

Table S1. Mathematical formulas for calculating all CV values:

$CV_{\text{FULL}} = (CV_{\text{FULL-EXTRACTION}}^2 + CV_{\text{FULL-SPOT}}^2)^{1/2}$
$CV_{\text{IN-TARGET}} = (CV_{\text{INTARGET-EXTRACTION}}^2 + CV_{\text{INTARGET-SPOT}}^2)^{1/2} = (CV_{\text{INTARGET-EXTRACTION}}^2 + 0)^{1/2} = CV_{\text{INTARGET-EXTRACTION}}$
$CV_{\text{TOTAL}} = (CV_{\text{EXTRACTION}}^2 + CV_{\text{MALDI}}^2)^{1/2}$
$BV_{\text{INTRA}} = (CV_{\text{TOTAL-Day1}} + CV_{\text{TOTAL-Day2}} + CV_{\text{TOTAL-Day3}}) / 3$
$BV_{\text{INTER}} = CV_{\text{TOTAL-ALLDAYS}}$

Table S2. Procedures for bacterial protein extraction

"In-Target" extraction:	"Full" extraction:
1. Spot a tiny portion of a colony in a spot	1. Suspend colony in 300 μ l of H ₂ O in an Eppendorf tube.
2. Overlay it with 1 μ l of pure formic acid (Sigma Aldrich, Germany) and wait until drying.	2. Vortex until the suspension is completely homogeneous.
3. Apply 1 μ l of IVD Matrix HCCA-portioned (Bruker Daltonics, Germany) dissolved in 50% acetonitrile, 47.5% water and 2.5 % trifluoroacetic acid.	3. Add 900 μ l of ethanol.
4. Measurement is performed after drying.	4. Invert the tube several times and centrifuge for 2 min at 14,000 rpm.
	5. Pipette and discard supernatant.
	6. Spin until all the remaining ethanol remains at the bottom of the tube.
	7. Pipette again to discard all ethanol and allow to dry completely (5 min).
	8. Add formic acid (AF, 70%), about ~15 μ l, depending on the size of the pellet. Pipette up and down until homogenized and wait for 2 min.
	5. Add the same amount of acetonitrile (ACN), pipette up and down until homogenized.
	6. Centrifuge for 2 min at 14.000 rpm.
	7. Add a drop of supernatant to the MALDI target plate.
	8. Allow to dry and add 1 μ l of matrix (HCCA).

Table S3. Characteristics of the carbapenemase-producing *K. pneumoniae* isolates used in the study.

Strain	ST	Resistance gene	Hospital
AI2818^a	15	OXA-48	Hospital Universitario Gregorio Marañón (Madrid)
AI2819	15	OXA-48	Hospital Universitario Gregorio Marañón (Madrid)
AI2594	15	OXA-48	Hospital Universitario Gregorio Marañón (Madrid)
AI2612^a	15	OXA-48	Hospital Arquitecto Marcide (Ferrol)
AI2829^a	15	OXA-48	Hospital Universitario Lucus Augusti (Lugo)
AI2627^a	512	KPC-3	Hospital Puerta del Mar (Cádiz)
AI2628^a	512	KPC-3	Hospital Puerta del Mar (Cádiz)
AI2629	512	KPC-3	Hospital Puerta del Mar (Cádiz)
AI2630^a	15	OXA-48	Hospital Puerta del Mar (Cádiz)
AI2631	512	KPC-3	Hospital Puerta del Mar (Cádiz)
AI2836	512	KPC-3	Hospital Puerta del Mar (Cádiz)
AI2632	512	KPC-3	Hospital Puerta del Mar (Cádiz)
AI2837	512	KPC-3	Hospital Puerta del Mar (Cádiz)
AI2873^a	11	OXA-48	Hospital Universitario Ramón y Cajal (Madrid)
AI2874^a	11	OXA-48	Hospital Universitario Ramón y Cajal (Madrid)
AI2875	11	OXA-48	Hospital Universitario Ramón y Cajal (Madrid)
AI2876	11	OXA-48	Hospital Universitario Ramón y Cajal (Madrid)
AI2877	11	OXA-48	Hospital Universitario Ramón y Cajal (Madrid)
AI2878	11	OXA-48	Hospital Universitario Ramón y Cajal (Madrid)
AI2879	11	OXA-48	Hospital Universitario Ramón y Cajal (Madrid)
AI2886^a	15	OXA-48	Hospital Universitario Ramón y Cajal (Madrid)
AI2891	11	OXA-48	Hospital Universitario Ramón y Cajal (Madrid)
AI2892	11	OXA-48	Hospital Universitario Ramón y Cajal (Madrid)
AI2895	11	OXA-48	Hospital Universitario Ramón y Cajal (Madrid)
AI2665	15	OXA-48	Hospital Universitario Central de Asturias (Oviedo)
AI2669	15	OXA-48	Hospital Universitario Central de Asturias (Oviedo)
AI2698	15	OXA-48	Hospital Universitario Central de Asturias (Oviedo)
AI3042	11	KPC-2	Hospital Virgen de la Salud (Toledo)
AI3048	11	KPC-2	Hospital de Guadalajara (Guadalajara)
AI3056	1961	KPC-2	ND*
AI2700	1961	KPC-2	Complejo Hospitalario de Pontevedra (Pontevedra)
AI2701	273	KPC-2	Hospital Universitario Gregorio Marañón (Madrid)
AI2979	1961	KPC-2	Complejo Hospitalario de Pontevedra (Pontevedra)
AI2706^a	512	KPC-3	Hospital General La Mancha Centro (Ciudad Real)
AI2980	1961	KPC-2	Complejo Hospitalario de Pontevedra (Pontevedra)
AI2982	1961	KPC-2	Complejo Hospitalario de Pontevedra (Pontevedra)
AI2713	273	KPC-2	Hospital Universitario Gregorio Marañón (Madrid)

AI2717	1961	KPC-2	Complejo Hospitalario de Pontevedra (Pontevedra)
AI2985	1961	KPC-2	Complejo Hospitalario de Pontevedra (Pontevedra)
AI3060	1961	KPC-2	Complejo Hospitalario de Pontevedra (Pontevedra)
AI2739^a	11	OXA-48	Hospital Universitario Marqués de Valdecilla (Santander)
AI2742^a	11	OXA-48	Hospital Universitario Marqués de Valdecilla (Santander)
AI2743	11	OXA-48	Hospital Universitario Marqués de Valdecilla (Santander)
AI2989	11	OXA-48	Hospital Universitario Marqués de Valdecilla (Santander)
AI2745	11	OXA-48	Hospital Universitario Marqués de Valdecilla (Santander)
AI2748	11	OXA-48	Hospital Universitario Marqués de Valdecilla (Santander)
AI2763	147	OXA-48	Hospital Univeristario de Bellvitge (Barcelona)
AI2764	147	OXA-48	Hospital Univeristario de Bellvitge (Barcelona)
AI2765	147	OXA-48	Hospital Univeristario de Bellvitge (Barcelona)
AI2766	147	OXA-48	Hospital Univeristario de Bellvitge (Barcelona)
AI2767	147	OXA-48	Hospital Univeristario de Bellvitge (Barcelona)
AI2993	147	OXA-48	Hospital Univeristario de Bellvitge (Barcelona)
AI2768	147	OXA-48	Hospital Univeristario de Bellvitge (Barcelona)
AI2769	147	OXA-48	Hospital Univeristario de Bellvitge (Barcelona)
AI2770	147	OXA-48	Hospital Univeristario de Bellvitge (Barcelona)
AI2996	147	OXA-48	Hospital Univeristario de Bellvitge (Barcelona)
AI2998	147	OXA-48	Hospital Univeristario de Bellvitge (Barcelona)
AI2773	307	OXA-48	Hospital Univeristario de Bellvitge (Barcelona)
AI2775	147	OXA-48	Hospital Univeristario de Bellvitge (Barcelona)
AI3001	307	OXA-48	Hospital Universitario Valencia Tres cruces (Valencia)
AI2776^a	11	OXA-48	Hospital Universitario Valencia Tres cruces (Valencia)
AI2777	307	OXA-48	Hospital Universitario Valencia Tres cruces (Valencia)
AI2778	11	OXA-48	Hospital Universitario Valencia Tres cruces (Valencia)
AI2780	11	OXA-48	Hospital Universitario Valencia Tres cruces (Valencia)
AI2781	307	OXA-48	Hospital Universitario Valencia Tres cruces (Valencia)
AI3002	307	OXA-48	Hospital Universitario Valencia Tres cruces (Valencia)
AI3003	307	OXA-48	Hospital Universitario Valencia Tres cruces (Valencia)
AI3004	307	OXA-48	Hospital Universitario Valencia Tres cruces (Valencia)
AI3006	307	OXA-48	Hospital Universitario Valencia Tres cruces (Valencia)
AI2789	307	OXA-48	Hospital Universitario Valencia Tres cruces (Valencia)
AI2791	307	OXA-48	Hospital Universitario Valencia Tres cruces (Valencia)
AI3009	307	OXA-48	Hospital Universitario Valencia Tres cruces (Valencia)
AI2794	307	OXA-48	Hospital Universitario Valencia Tres cruces (Valencia)
AI2805^a	512	KPC-3	Hospital Reina Sofía (Córdoba)
AI2806^a	512	KPC-3	Hospital Reina Sofía (Córdoba)
AI2807	512	KPC	Hospital Reina Sofía (Córdoba)
AI2808	512	KPC-3	Hospital Reina Sofía (Córdoba)

AI2809	512	KPC-3	Hospital Reina Sofía (Córdoba)
AI2810	512	KPC-3	Hospital Reina Sofía (Córdoba)
AI2811	512	KPC-3	Hospital Reina Sofía (Córdoba)
AI2812	512	KPC-3	Hospital Reina Sofía (Córdoba)
AI2813	512	KPC-3	Hospital Reina Sofía (Córdoba)
AI2814	512	KPC-3	Hospital Reina Sofía (Córdoba)
AI2815	512	KPC-3	Hospital Reina Sofía (Córdoba)
AI2816	512	KPC-3	Hospital Reina Sofía (Córdoba)
AI2817	512	KPC-3	Hospital Reina Sofía (Córdoba)
1	273	KPC-2	U*
2^a	11	KPC-2	U*
3^a	11	KPC-2	U*
4	101	KPC-2	U*
5	101	KPC-2	U*
6	512	KPC-3	U*
7	512	KPC-3	U*
8	512	KPC-3	U*
9	512	KPC-3	U*
10	512	KPC-3	U*
11	101	KPC-2	U*
12^a	11	KPC-2	U*
13^a	11	KPC-2	U*
14	15	OXA-48	Hospital Universitario A Coruña (CHUAC)
15	15	OXA-48	Hospital Universitario A Coruña (CHUAC)
16	405	OXA-48	U*
17	11	OXA-48	U*
AI2830	147	NDM-1	Hospital Universitario Lucus Augusti (Lugo)
AI2880	101	NDM-1	Hospital Universitario Ramón y Cajal (Madrid)
AI2898	101	NDM-1	Hospital Universitario Ramón y Cajal (Madrid)
AI2934	395	NDM-1	Hospital Virgen de la Macarena (Sevilla)
AI2779	437	NDM-23	Hospital Universitario Valencia Tres cruces (Valencia)
AI3007	101	NDM-1	Hospital Universitario Valencia Tres cruces (Valencia)
AI2793	437	NDM-1	Hospital Universitario Valencia Tres cruces (Valencia)
18	512	KPC-3	U*
19	11	OXA-48	U*
20	.. ^b	.. ^b	Hospital Universitario A Coruña (CHUAC)
21	-	-	Hospital Universitario A Coruña (CHUAC)
22	-	-	Hospital Universitario A Coruña (CHUAC)
23	-	-	Hospital Universitario A Coruña (CHUAC)
24	-	-	Hospital Universitario A Coruña (CHUAC)
25	-	-	Hospital Universitario A Coruña (CHUAC)
26	-	-	Hospital Universitario A Coruña (CHUAC)

27	-	-	Hospital Universitario A Coruña (CHUAC)
28	-	-	Hospital Universitario A Coruña (CHUAC)
29	-	-	Hospital Universitario A Coruña (CHUAC)
30	-	-	Hospital Universitario A Coruña (CHUAC)
31	-	-	Hospital Universitario A Coruña (CHUAC)
32	-	-	Hospital Universitario A Coruña (CHUAC)
33	-	-	Hospital Universitario A Coruña (CHUAC)
34	-	-	Hospital Universitario A Coruña (CHUAC)
35	-	-	Hospital Universitario A Coruña (CHUAC)
36	-	-	Hospital Universitario A Coruña (CHUAC)
37	-	-	Hospital Universitario A Coruña (CHUAC)
38	-	-	Hospital Universitario A Coruña (CHUAC)
39	-	-	Hospital Universitario A Coruña (CHUAC)
40	-	-	Hospital Universitario A Coruña (CHUAC)
41	-	-	Hospital Universitario A Coruña (CHUAC)
42	-	-	Hospital Universitario A Coruña (CHUAC)
43	-	-	Hospital Universitario A Coruña (CHUAC)
44	-	-	Hospital Universitario A Coruña (CHUAC)
45	-	-	Hospital Universitario A Coruña (CHUAC)
46	-	-	Hospital Universitario A Coruña (CHUAC)
47	-	-	Hospital Universitario A Coruña (CHUAC)
48	-	-	Hospital Universitario A Coruña (CHUAC)
49	-	-	Hospital Universitario A Coruña (CHUAC)
50	-	-	Hospital Universitario A Coruña (CHUAC)
51	-	-	Hospital Universitario A Coruña (CHUAC)
52	-	-	Hospital Universitario A Coruña (CHUAC)
53	-	-	Hospital Universitario A Coruña (CHUAC)
54	-	-	Hospital Universitario A Coruña (CHUAC)
55	-	-	Hospital Universitario A Coruña (CHUAC)
56	-	-	Hospital Universitario A Coruña (CHUAC)
57	-	-	Hospital Universitario A Coruña (CHUAC)
58	-	-	Hospital Universitario A Coruña (CHUAC)
59	-	-	Hospital Universitario A Coruña (CHUAC)
60	-	-	Hospital Universitario A Coruña (CHUAC)
61	-	-	Hospital Universitario A Coruña (CHUAC)
62	-	-	Hospital Universitario A Coruña (CHUAC)
63	-	-	Hospital Universitario A Coruña (CHUAC)
64	-	-	Hospital Universitario A Coruña (CHUAC)
65	-	-	Hospital Universitario A Coruña (CHUAC)
66	-	-	Hospital Universitario A Coruña (CHUAC)
67	-	-	Hospital Universitario A Coruña (CHUAC)
68	-	-	Hospital Universitario A Coruña (CHUAC)
69	-	-	Hospital Universitario A Coruña (CHUAC)
70			Hospital Universitario A Coruña (CHUAC)

U* Unknown origin

^a Strains processed in the inter-laboratory study.

^b Isolates were screening for carbapenemase production according to the screening cut-off values recommended by EUCAST, that is having a meropenem or ertapenem MIC > 0,125 mg/L. Carbapenemase non-producing isolates *K. pneumoniae* were not genotypically characterized.

Table S4. Results of the final validation test

Isolate	Resistance gene ¹	RF ²			
		M ^{THRESHOLD} raw ³	M ^{THRESHOLD} norm ⁴	M ^{LINEAR} raw	M ^{LINEAR} norm
AI3042	KPC	OXA-48	OXA-48	OXA-48	OXA-48
AI3048	KPC	OXA-48	OXA-48	OXA-48	OXA-48
AI3056	KPC	OXA-48	OXA-48	OXA-48	OXA-48
AI2979	KPC	OXA-48	OXA-48	OXA-48	OXA-48
AI2805	KPC	KPC	KPC	KPC	KPC
AI2807	KPC	OXA-48	KPC	KPC	KPC
AI2808	KPC	KPC	KPC	OXA-48	KPC
AI2809	KPC	KPC	KPC	KPC	KPC
AI2810	KPC	KPC	KPC	KPC	KPC
AI2811	KPC	OXA-48	KPC	KPC	KPC
AI2813	KPC	KPC	KPC	KPC	KPC
AI2814	KPC	-	KPC	OXA-48	KPC
AI2816	KPC	KPC	KPC	KPC	KPC
AI2817	KPC	KPC	KPC	KPC	KPC
AI2700	KPC	KPC	KPC	OXA-48	KPC
AI2701	KPC	KPC	KPC	KPC	KPC
AI2706	KPC	KPC	KPC	KPC	KPC
AI2980	KPC	KPC	KPC	KPC	KPC
AI2713	KPC	KPC	KPC	KPC	KPC
AI2717	KPC	KPC	KPC	KPC	KPC
AI2985	KPC	KPC	KPC	KPC	KPC
AI3060	KPC	KPC	KPC	KPC	KPC
2	KPC	KPC	-	KPC	KPC
3	KPC	KPC	OXA-48	OXA-48	OXA-48
4	KPC	KPC	KPC	OXA-48	OXA-48
5	KPC	OXA-48	OXA-48	OXA-48	OXA-48
7	KPC	OXA-48	OXA-48	OXA-48	OXA-48
8	KPC	KPC	KPC	KPC	KPC
9	KPC	KPC	KPC	KPC	KPC
10	KPC	KPC	KPC	KPC	KPC
11	KPC	KPC	OXA-48	OXA-48	OXA-48
12	KPC	OXA-48	OXA-48	OXA-48	OXA-48
13	KPC	KPC	OXA-48	OXA-48	OXA-48
AI2628	KPC	KPC	KPC	KPC	KPC
AI2629	KPC	KPC	KPC	KPC	KPC
AI2836	KPC	KPC	KPC	KPC	KPC
AI2898	NDM	NDM	NDM	OXA-48	NDM
AI2779	NDM	KPC	KPC	KPC	OXA-48
AI2793	NDM	OXA-48	OXA-48	OXA-48	NDM
AI2819	OXA-48	OXA-48	OXA-48	OXA-48	OXA-48
AI2874	OXA-48	KPC	-	OXA-48	OXA-48
AI2875	OXA-48	OXA-48	OXA-48	OXA-48	OXA-48
AI2876	OXA-48	OXA-48	OXA-48	OXA-48	OXA-48
AI2878	OXA-48	KPC	-	KPC	OXA-48
AI2879	OXA-48	OXA-48	OXA-48	OXA-48	OXA-48
AI2594	OXA-48	KPC	-	OXA-48	OXA-48
AI2886	OXA-48	OXA-48	OXA-48	OXA-48	OXA-48
AI2892	OXA-48	OXA-48	OXA-48	OXA-48	OXA-48
AI2895	OXA-48	KPC	OXA-48	KPC	OXA-48

AI2665	OXA-48	OXA-48	OXA-48	OXA-48	OXA-48
AI2669	OXA-48	OXA-48	OXA-48	OXA-48	OXA-48
AI2698	OXA-48	OXA-48	OXA-48	OXA-48	OXA-48
AI2739	OXA-48	OXA-48	OXA-48	OXA-48	OXA-48
AI2742	OXA-48	OXA-48	OXA-48	OXA-48	OXA-48
AI2743	OXA-48	OXA-48	OXA-48	OXA-48	OXA-48
AI2745	OXA-48	OXA-48	OXA-48	OXA-48	OXA-48
AI2763	OXA-48	OXA-48	KPC	OXA-48	OXA-48
AI2764	OXA-48	OXA-48	OXA-48	OXA-48	OXA-48
AI2765	OXA-48	OXA-48	OXA-48	OXA-48	NDM
AI2766	OXA-48	OXA-48	OXA-48	OXA-48	NDM
AI2767	OXA-48	KPC	OXA-48	OXA-48	OXA-48
AI2993	OXA-48	OXA-48	OXA-48	OXA-48	NDM
AI2768	OXA-48	OXA-48	OXA-48	OXA-48	OXA-48
AI2770	OXA-48	OXA-48	OXA-48	OXA-48	OXA-48
AI2773	OXA-48	OXA-48	OXA-48	OXA-48	OXA-48
AI2775	OXA-48	OXA-48	OXA-48	OXA-48	OXA-48
AI3001	OXA-48	OXA-48	OXA-48	OXA-48	OXA-48
AI2776	OXA-48	OXA-48	OXA-48	OXA-48	OXA-48
AI2777	OXA-48	OXA-48	OXA-48	OXA-48	OXA-48
AI2780	OXA-48	OXA-48	OXA-48	OXA-48	OXA-48
AI3002	OXA-48	OXA-48	OXA-48	OXA-48	OXA-48
AI3003	OXA-48	OXA-48	OXA-48	OXA-48	OXA-48
AI3004	OXA-48	KPC	OXA-48	OXA-48	OXA-48
AI3006	OXA-48	OXA-48	OXA-48	OXA-48	OXA-48
AI2612	OXA-48	KPC	OXA-48	KPC	OXA-48
AI2789	OXA-48	OXA-48	OXA-48	OXA-48	OXA-48
AI3009	OXA-48	OXA-48	OXA-48	OXA-48	OXA-48
AI2794	OXA-48	OXA-48	OXA-48	OXA-48	OXA-48
AI2630	OXA-48	KPC	OXA-48	KPC	OXA-48
14	OXA-48	OXA-48	OXA-48	OXA-48	OXA-48
15	OXA-48	OXA-48	OXA-48	OXA-48	OXA-48
16	OXA-48	OXA-48	OXA-48	OXA-48	OXA-48
17	OXA-48	KPC	-	OXA-48	OXA-48
AI2818	OXA-48	OXA-48	OXA-48	OXA-48	OXA-48
20	non CPK	-	OXA-48	-	-
21		-	-	-	-
23		-	-	-	-
24		-	-	-	-
25		-	-	-	-
27		-	-	-	-
30		-	-	-	-
31		-	-	-	-
32		-	-	-	-
33		-	-	-	-
34		-	-	-	-
36		-	-	-	-
37		-	-	-	-
38		OXA-48	OXA-48	-	-
39		-	-	-	-
40		-	-	-	-
41	-	-	-	-	
43	-	-	-	-	

45	-	-	-	-
47	-	-	-	-
48	-	-	-	-
49	-	-	-	-
50	-	-	-	-
53	-	-	-	-
54	-	-	-	-
55	-	-	-	-
56	-	-	-	-
57	-	OXA-48	-	-
58	-	-	-	-
59	-	-	-	-
60	-	-	-	-
63	-	-	-	-
64	-	-	-	-
65	-	-	-	-
66	-	-	-	-
67	-	-	-	-
68	-	-	-	-
69	-	-	-	-

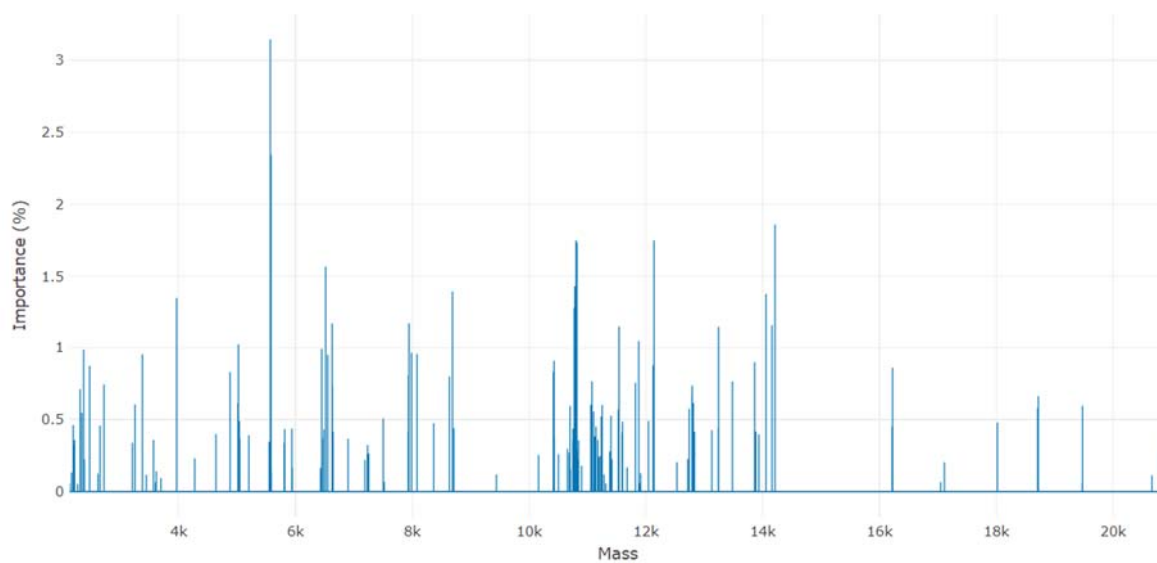
¹ Resistance gene obtained by the molecular characterization of the isolates

² Random Forest (RF) analysis

³ Results obtained with the raw spectra

⁴ Results obtained with the normalized spectra

Figure 1. RF-M_{LINEAR} features importance



The graph represents the importance of each mass peak for the result of the analysis. On the X-axis, the individual sample masses are represented and on the Y-axis the percentage of importance of each mass peak for the decision making in the allocation of each isolate into one of the categories of the analysis (OXA-48, KPC, NDM, non CPK) is represented.