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Evaluation of Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry for Identification of KPC-Producing *Klebsiella pneumoniae*

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We evaluated a real-time single-peak (11.109-Da) detection assay based on matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) for the identification of *Klebsiella pneumoniae* carbapenemase (KPC)-producing *K. pneumoniae*. Our results demonstrated that the 11.109-Da peak was detected in 88.2% of the KPC producers. Analysis of bla_{KPC}-producing *K. pneumoniae* showed that the gene encoding the 11.109-Da protein was commonly (97.8%) associated with the Tn*4401*a isoform.

In the last decade, an emerging spread of carbapenemase-producing *Enterobacteriaceae* (CPE) was observed worldwide, especially in *Klebsiella pneumoniae* strains. As a result, *K. pneumoniae* carbapenemase (KPC)-producing *K. pneumoniae* has become endemic in several countries, including Italy, Greece, and the United States (1).

Rapid detection of KPC producers is mandatory to limit the spread of this pathogen and for clinical management of complicated infection due to KPC-producing *K. pneumoniae*. Numerous phenotypic (e.g., Carba NP, modified Hodge test, disk-diffusion synergy test) and genotypic (e.g., single and multiplex PCR) assays were developed for rapid identification of CPE (2, 3). Recently, matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) was proposed to detect carbapenemase production in Gram-negative organisms (4).

Here, we propose a simple approach based on MALDI Biotyper RTC software to identify KPC-producing *K. pneumoniae* clinical strains. The aim of this study was to evaluate the sensitivity and specificity of an innovative MALDI-TOF approach using a large population of well-characterized unrelated *K. pneumoniae* strains collected in Italy.

β-Lactamase genes and the clonal relatedness of bacterial isolates were investigated by PCR and multilocus sequence typing (MLST) analysis (3, 5). For MALDI-TOF MS analysis, strains were evaluated as previously described (6).

Dendrogram analysis revealed two main separate clusters, representing KPC-producing and non-KPC-producing *K. pneumoniae* strains, which included extended-spectrum β-lactamase (ESBL) producers and wild-type strains (Fig. 1). In order to evaluate the major significant differences between the protein patterns of these two groups, spectra were analyzed by ClinProtTools software (v3.0; Bruker Daltonics, Inc.). Single-peak analysis identified a total of 10 peaks (*m/z* of 5.555, 6.082, 6.152, 7.384, 7.663, 7.704, 7.763, 7.851, 11.109, and 11.185) that potentially differentiate between KPC-positive and KPC-negative *K. pneumoniae* strains. In order to identify group-specific peaks as candidate biomarkers for KPC-producing *K. pneumoniae* strains, the presence of different peaks were manually investigated by ClinProtTools software. Our analysis demonstrated that an 11.109-Da peak was detected in most of the spectra obtained from KPC-positive strains, whereas it was absent in all of the KPC-negative isolates. At the same time, the remaining eight peaks (excluding the 5.555-Da peak as a double charged 11.109-Da peak) were found in both KPC-producing and non-KPC-producing *K. pneumoniae* strains, suggesting a low discriminatory power. Our data are consistent with previous findings that demonstrated a correlation between the 11.109-Da peak, representing a cleavage product of a hypothetical protein named pKpQIL_p019 (p019), and the bla_{KPC}-harboring pKpQIL-like plasmids (7). Moreover, Youn and colleagues (8) recently proposed an in-house script developed in flexAnalysis software for rapid detection of the 11.109-Da peak in KPC-producing enterobacterial clinical strains.

In our MALDI-TOF analysis, the 11.109-Da peak was detected in 30 (88.2%) of 34 KPC-producing *K. pneumoniae* strains, while only 4 KPC-producing *K. pneumoniae* strains were negative (Table 1). MLST analysis showed that the 11.109-Da peak was found in KPC-producing *K. pneumoniae* belonging to a different sequence type (ST), as shown in Table 1. At the same time, all of the 11.109-peak-negative KPC-producing *K. pneumoniae* belonged to ST512.

We then evaluated the performance of the MALDI Biotyper RTC software to detect the 11.109-Da peak in well-characterized *K. pneumoniae* strains. For this purpose, we edited the main spectra of a representative ST258 KPC-producing *K. pneumoniae* strain by removing all peaks except the *m/z* 11.109-Da peak. Detection of the 11.109-Da peak was performed by real-time identi-

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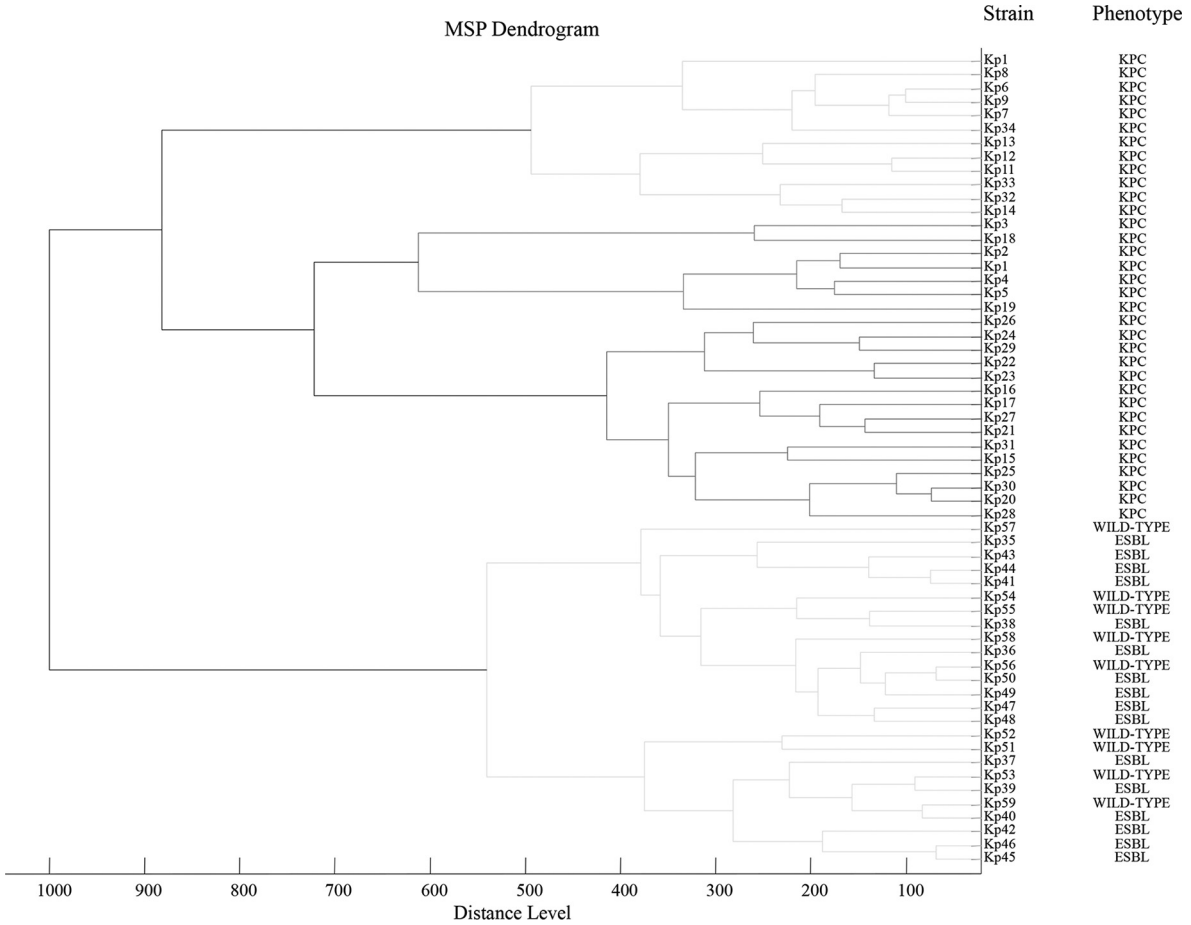

FIG 1 Score-oriented dendrogram based on main spectra (MSP) of 59 *K. pneumoniae* strains included in this study. Phenotype of *K. pneumoniae* strains is shown on the right. Cluster analysis of MSP analyzed by Bruker Biotyper software is displayed in relative units.

TABLE 1 Summary of the single-peak (11.109-Da) detection analysis, *p019* gene, and sequence type of 59 *K. pneumoniae* strains included in study

K. pneumoniae strain	No. of isolates	~11,109-Da peak (*n* [%])	Sequence type (ST)
KPC producers	20	16 (80)	512
	7	7 (100)	258
	4	4 (100)	554
	1	1 (100)	307
	2	2 (100)	101
ESBL[a] producers	1		307
	6		37
	1		395
	2		323
	1		147
	3		15
	1		512
	1		45
Wild type	1		1628
	1		416
	1		1243
	2		35
	1		466
	1		405
	1		160
	1		37

[a] ESBL, extended-spectrum β-lactamase.

fication of KPC-producing *K. pneumoniae* with the following parameters: desired mass tolerance of the adjusted spectrum 500, parameter of the intensity correction function 1, and result thresholds of 0.5 (good matching) and 0.3 (moderate matching). The Biotyper RTC software identified the 11.109-Da peak in 333 (97.9%) of 340 technical replicates of the 11.109-Da-peak-positive KPC-producing *K. pneumoniae* strains. Moreover, the 11.109-Da peak was negative in 212 (99.1%) of 214 KPC-negative *K. pneumoniae* strains. Therefore, the peak detection method implemented in Biotyper RTC software showed 97.94% sensitivity (95% confidence interval [CI], 95.80% to 99.17%) and 99.07% specificity (95% CI, 96.66% to 99.89%) in 11.109-Da-peak-positive clinical isolates. The positive predictive value was 99.4% (95% CI, 97.86% to 99.93%).

Although detection of an 11.109-Da peak has been considered a promising and helpful assay for the rapid detection of KPC producers by MALDI-TOF, a recent detailed genetic analysis showed that a gene (*p019*) encoding the 11.109-Da protein is part of an insertion sequence (IS*Kpn31*) into which Tn*4401* was inserted (9).

Based on these findings, we investigated the presence of a *p019* gene in bla_{KPC}-harboring plasmids in order to evaluate the application of the 11.109-Da-peak detection assay in KPC-producing *K. pneumoniae*. Analysis of bla_{KPC}-bearing plasmids showed that the *p019* gene present in plasmids belonged to different incompat-

TABLE 2 Characteristics and genetic analysis of the bla_{KPC}-harboring plasmids in *K. pneumoniae* strains

Plasmid name	Accession no.	Area of isolation	Yr	Sequence type (ST)	Plasmid size (kb)	Inc plasmid	bla_{KPC}	Tn*4401* isoform	*p019* gene	IS*Kpn31*	Reference
pBK30661	KF954759	USA	2010	258	73.6	FIA	KPC-3	Tn*4401*d	No	No	10
pNJST258N2	CP006926	USA	2010	258	73.6	FIA	KPC-3	Tn*4401*d	No	No	11
pBK30683	KF954760	USA	2010	963	139.9	FIA	KPC-3	Tn*4401*d	No	No	10
pKPC-63d	CP009773	USA	2013	258	75.6	FIA	KPC-3	Tn*4401*d	No	No	12
pKPC-def	CP009776	USA	2013	258	115.3	FIA	KPC-3	Tn*4401*d	No	No	12
pKPC-LK30	KC405622	Taiwan	2012	11	86.5	FIIK	KPC-2	NTE[f]	No	No	13
p1-JM45	CP006657	China	2010	11	317.1	FIIK1	KPC-2	NTE[f]	No	No	
pBK32179	JX430448	USA	2010	258	165.2	FIIK1	KPC-2	Tn*4401*a	Yes	Yes	14
pKPN101-IT	JX283456	Italy	2011	111	107.7	FIIK1, R	KPC-2	Tn*4401*a	Yes	Yes	15
pSLMT	HQ589350				21.1	FIIK2	KPC-2	Tn*4401*a	Yes	Yes	
pKpQIL	GU595196	Israel	2006	258	113.6	FIIK2[a]	KPC-3	Tn*4401*a	Yes	Yes	16
pKpQIL-IT	JN233705	Italy	2010	258	115.3	FIIK2[a]	KPC-3	Tn*4401*a	Yes	Yes	17
pGR-1780	KF874497	Greece			113.6	FIIK2[a]	KPC-2	Tn*4401*a	Yes	Yes	18
pGR-1504	KF874496	Greece			113.6	FIIK2[a]	KPC-2	Tn*4401*a	Yes	Yes	18
pGR-1870	KF874498	Greece			116	FIIK2[a]	KPC-2	Tn*4401*a	Yes	Yes	18
pGR-3913	KF874499	Greece			113.6	FIIK2[a]	KPC-2	Tn*4401*a	Yes	Yes	18
pKpQIL-10	KJ146687	USA	2010	258	113.6	FIIK2[a]	KPC-2	Tn*4401*a	Yes	Yes	19
pKpQIL-6e6	CP008830	USA	2011	258	113.6	FIIK2[a]	KPC-3	Tn*4401*a	Yes	Yes	20
pKpQIL-234	KJ146689	USA	2009	234	114.4	FIIK2[a]	KPC-2	Tn*4401*a	Yes	Yes	19
pKpQIL-531	CP008833	USA	2012	258	113.6	FIIK2[a]	KPC-2	Tn*4401*a	Yes	Yes	
pVGH151	KJ721790	Taiwan	2013		113.6	FIIK2[a]	KPC-2	Tn*4401*a	Yes	Yes	
pKpQIL-LS6	NC_021655	Italy	2011	258	78.2	FIIK2[a,c]	KPC-3	Tn*4401*a	Yes	Yes	21
pKPHS2	CP003224	China	2011	11	111.1	FIIK2, R	KPC-2	NTE[f]	No	No	
pKP048	FJ628167	China	2006-2007		151.1	FIIK2, R	KPC-2	NTE[b]	No	No	22
pBK15692	KC845573	USA	2005	258	77.8	I2	KPC-3	Tn*4401*b	No	No	23
p38544-85,403kb	CP010362				85.4	R, colRNAI	KPC-2	Tn*4401*a	Yes	Yes	
p32192	CP010575	USA	2010	258	72.8	R	KPC-2	Tn*4401*a	Yes	Yes	
p34618-71.572kb	CP010396	USA	2011	258	71.5	R	KPC-2	Tn*4401*a	Yes	Yes	
pKPC-484	CP008798	USA	2012	258	85.4	R	KPC-2	Tn*4401*a	Yes	Yes	
pKP1433	JX397875	Greece	2009-2010	340		N[d]	KPC-2	Tn*4401*b	No	No	24
pKPC_FC13/05	CP004366	Brazil	2005	442	53.0	N	KPC-2	Tn*4401*b	No	No	25
pKPC_FCF/3SP	CP004367	Brazil	2009	442	54.6	N	KPC-2	Tn*4401*b	No	No	25
pKo6	KC958437	China			51.0	N	KPC-2	NTE	No	No	
p9	FJ223607	USA	2005		70.5	N	KPC-2	Tn*4401*b	No	No	26
p12	FJ223605	USA	2005		75.6	N	KPC-3	Tn*4401*b	No	No	26
pBK31551	JX193301	USA	2005	834	83.7	N	KPC-4	Tn*4401*b	No	No	27
pKPC-e4e	CP009864	USA	2013	1518	62.5	N	KPC-3	Tn*4401*b	No	No	12
pHS062105-3	KF623109	China	2006		42.8	P	KPC-2	NTE	No	No	
pKPC-NY79	JX104759	USA	2011	258	42.4	X3	KPC-2	Tn*4401*a	Yes	Yes	28
pKP13d	CP003997	Brazil	2009	442	45.5	X3	KPC-2	NTE[f]	No	No	29
pKpS90	JX461340	France	2009	258	53.2	X3	KPC-2	Tn*4401*a	No	No	30
pBK31567	JX193302	USA	2006	429	47.3	X5	KPC-5	Tn*4401*b	No	No	27
p15S	FJ223606	USA	2005		23.7	ColE1	KPC-2	Tn*4401*a	No	No	26
pNJST258C2	CP006919	USA	2010	258	25.2	ColE1	KPC-3	Tn*4401*b	No	No	11
pKEC-dc3	CP007732	USA	2012	34	268.3	A/C	KPC-2	NTE[d]	No	No	
pKP53IL	NC_021356				33.7	[e]	KPC-2	NTE[f]	No	No	
pKPC_UVA01	CP009465	USA	2007	45	43.6	[e]	KPC-2	Tn*4401*b	No	No	31
pKPC_CAV1193	CP013325	USA	2010	941	49.5	[e]	KPC-2	Tn*4401*b	No	No	32

[a] pKpQIL-like plasmid.
[b] Partial Tn4401 transposon (IS*Kpn27* and IS*Kpn6*-like element).
[c] Lack of *repA* replicon gene.
[d] Partial Tn4401 transposon (ISKpn6 element).
[e] Nontypeable (presence of *repA* gene).
[f] bla_{KPC}-bearing non-Tn*4401* element (NTE).

ibility (Inc) groups, such as IncFIIK, IncFIIK1, IncFIIK2 (including pKpQIL-like and less-related plasmid), R, and X3 (Table 2). Detailed analysis of *K. pneumoniae* plasmids showed that the *p019* gene was present in 20 (95.2%) of 21 plasmids harboring the transposon Tn*4401*a, whereas it was absent in plasmids carrying other Tn*4401* isoforms (i.e., Tn*4401*b and Tn*4401*d).

In order to evaluate the distribution and genetic context of the *p019* gene in the population of bla_{KPC}-producing *K. pneumoniae* strains, we analyzed the whole genomes of 159 KPC-producing *K. pneumoniae* strains isolated from different countries (see data set S1 in the supplemental material) and available in public databases. Our analysis demonstrated that *p019* was present in 135 (97.8%)

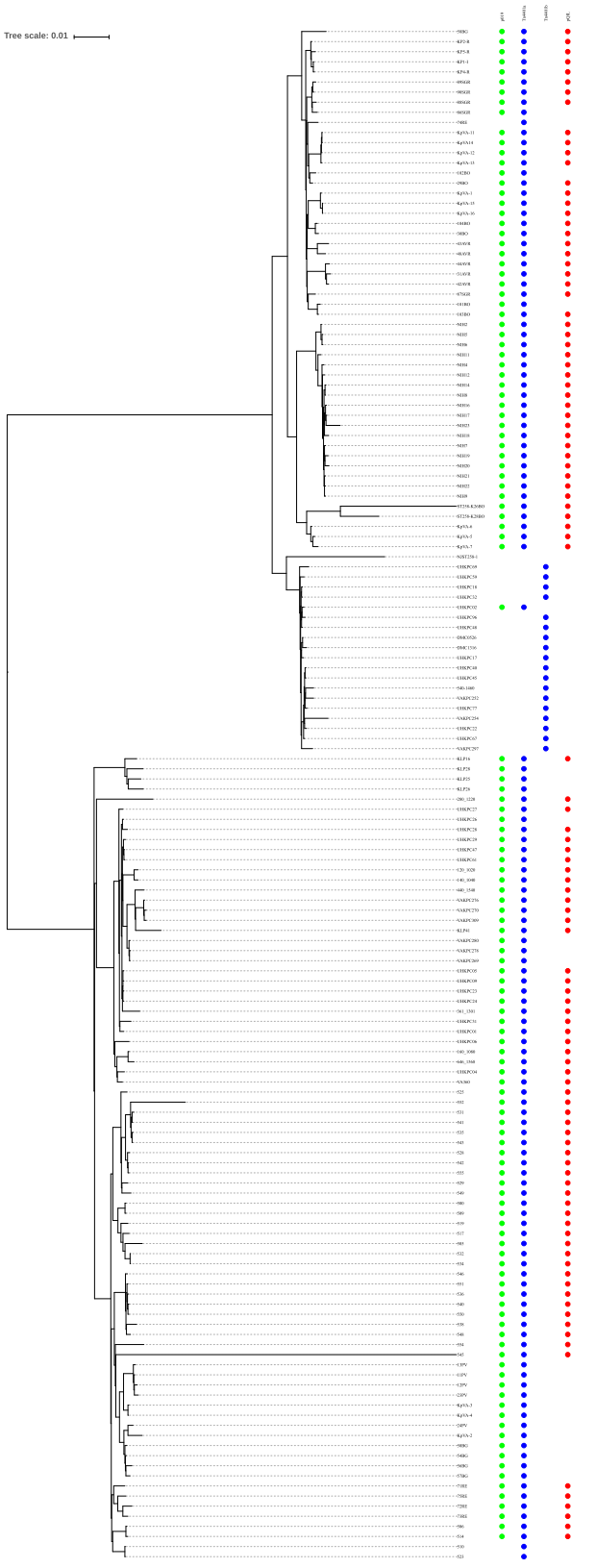

FIG 2 Maximum-likelihood phylogeny based on single nucleotide polymorphisms in the core genomes of the ST258 and ST512 *K. pneumoniae* strains. Phylogeny relationships between 153 genomes were estimated using the parsnp software program (33). The presence of the Tn*4401*a, Tn*4401*b, IncFIB (pQIL) replicon type, and *p019* gene is indicated by colored circles.

of 138 genomes harboring Tn*4401*a and absent in 20 KPC-producing *K. pneumoniae* genomes harboring Tn*4401*b. In detail, whole-genome analysis showed that in 111 (82.2%) of 135 Tn*4401*a-positive genomes carrying the *p019* gene possessed the IncFIB (pQIL) plasmid replicon type. At the same time, a p019-harboring IS*Kpn31* region was present in 24 (50%) of 48 KPC-producing *K. pneumoniae* genomes negative for the pQIL plasmid replicon type (see data set S1 in the supplemental material).

Deeper examination into the ST258 and ST512 genomes showed that strains belonging to clade 1 always carried the transposon Tn*4401*a and that 79 (98.7%) of 80 of the strains carried the *p019* gene (Fig. 2). At the same time, both the Tn*4401*a and Tn*4401*b isoforms were present in strains belonging to clade 2, while the *p019* gene was associated with the Tn*4401*a isoform.

In conclusion, the present study described and validated an innovative, rapid, cost-effective, and reliable MALDI-TOF MS method for rapid identification of KPC-producing *K. pneumoniae* clinical strains. Our results demonstrated that the *p019* gene was found in a wide range of Inc group plasmids and that it was commonly associated with the Tn*4401*a isoform in *K. pneumoniae*. A major limitation of the developed method was the absence of a p019 product protein in different bla_{KPC}-harboring plasmids in *K. pneumoniae*. However, the high positive predictive value of this approach suggests that our method is suitable as a first step for direct screening of KPC-producing *K. pneumoniae*, while negative results should be confirmed by additional confirmatory tests (34).

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