in conjunction with eCIM ($p <= 0.05$) (95%CI); In addition, ROC curves and comparisons were made for each of these tests. Results: The 11.109 Da peak was detected in 100% of KPC Tn*4401a* positive isolates reaching sensitivity values of 100% (CI 98.53 - 100), specificity of 94.78% (CI 90.64 - 98.92), PPV of 82.93% (CI 70.19 - 95.66), NPV of 100% (CI 99.61- 100) and PVR of 19.14% (CI 9.31- 39.37). An agreement between the three diagnostic tests of 92.3% and a Kappa index of 0.8877 (CI 0.8112-0.9642) was obtained. Comparison of the ROC curves of the three tests showed AUC of 0.9714, 0.9786 and 0.971 for MALDI TOF, RAPIDEC® CARBA NP and inactivation respectively. Conclusion: Detection of the 11.109 Da peak confirms the presence of KPC-type carbapenemase, thus saving time and costs by detecting KPC-type carbapenemases simultaneously with

identification; a negative result does not rule out the presence of the enzyme. The circulation of transposons other than Tn*4401a* requires the use of additional tests.

1. Introduction

The emergence and spread of carbapenemase-producing Enterobacteriaceae (CPE) has become a public health threat for the prevention, management and control of infectious diseases (1,2). Their rapid dissemination, difficult control, and the limited availability of therapeutic options are associated with an increase in morbidity and mortality rates in patients infected by these microorganisms along with an increase in health care costs (1,3,4).

KPC-type carbapenemases (*Klebsiella pneumoniae* carbapenemase), are enzymes that present the highest frequency and dissemination capacity worldwide and are endemic in many countries, including Colombia. (5,6). The success of this propagation is related to the transmission of the bla_{KPC} gene associated to mobile segments such as the Tn*4401* transposon included in IncFII plasmids, pKpQIL is the plasmid being the most common vehicle for bla_{KPC} gene transmission (7). In this case, using technologies that allow the rapid detection of these elements and their association with the presence of this type of carbapenemases is crucial to guide timely an effective treatment, improving the chances of survival of patients and the control of in-hospital dissemination of carbapenemase-producing bacteria (7,8).

Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry-Based (MALDI-TOF MS MS) has been successfully included in the microbiology laboratory offering a fast and reliable alternative for the identification of microorganisms by analyzing proteins, mainly ribosomal, through the creation of a mass spectrum that is specific to each genus and species. (9–11). This tecnology has been described in the detection of carbapenemase enzymes through different methodologies, by monitoring and analyzing mass spectra of intact antibiotics versus the molecule degraded upon exposure to carbapenemase-producing bacteria or through the detection of characteristic peak patterns related to resistance determinants (12).

Recent studies have described the correspondence between the production of KPC-type carbapenemases with the presence in the MALDI-TOF MS mass spectrum of a peak of 11.109 Da corresponding to the pKpQIL_p019 protein (p019) (13–15). The p019 gene is associated with the Tn*4401a* transposon isoform as part of an insertion sequence that is only present in plasmids harboring the KPC gene (13), thus confirming the direct relationship between these characteristic spectra and the presence of KPC-type carbapenemases.

Few studies have explored this application in the VITEK MS system, using a small variety of isolates limited to one or two health institutions, without ruling out the potential clonality of isolates which could introduce a bias when comparing the performance of the test, and the sensitivity results of these studies would not apply to other epidemiological settings (16,17).

In Colombia a high circulation of KPC-producing bacteria has been described (5,18) along with this, there is an increase in the use of MALDI TOF MS in clinical laboratories. It is importance to have routine diagnostic tools in clinical microbiology laboratories that allow timely patient management and prevent the spread of this resistance mechanism. This study was performed with the aims to determine the diagnostic validity of the MALDI-TOF Vitek MS technique in the detection of KPC-type carbapenemases through the identification of the characteristic peak 11.109 Da in a collection of carbapenem-resistant *Enterobacterales*. In addition, to determine the agreement between MALDI TOF Vitek MS and other phenotypic tests conventionally used for the detection of KPC-type carbapenemasas.

2. **Methods and materials**

A validation study of a MALDI TOF MS method was performed for the detection of KPC-type carbapenemases using PCR (Polymerase Chain Reaction) as a reference method and the ability of MALDI TOF MS to detect the 11.109 Da peak in isolates with unknown plasmid background was described. Additionally, the diagnostic performance for detection of carbapenemases by other conventional methods was determinate The concordance between MALDI TOF Vitek MS, RAPIDEC® CARBA NP (bioMérieux, Marcy l'Etoile, France) and the modified Carbapenemase Inactivation Method (mCIM) in conjunction with

eCIM was evaluated; ROC curve was performed for each of the tests with their subsequent comparison.

2.1. Bacterial isolates

We used a collection of 210 isolates collected during two research projects conducted in the years 2012 to 2015 from hospitalized patients in 5 institutions of third level of complexity in the city of Medellin, Colombia. The collection of isolates were characterized using PCR for detecting carbapenemase genes according to the protocols developed by Poirel et al (19). From this collection we selected thirty-four positive controls isolates consisting of carbapenemase-producing *Klebsiella pneumoniae* strains positive for the bla_{KPC} gene, the pKpQIL plasmid and the Tn*4401a* transposon and 146 negative controls including *Enterobacterales* and non-fermentative Gram-negative bacilli (NFGNB) carrying different resistance mechanisms including KPC-type carbapenemases not having pKpQIL plasmid neither the Tn*4401a* transposon and carbapenemases other than KPC. Additionality thirty *Enterobacterales* isolates positive for the bla_{KPC} gene but with an unknown plasmid background were evaluated exclusively by MALDI TOF for the sole purpose of describing the ability of this technique to detect the 11,109 Da peak in isolates in which the presence of the Tn*4401a* transposon is unknown. (Figure 1).

2.2. Description of procedure

2.2.1. Bacterial identification

The isolates stored at -70°C were activated after two subcultures on blood agar for 18-24 h at 35°C (20), and re-identified using the MALDI-TOF Vitek® MS system (bioMérieux, Marcy l'Etoile, France) before use. Carbapenemase production control was carried out by checking the susceptibility profile of the isolates together with phenotypic testing for carbapenemases detection, in which the presence of a sensitive profile and a negative phenotypic test result following the activation process of an isolate previously characterized as carbapenemase-producing suggested loss of the plasmid.

2.2.2. Antimicrobial susceptibility

Antibiotic susceptibility was determined using the VITEK® 2 automated system (bioMérieux, Marcy l'Etoile, France) (21), to determine the sensitivity profile of the isolates

and to confirm the presence or not of carbapenem resistance. The antibiotics susceptibility was interpreted according to *The clinical and Laboratory Standards Institute* (CLSI) (22). An isolate was considered resistant to carbapenems when an intermediate or resistant result to one of the carbapenems (Ertapenem, , Imipenem or Meropenem) was recorded (23).

2.2.3. Determination of the diagnostic validity of the MALDI-TOF VITEK MS technique in the detection of KPC-type carbapenemases.

The diagnostic validation of MALDITOF vitek MS was performed using 34 positive controls and 134 negative controls. An initial pilot test was performed to determine the best quality of the spectra to be analyzed, and the best method for the detection of the 11.109 Da peak. After the validation process, KPC-producing isolates, but with unknown plasmid background, were processed by MALDI TOF to describe the 11.109 Da peak in isolates in which the presence of the Tn*4401a* transposon is unknown.

2.2.3.1. Detection of the 11.109 Da peak by MALDI-TOF Vitek® MS

2.2.3.1.2 Sample Preparation

The pilot test was performed with 30 isolates molecularly characterized as positive for the *bla*$_{KPC}$ gene and carriers of the Tn*4401a* transposon, three methods were evaluated: Direct method (direct deposition of the colony in the well); Formic acid method (direct deposition of the colony in the well plus 1uL of formic acid); and sample extraction method described by Rocco et al (24) for both the Vitek MS RUO and Vitek MS IVD systems (Supplement 1).

2.2.3.1.3. Spectrum acquisition and analysis

To obtain the spectra, the MALDI-TOF Vitek® MS system (bioMérieux, Marcy l'Etoile, France) was used in its two configurations RUO and IVD in a positive linear mode in the mass range 2000-20000 m/z, without changing the instrument parameters. Results were analyzed using the SaramisTM database (Spectral File and Microbe Identification System) (Version 4.10, AnagnosTec, Potsdam, Germany) and Shimadzu Biotech Launchpad® software (Shimadzu Corporation, Kyoto, Japan) (24).

Data analysis was performed according to the manufacturer's instructions; the identification of the microorganism at the species level was considered valid in the range of percentages between 75% and 99.9%, using *Escherichia coli* ATCC® 8739 as a control strain. The detection of the 11.109 Da peak was performed by visual inspection of the spectra obtained by MALDI-TOF MS using SaramisTM software and a record of the corresponding spectrum was made. The 11.109 Da peak was considered as positive in a range of 10 Da above or below this value (11.099 Da -11.119 Da) and negative between values greater than 11.119 Da and less than 11.099 Da.

K. pneumoniae ATCC BAA 1705 (*bla*$_{KPC}$-positive) and *K. pneumoniae* ATCC BAA 1706 (*bla*$_{KPC}$-negative) strains were used as positive and negative controls, respectively.

2.2.4. Diagnostic performance for detection of carbapenemases by other conventional methods.

Diagnostic performance for detection of carbapenemasas by RAPIDEC® CARBA NP method and the modified Carbapenemase Inactivation Method (mCIM) in conjunction with eCIM was performed using PCR as the reference method. Based on the ability of these tests to detect any type of carbapenemases, 98 and 96 positive controls and 70 negative controls were used, respectively.

The RAPIDEC® CARBA NP technique (bioMérieux, Marcy l'Etoile, France) was used according to the manufacturer (25). The results were read by three observers blind to the results obtained for other methods of carbapenemase detection.

Modified carbapenemase inactivation method (mCIM) in conjunction with eCIM was performed following the standard method described by *The Clinical and Laboratory Standards Institute (CLSI)* (23,26).

2.2.5. Concordance

The concordance between MALDI TOF vitek MS, RAPIDEC® CARBA NP and the modified Carbapenemase Inactivation Method (mCIM) in conjunction with eCIM was established using the 34 isolates of KPC-producing isolates (Tn*4401a* positive) as positive control and 70 negative controls.

ROC curve was performed for each of the tests and the subsequent comparison of these to establish the ability of the tests to differentiate a KPC-producing isolate (Tn*4401a* positive), using only the 34 isolates of KPC-producing isolates (Tn*4401a* positive).

3. Statistical analysis

Categorical variables were described in relative and absolute frequencies, quantitative variables were described by reporting mean and standard deviation or medians with their interquartile range according to the data distribution. Diagnostic sensitivity and specificity values, positive and negative predictive values and likelihood ratios were performed for detection of 11.109 Da by Vitek MS as marker for KPC presence.

Percent of agreement and nominal Cohen's Kappa coefficient (95%CI) were calculated to establish agreement between the three tests. For each of the tests, a ROC curve, and a subsequent comparison of these was performed to select the test with the best ability to differentiate between KPC-producing bacteria carrying the Tn*4401a* transposon. Values of $p \leq 0.05$ were considered statistically significant. Statistical analyses were performed using EPIDAT 4.2 (27).

4. Results

4.1. Description of the isolates

Among the 210 initial isolates, 15 were excluded due to possible plasmid loss, confirmed by negative phenotypic tests for carbapenemase detection and antibiogram with a sensitive profile, leaving a total of 195 isolates. Two additional isolates were excluded for the analysis of the diagnostic validation of the inactivation method (Figure 1). The 168 microorganisms studied for MALDI TOF Vitek MS diagnostic validation were distributed as: *Klebsiella pneumoniae* n=80 (47.6%), *Escherichia coli* n=29 (17.3%), *Enterobacter cloacae* n=27 (16.1%), *Pseudomonas aeruginosa* n=27 (16.1%) and n=5 (3%) of other Gram-negative bacterial species (Table 1). The previously known resistance mechanisms associated with the different species are described in figure 2.

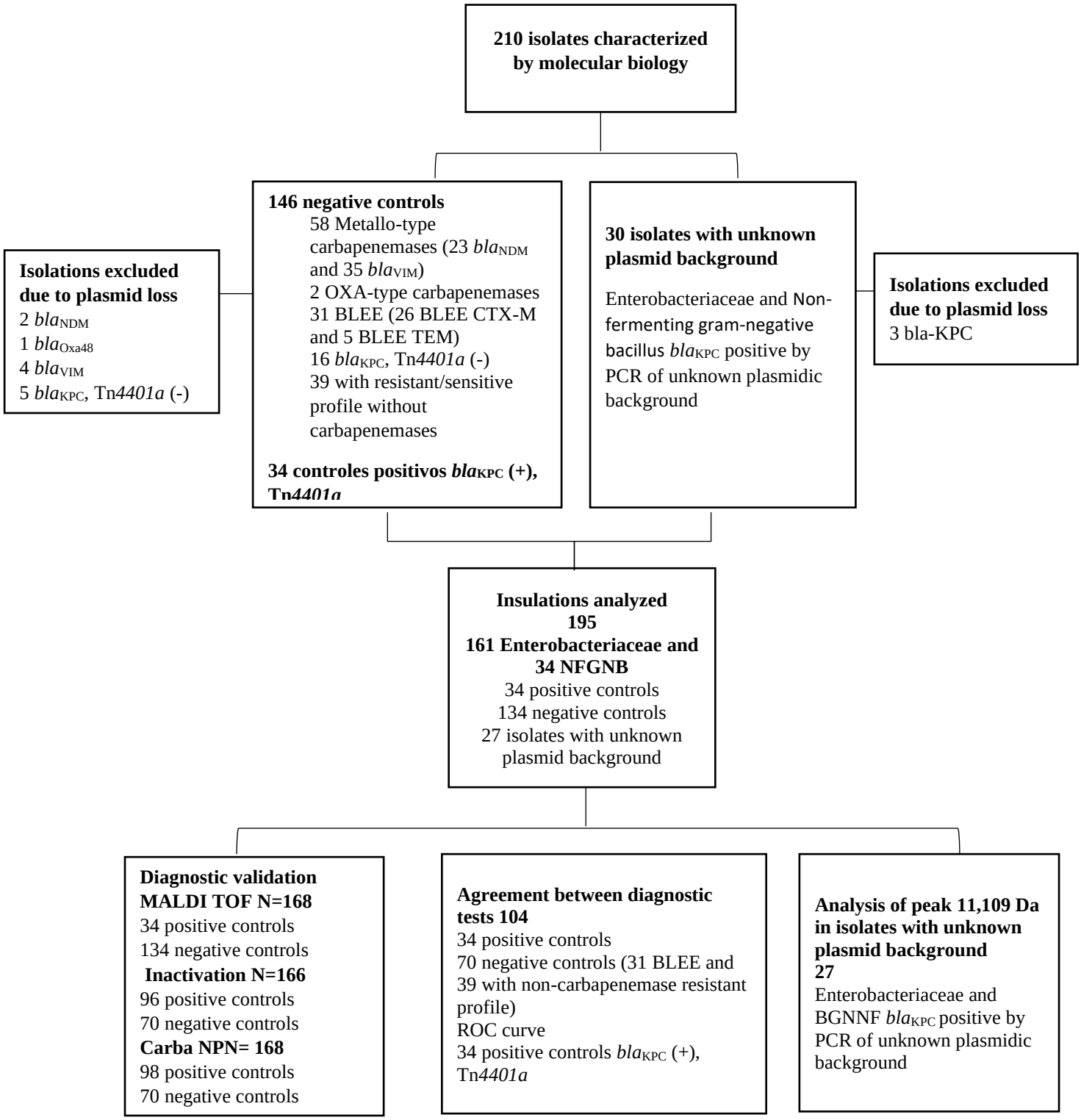

Figure 1. Selection of isolates. *From a total of 210 initial isolates, 15 isolates were excluded due to plasmid loss for a total of 195 microorganisms analyzed.*Non-fermenting gram-negative bacillus*

Table 1. General description of the presence of peak 11.109 Da according to the type of microorganisms and previously known resistance mechanisms

Variables			Confirmed identification by Vitek MS	
	n (168)	**%**	**Positive (n) peak 11.099 -11.119 Da**	**Negative (n) peak < 11.099 or > 11.119 Da**
Microorganism				
Klebsiella pneumoniae	80	47,6	37	43
Escherichia coli	29	17,3	2	27
Enterobacter cloacae	27	16,1	1	26
Pseudomonas aeruginosa	27	16,1	1	26
Klebsiella oxytoca	2	1,2	0	2
Providencia stuartii	1	0,6	0	1
Citrobacter freundii	1	0,6	0	1
Acinetobacter baumanii complex	1	0,6	0	1
Characterization by molecular biology				
bla_{KPC}, Tn4401a	34	20,2	34	0
bla_{VIM}	31	18,5	1	30
BLEE CTX-M*	26	15,5	2	24
With sensitive profile without carbapenemases	21	12,5	1	20
bla_{NDM}	21	12,5	1	20
With resitent profile without carbapenemases	18	10,7	1	17
bla_{KPC}, Tn4401a (-)	11	6,5	1	10
*BLEE TEM**	5	3	0	5
bla_{Oxa}	1	0,6	0	1

**BLEE: Extended spectrum beta-lactamase*

Figure 2: Resistance mechanisms by species of isolate selected. *In the study. K. pneumoniae was described carrying all blaKPC (+), Tn4401a and blaKPC (+), Tn4401a (-) genes. Pseudomonas aeruginosa was described as carrying only blaVIM-type metallocarbapenemases and Escherichia coli was described exclusively as a BLEE producer.*

4.2. **MALDI-TOF MS diagnostic validation**

The Vitek® MS IVD system was used through direct methodology for the rapid identification of the 11.109 Da peak, which was detected in 34/34 (100%) bacteria carrying the *bla*$_{KPC}$ (+) gene, Tn*4401a* and in 7/134 (5%) of the negative controls (Table 1). Therefore, the detection of this peak reached sensitivity values of 100 % (CI 98.53 - 100), specificity of 94.78 % (CI 90.64 - 98.92), positive predictive value of 82.93% (CI 70.19 - 95.66), negative predictive value of 100% (CI 99.61- 100) and positive likelihood ratio of 19.14% (CI 9.31- 39.37) (Table 3).

Table 3. Diagnostic evaluation of MALDI TOF MS using PCR as a reference method.

Performance parameters		
	IC 95%	
Sensitivity	**LL**	**UL**
100	98,53	100
Specificity		
94,78	90,64	98,92
Validity index		
95,83	92,51	99,15
VPP		
82,93	70,19	95,66
VPN		
100	99,61	100
RV +		
19,14	9,31	39,37

PPV: Positive Predictive Value, NPV: Negative Predictive Value, PLR +: Likelihood Ratio, IC 95%: 95% confidence interval, UL: Upper limit, LL: Lower limit

4.3. **MALDITOF MS in the detection of the 11.109 Da peak in KPC-producing isolates with unknown plasmid background.**

Among the of 27 KPC-producing isolates with unknown plasmid background, the Tn*4401a* transposon was detected through the identification of the 11.109 Da peak in the mass spectra of 6/27 (22%) isolates.

4.4. **Diagnostic performance for detection of carbapenemases of mCIM carbapenemics inactivation test in association with eCIM and the RAPIDEC® CARBA NP test**

The inactivation tests for carbapenems mCIM in association with eCIM and RAPIDEC® CARBA NP using PCR as a reference test showed sensitivity values of 89.58% (CI 82.95 - 96.21) and 100% (CI 99.49 - 100) and specificity values of 97.14% (CI 92.53 - 100) and 95.71% (CI 90.26 - 100) respectively (Table 5). Of the 168 isolates evaluated by RAPIDEC® CARBA NP, a discrepancy was observed in 10 (16.8%) observers yielding a Kappa of 0.9078 (CI 0.8549-0.9607) p-value: ≤ 0.05. Discrepancies occurred in non-carbapenemase-producing bacteria, 8 ESBL-producing isolates and 2 carbapenemase-resistant but non-carbapenemase-producing isolates. In all cases one of the three observers erroneously qualified the result as positive.

Table 5. Performance evaluation measures Modified Carbapenemase Inactivation Method (mCIM) in conjunction with eCIM and CARBA NP using PCR as a reference method.

Performance parameters						
		IC 95%			IC 95%	
	Inactivation	LL	UL	Carba NP	LI	UL
Sensitivity	89,58	82,95	96,21	100	99,49	100
Specificity	97,14	92,53	100	95,71	90,26	100
Validity index	92,77	88,53	97,01	98,21	95,91	100
VPP	97,73	94,05	100	97,03	93,22	100
VPN	87,18	79,12	95,24	100	99,25	100

RV +	31,35	7,99	123,1	23,33	7,71	70,59
RV-	0,11	0,06	0,19			

PPV: Positive Predictive Value, NPV: Negative Predictive Value, PLR +: Positive Likelihood Ratio, PLR- Negative Likelihood Ratio, IC 95%: 95% confidence interval, UL: Upper limit, LL: Lower limit

4.5. **Concordance between the three diagnostic tests.**

There was an agreement between the three diagnostic tests of 92.3% and a Kappa index of 0.8877 (CI 0.8112-0.9642), p-value: ≤ 0.05. The ROC curve for each of the tests yielded values of area under the curve (AUC) of 0.9739 (CI: 0.9550-0.9928), 0.9336 (CI: 0.8972-0.9701) and 0.9786 (CI: 0.9547 -1.0025) for MALDI TOF, inactivation and RAPIDEC® CARBA NP respectively. Comparison of the ROC curves of the three tests showed AUC of 0.9714, 0.9786 and 0.971 for MALDI TOF, RAPIDEC® CARBA NP and inactivation respectively (Figure 3).

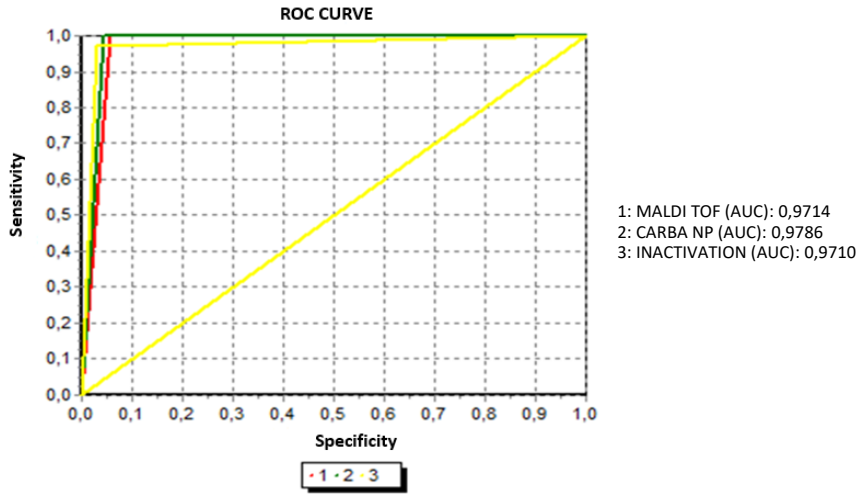

Figure 3. *ROC curve comparison of the three diagnostic tests: Comparison of the ROC curves of the three tests showed AUC of 0.9714, 0.9786 and 0.971 for MALDI TOF, RAPIDEC® CARBA NP and inactivation respectively*

4.6. Susceptibility profile

All *bla*$_{KPC}$ Tn*4401a* (+)/ Tn*4401a* (-), *bla*$_{NDM,}$ *bla*$_{VIM}$ and *bla*$_{OXA}$ isolates presented a resistant or intermediate susceptibility profile for all three carbapenemics evaluated (Table 6). All isolates carrying peak 11.109 Da had Meropenem, Ertapenem and Imipenem minimum inhibitory concentrations of >=16 ug/mL >=8 ug/mL and >=16 ug/mL respectively.

Table 6. Carbapenemics susceptibility profile according to resistance genes

	Interpretation Meropenem (n)			**Interpretation Imipenem (n)**			**Interpretation Ertapenem (n)**		
Resistance gene	**Resistant**	**Intermediate**	**Sensible**	**Resistant**	**Intermediate**	**Sensible**	**Resistant**	**Intermediate**	**Sensible**
bla$_{KPC}$, Tn*4401a*	34	0	0	34	0	0	34	0	0
bla$_{NDM}$	21	0	0	20	1	0	20	0	0
bla$_{VIM}$	25	5	0	31	0	0	3	0	0
bla$_{Oxa}$	1	0	0	1	0	0	1	0	0
bla$_{CTX-M}$	1	0	25	1	0	25	1	0	25
bla$_{TEM}$	0	0	5	0	0	5	0	0	5
Sensitive profile*	0	0	21	0	0	21	0	0	20
resistant profile	3	1	14	5	2	11	8	4	2
bla-KPC, Tn*4401a* (-)	11	0	0	11	0	0	8	1	0

**With carbapenemase-free Sensitive profile, ** With carbapenemase-free resistant profile*

Among the KPC-types carbapenemase-producing bacteria, 41/45 (91%) showed a sensitive profile to Ceftazidime/avibactam; the only Oxa-type carbapenemase showed a sensitive profile and 45/52 (87%) of the Metalocarbapenemases showed a resistant profile to this antibiotic. As for aztreonam, 44/45 (98%) of the KPC-type carbapenemases showed a resistant profile; 25/52 (49%) Metalocarbapenemases showed a resistant result, 9/52 (18%) a sensitive profile and 17/52 (33%) an intermediate result.

5. Discussion

The worldwide dissemination of KPC-type carbapenemase-producing Enterobacteriaceae requires rapid identification methods that allow timely decision making in the critically ill

patient. The use of MALDI TOF MS through the detection of the 11.109 Da peak provides a novel and rapid option for the detection of these enzymes.

Vitek MS diagnostic performance found in the present study showed sensitivity values of 100% and specificity of 94.7%, which are similar to the values reported in other studies who have described sensitivities ranging from 85.1% to 98.7% and specificities ranging from 99% to 100% (14,17,24,28,29). The difference of our study with others that have evaluated the MALDI TOF VITEK MS is that the isolates we used are derived from different institutions decreasing the possibility of a bias effect due to the clonality of the isolates studied (17,24).

The results of this study confirmed that Vitek MS provides quick answers to the clinician, due to the joint identification of the microorganism and the presence of the 11,109 Da peak indicating the presence of carbapenemase producing microorganisms. This finding has direct impact on therapeutic decision-making and the initial management of these (30), dereasing time of hospitalization with significant savings in time and costs of diagnosis (3,8).

The *p019* gene encodes the protein P019 of mass 11,109 Da that is part of the ISKpn31 insertion sequence into which the Tn*4401a* transposon was inserted (13,31). Plasmids carrying bla_{KPC} that do not have this genetic environment and in particular the p019 gene, will not be able to be detected, nor can the other mechanisms of carbapenem resistance ($bla_{NDM,}$ bla_{VIM}, efflux pumps and porin closure). Additional resistance confirmation techniques and a precise knowledge of the local genomic epidemiology of carbapenem resistance must be available (31). In our study 7 false positives were identified by Vitek MS, probably generated by the presence of another protein similar to P019 that generates an analogous peak in the spectrum. A less likely reason is that the P019 protein, being part of the mobile element (Tn*4401a*), could insert itself without a specific target in the bacterial chromosome, not necessarily being associated with the bla_{KPC} gene, and generate the 11,109 Da peak in the spectrum (31,32) Several studies in Colombia have described the co-circulation of bla_{KPC} genes associated with both the Tn*4401* transposon isoform a and b and with elements other than Tn*4401* (NTEKPC) (2,33–35), that could be the explanation for the results obtained in the small set of isolates KPC with unknown plasmid background

evaluated in which only 22% of isolates were positive for peak 11,109 Da indicating that the remaining 78% of KPC carbapenemase-producing strains carried transposons other than Tn*4401a*. This scenario suggests the need to interpret the presence or absence of the 11,109 Da peak in the MALDI TOF MS spectrum according to the epidemiological setting of the *bla*$_{KPC}$ gene associated with the Tn*4401a* transposon and the performance of studies evaluating the MADI-TOF Vitek MS method in a prospective way. In a high prevalence environment, the test would have high PPV and NPV, but in settings where resistance to carbapenemics is mediated by other mechanisms or there is circulation of different transposons carrying *bla*$_{KPC}$ it would have high PPV and low NPV, in this case the presence of the 11,109 Da peak confirms the presence of KPC-type carbapenemases, but a negative result does not rule out this resistance and further confirmatory studies are needed.

The evaluation of the diagnostic performance for mCIM carbapenemics inactivation test in association with eCIM showed sensitivity of 89.58% and specificity of 97.14%. Other studies have reported values ranging from 99 to 100% and 96 to 100% respectively (36). However, although this technique has the benefit to differentiate metalocarbapenemases from other types of enzymes and is more economical (23), it has a laborious processing, involving a 4-hour incubation during the inactivation step and subsequent overnight incubation that makes the application of this test in the clinic more difficult (36).

RAPIDEC® CARBA NP presented sensitivity values of 100% and specificity values of 95.7% compared to those described in other studies where values of 94 to 98% and 99 to 100%, respectively, have been obtained (36). This performance values together with the rapid differentiation of carbapenemase-producing bacteria from non-producing bacteria, make this test a good option in the clinical routine for an initial approach to diagnosis; however, since this technique does not differentiate the type of enzyme, it requires the use of additional confirmatory tests (37). Likewise, it has a low sensitivity to identify OXA48 type carbapenemases and being of visual interpretation based on color variation, it has an important degree of subjectivity (36–38). In our case, in 10 isolates there was no agreement between the observers, despite this, the kappa index presented a value of 0.9078, which is equivalent to an almost perfect agreement.

An almost perfect agreement was obtained among the three tests for the detection of the production of KPC-type carbapenemases, in addition, when comparing the AUC of the three ROC curves, similar values were identified.

For a rational use of diagnostic tests and considering the characteristics of the evaluated tests a diagnostic algorithm is suggested to determine the presence of carbapenemase producing isolates: the initial test should be Vitek MS which allows the detection of the 11,109 Da pick together with the identification of the bacteria at no additional cost. A positive result confirms the presence of a KPC type carbapenemases while a negative result should be follows by performing carba NP test which allows detecting the presence or not of carbapenemases in a maximum time of 2 hours, a negative result rules out the presence of any type of enzyme; a positive result, should follows by an Inactivation test eCIM/mCIM in order to differentiate metallo type carbapenemases from other types of carbapenemases or the use of rapid carbapenemases detection methods such as the NG test CARBA 5® , which in 15 minutes allows the identification of 5 different carbapenemases (39)

The sensitivity profile observed for carbapenem antibiotics according to the mechanism of resistance showed a behavior consistent with that reported in other studies (40,41). At this point, all carbapenemase-producing bacteria and in particular the KPC-type bacteria carrying the Tn*4401a* transposon showed a complete hydrolysis profile of all carbapenemics (Meropenem, Ertapenem and Imipenem MICs of >=16 ug/mL >=8 ug/mL and >=16 ug/mL respectively) (23). This resistance profile associated with the presence of the 11,109 Da peak could quickly guide treatment to a particular therapeutic option.

Ceftazidime avibactam and aztreonam are therapeutic alternatives for treating infections caused by carbapenemase producing microorganisms. As expected, most of the KPC and Oxa carbapenemase-producing isolates evaluated showed a sensitive profile(42). Nevertheless, 9% of the isolates presented a resistant profile which suggest the presence of additional resistance mechanisms such as mutations in AmpC or in carbapenemase enzymes mainly in *bla*$_{\text{KPC-3}}$ carriers of omega-loop mutations. (43–46), changes in PBPs, decreased drug permeability or increased efflux pumps (47,48). Among Metalocarbapenemases evaluated, 13% presented an unusual profile of sensitivity to

ceftazidime avibactam, considering that the most common mechanism of acquired resistance to this antibiotic is the production of avibactam-refractory beta-lactamases such as class B enzymes (43).

Aztreonam presented a resistant profile for most of the isolates carrying the *bla*$_{KPC}$ gene, as expected, while the metallocarbapenemases presented a more heterogeneous profile indicating that although aztreonam is not hydrolyzed by metallocarbapenemases, its action may be affected by the existence of additional serine-type beta-lactamases (42,49). The concomitant use of aztreonam with the new combinations of beta-lactams with beta-lactamase inhibitors such as ceftazidime avibactam can restore its action against metallocarbapenemases due to the inhibition of serine-type beta-lactamases by avibactam, (42,50,51).

In conclusion, the present study described and validated MALDI-TOF technology as an innovative, rapid, and efficient method for the rapid identification of KPC-producing *Enterobacterales* carrying the Tn*4401a* transposon. Our results demonstrated the good diagnostic performance of this system in the detection of the 11,109 Da peak; furthermore, its inclusion as part of the diagnostic algorithm of the institutions that have this technology would allow savings in time and costs by detecting KPC-type carbapenemases simultaneously with the identification allowing for rapid treatment targeting. An important limitation of the method evaluated is its inability in the detection of other types of carbapenemases other than those carrying Tn*4401a* transposons, a situation that is conditioned by the prevalence of the KPC gene associated with the "a" isoform of the Tn*4401* transposon, so that a positive result can only be used as a first step for the direct detection of KPC-producing *Enterobacterales*, while negative results must be confirmed by an additional confirmatory test.

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