Supplemental Data

Rapid Identification of New Delhi Metallo-β-Lactamase (NDM) Using Tryptic Peptides and LC-MS/MS

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MRM assay for mutated form of SLGNLGDADTEHYAASAR

Labeled peptides SLGNL***D***DADTEHYAASAR for NDM-12, SLGNLGDADTEHYAAS***V***R for NDM-6, and NDM-15 containing heavy isotopic labels in R (U-13C6; U-15N4) or K (U-13C6; U-15N2) C-terminus amino acids were purchased (JPT, Berlin, Germany). Bolded letters ***D*** and ***V*** indicate mutations of G6D and A17V. The peptides and transitions for all three peptides are listed below. The collision energy for all transitions was set to 23 eV. The MRM assay was run separately on an Agilent CubeChip 6495 QQQ with a high capacity chip as described in the main text for sample L063.

Peptide	Charge	Precursor	T1	T2	T3	T4	T5	T6
SLGNLGDADTEHYAASAR	3+	616.6221	Y6+	Y16++	Y14++	Y13++	Y12++	Y11++
SLGNL***D***DADTEHYAASAR	3+	635.9573	Y16++	Y14++	Y13++	Y12++	Y11++	
SLGNLGDADTEHYAAS***V***R	3+	625.9659	Y16++	Y14++	Y13++	Y12++	Y11++	

Total proteomics for two preparations of samples L092 and L099

In the 100-sample assay validation experiment, no NDM protein was detected in sample numbers L092 and L099. However, triplicate testing of protein extractions from repeat cultures of these two isolates revealed highly expressed NDM protein. To rule out the possibility of plasmid loss or sample mishandling, we used a total proteomics approach to investigate the plasmid proteins in two preparations of these two samples that had initially demonstrated no NDM peptides.

1 μg of the leftover digests used for MRM was injected to Orbitrap Lumos LC-MS/MS for total proteomics with 2 hr gradient using the same protocol described in the main text for protein identification. To search for plasmid proteins, LC-MS/MS data for two preparations of sample L092 were searched against the protein database of CDC_0118 (the corresponding genomic sequence for L092), which has 5346 proteins including 394 plasmid proteins (downloaded from NCBI on 10/2018). LC-MS/MS data for two sample preparations of L099 were searched against the protein database of CDC_0128 (the corresponding genomic sequence for L099), which has 5165 proteins including 395 plasmid proteins (downloaded from NCBI on 10/2018). Label-free quantification was analyzed by Proteome Discoverer 2.2 (Thermal Scientific) to compare the plasmid protein expression of the two preparations of sample L092 and the two preparations of sample L099 respectively.

A total of 1349 to 1562 (including 30-48 plasmid proteins for each sample) high confidence proteins (FDR < 0.1) with minimum of 2 unique peptides were detected for these 4 samples. CDC_0118 (L092) contains three probable plasmid contigs: plasmid unitig_1 (encoding 165 proteins), plasmid unitig_2 (encoding 214 proteins) and plasmid unitig_3 (encoding 105 proteins). CDC_0128 (L099) also contains three probable plasmid contigs: plasmid tig00000792 (encoding 156 proteins), plasmid tig00000793 (encoding 222 proteins) and plasmid tig00000856 (encoding 7 proteins) with 156, 222 and 7. In L092,

the *bla*$_{NDM}$ gene is located on plasmid unitig_1. A total of 22 high confidence proteins encoded by plasmid unitig_1 were detected in the first preparation of sample L092 and 20 were detected in the repeated sample preparation of sample L092, indicating that this presumptive plasmid was not lost, even though NDM was not detected in the first preparation of the sample L092.

A similar result was found for the two preparations of the sample L099. The *bla*$_{NDM}$ gene is located on presumptive plasmid tig00000793. A total of 12 high confidence plasmid proteins encoded by tig00000793 were detected in the first preparation of L099, and 16 proteins from this contig were detected in the repeat preparation of the sample L099, indicating that this presumptive plasmid was not lost, even though NDM protein was not detected. The results are summarized in the supplemental Table 5a and 5b.

Targeted LC-MS/MS: Targeted LC-MS/MS was performed on Orbitrap Fusion Tribrid mass spectrometer-based (Thermo Fisher Scientific) nano LC-MS system. Briefly, 6 μL of 5 fm/μL labeled peptide mix was added to 24 μL of 0.1 μg/μL tryptic digests and 10 μL of the resulting mixture was injected into the mass spectrometer and separated on an EasySpray column (Thermo Fisher ES803, 50 cm x 75 μm ID packed with PepMap RSLC C18 2 μm particles) using a 40-min linear gradient of 5-35% ACN in 0.1% FA at a flow rate of 300 nL/min. Mass analysis was carried out in Targeted MS2 (tMS2) mode. Selected precursors and their corresponding labeled peptides were isolated at 0.8 m/z window and fragmented in HCD cell with previously optimized energy which was 28, 28 and 24 eV for SLGNLGDADTEHYAASAR, ASMIVMSHSAPDSR and AFGAAFPK respectively. Resulting fragment ions were detected in the Orbitrap cell at 30K resolution. The maximum acquisition time and gain for each targeted MS/MS spectrum was 120 ms and $2x10^5$ respectively. Skyline 4.2 software package (MacCross lab) was used for quantitative and relative spectral intensity comparisons. The same tryptic peptide digests and labeled peptides used in MRM validation were used in this tMS2 experiment.

Supplemental Figure 4 shows the comparison of LC-MS/MS chromatograms of five samples on three NDM peptide markers by targeted LC-MS/MS using a high mass resolution mass spectrometer (Orbitrap Lumos) and MRM assay using Agilent 6495 Chip Cube QQQ. Five samples were selected for this experiment: L017, L021 (as negative control), L024 (as positive control), L017 L075 and L100. L017 had very low NDM expression and the Chip Cube QQQ MRM assay failed to detect NDM peptide markers in this sample. L075 and L100 had low NDM abundance and were the only two samples whose spectra were manually identified as NDM-positive. L024 had medium NDM abundance. The use of the high resolution Orbitrap Lumos mass spectrometer and longer gradient reduced interference and improved the detection of the isolates with low NDM abundance, especially for AFGAAFPK. The gradient used in the Orbitrap Lumos was 40 min which is much longer than 10 min used in MRM. For L017, two NDM peptide markers, SLGNLGDADTEHYAASAR and AFGAAFPK, were detected by Orbitrap Lumos while ASMIVMSHSAPDSR was not detected. No interference was observed for AFGAAFPK. The MS/MS spectra for AFGAAFPK in L017 were clear and decisively positive. The R-ratio for L017, L100, L075 and L024 was 0.01, 0.08, 0.27 and 3.64 respectively for AFGAAFPK, which correctly correlated to the NDM abundance levels determined by MRM assay. Thus, AFGAAFPK could be the best peptide marker for the NDM protein using the high resolution mass spectrometer and longer gradient. Interferences were still observed for SLGNLGDADTEHYAASAR in sample L100. However, its MS/MS spectra acquired by the Orbitrap Lumos contained the full set of transitions. After removing the y8+, y8++, y9+, and y8++ transitions, the remaining transitions of the native peptide in L100 matched well with those in the labeled peptide, indicating positive peptide identification. The LC-MS chromatogram shown in Supplemental Figure 4 for this peptide in L100 is displayed with y8+, y8++, y9+ and y9++ transitions

removed. Acquisition of the full set of transitions is one of the advantages of the high resolution mass spectrometer.

References

1. Fusaro VA, Mani DR, Mesirov JP, Carr SA. 2009. Prediction of high-responding peptides for targeted protein assays by mass spectrometry. Nat Biotechnol 27:190-8.

Supplemental Table 1: *bla* $_{NDM}$-containing and negative control isolates used in assay development and

	Protein ID	Assay development		Validation	
Name	***bla*** $_{NDM}$ **containing**	**Negative control**	***bla*** $_{NDM}$ **containing**	**Negative control**	***bla*** $_{NDM}$ **containing**
Achromobacter sp.		1		2	
Acinetobacter baumannii			1		1
Acinetobacter ursingii				1	
Aeromonas sp.				1	
Citrobacter freundii complex		1		4	
Citrobacter koseri		1		1	
Citrobacter sp.					1
Chryseobacterium sp.				1	
Enterobacter cloacae complex		1		8	1
Escherichia coli		2	1	21	9
Enterococcus faecalis				1	
Klebsiella oxytoca		2		2	
Klebsiella oxytoca/Raoutella ornitholytica				1	
Klebsiella pneumoniae	1	2	2	11	8
Morganella morganii				1	1
Pseudomonas aeruginosa		4		8	
Proteus mirabilis				1	1
Providencia rettgeri					1
Pantoea sp.		1			
Rhizobium radiobacter				2	
Salmonella Senftenberg					1
Stenotrophomonas maltophilia		1		4	
Serratia liquifaciens				1	
Serratia marcescens				2	
Sphingomonas sp.				1	
Staphylococcus epidermidis				1	
Staphylococcus haemolyticus				1	
Total	**1**	**16**	**4**	**76**	**24**

Supplemental Table 2: Tryptic peptides for 15 NDM variants and ESP values per ESPPredictor

in silico digested tryptic peptide	n variants	aa position	Unique to NDM	Note (PD1.4)	ESP values[a]
GMVAAQHSLTFAANGWVEPATAPNFGPLK	1			ND	
MGGMDALHAAGIATYANALSNQLAPQK	1			ND	
QLAPNVWQHTSYLDMPSFGAVTSNGLIVR	1			ND	
SLGNLDDADTEHYAASAR	1			ND	
TDIAFGGCLIK	1			ND	
VFYPGPGHTSDNITVGIDR	1			ND	
VLLVDTAWTDDQTAQILNWIK	1			ND	
SLGNLGDADTEHYAASVR	2			ND	
SLGNLGDADTEHYAASAR	12	217-234	yes	strong peak	0.67
VLVVDTAWTDDQTAQILNWIK	12			ND	0.08
QLAPNVWQHTSYLDMPGFGAVASNGLIVR	14			ND	0.03
VFYPGPGHTSDNITVGIDGTDIAFGGCLIK	14			ND	0.16
AAITHTAR	15			ND	0.32
AFGAAFPK	15	235-242	yes	strong peak	0.49
ASMIVMSHSAPDSR	15	243-256	yes	strong peak	0.53
FGDLVFR	15	46-52	none specific	weak peak	0.38
MELPNIMHPVAK	15			ND	0.42
QEINLPVALAVVTHAHQDK	15			ND	0.22

[a]Enhanced signature peptide: prediction tool based on protein sequence as defined in Fusaro et al, 2009 (1)

Supplemental Table 3: R ratios and rdotp values for the second preparation of *bla* $_{NDM}$ positive L017, L092 and L099 isolates tested

Sample, followed by replicate number	SLGNLGDADTEHYAASAR	ASMIVMSHSAPDSR	AFGAAFPK	Assay result
L017-1	0.99/0.04[a]	0.93/0.08[b]	0.91/0.14[b]	Negative
L017-2	0.92/0.04	0.94/0.06	0.91/0.1[b]	Negative
L017-3	0.9/0.04	0.95/0.06	0.96/0.07[b]	Negative
L048	1.0/34.83	0.98/93.42	1.0/15.03	Positive
L075	0.99/0.26	0.95/0.33[c]	0.99/0.11	Positive
L092-1	1.0/18.39	0.98/31.65	1.0/8.49	Positive
L092-2	1.0/16.69	0.98/30.58	1.0/7.26	Positive
L092-3	1.0/18.69	0.98/35.17	1.0/7.5	Positive
L099-1	1.0/23.84	0.98/48.98	1.0/10.65	Positive
L099-2	1.0/26.35	0.98/50.54	1.0/10.96	Positive
L099-3	1.0/28.53	0.98/52.31	1.0/11.85	Positive

[a]Carryover

[b]Interference

[c]Values after interfering y11 transition was removed. Prior to removal, values were 0.54/1.22

Supplemental Table 4: Assay reproducibility on three separate colonies on the same plate

Isolate number	SLGNLGDADTEHYAASAR					ASMIVMSHSAPDSR					AFGAAFPK						
	Set 1	Set 2	Set 3	Ave	%CV	Set 1	Set 2	Set 3	Ave	%CV	Set 1	Set 2	Set 3	Ave	%CV		
L092	18.4	16.7	18.7	17.9	6.03	31.7	30.6	35.2	32.5	7.39	8.5	7.3	7.5	7.8	8.24		
L099	23.8	26.4	28.5	26.2	8.99	49	50.5	52.3	50.6	3.27	10.7	11	11.9	11.2	5.58	Ave	%CV
Ratio L099/L092				1.46					1.56					1.44		1.49	4.32

Supplemental Table 5a: Label-free quantification of detected plasmid proteins in two preparations of L092

Accession	*Description*	*Coverage [%]*	*# Unique Peptides*	*Ratio first and second preparations of L092*	*plasmid*[a]
ARA20636.1	cupin (plasmid) [Escherichia coli]	35.59	2	21.06	plasmid_unitig_2
ARA20543.1	hypothetical protein AM365_27365 (plasmid) [Escherichia coli]	52.22	4	6.00	plasmid_unitig_1
ARA20470.1	hypothetical protein AM365_26880 (plasmid) [Escherichia coli]	39.00	7	2.61	plasmid_unitig_1
ARA20624.1	WYL domain-containing protein (plasmid) [Escherichia coli]	13.58	3	2.48	plasmid_unitig_2
ARA20437.1	plasmid replication protein (plasmid) [Escherichia coli]	28.96	8	2.43	plasmid_unitig_1
ARA20637.1	DUF86 domain-containing protein (plasmid) [Escherichia coli]	39.10	3	1.69	plasmid_unitig_2
ARA20570.1	aminoglycoside N-acetyltransferase AAC(6')-Ib3 (plasmid) [Escherichia coli]	39.67	5	1.68	plasmid_unitig_1[b]
ARA20560.1	hypothetical protein AM365_27450 (plasmid) [Escherichia coli]	51.56	2	1.63	plasmid_unitig_1
ARA20674.1	cobyrinic acid ac-diamide synthase (plasmid) [Escherichia coli]	49.04	7	1.60	plasmid_unitig_2
ARA20485.1	sulfonamide-resistant dihydropteroate synthase Sul1 (plasmid) [Escherichia coli]	53.76	10	1.50	plasmid_unitig_1
ARA20561.1	hypothetical protein AM365_27455 (plasmid) [Escherichia coli]	36.15	4	1.50	plasmid_unitig_1
ARA20681.1	plasmid maintenance protein CcdB (plasmid) [Escherichia coli]	23.76	2	1.48	plasmid_unitig_2
ARA20452.1	DNA replication terminus site-binding protein (plasmid) [Escherichia coli]	43.64	10	1.36	plasmid_unitig_1
ARA20545.1	cobalamin biosynthesis protein CobQ (plasmid) [Escherichia coli]	55.94	10	1.30	plasmid_unitig_1
ARA20762.1	toxin (plasmid) [Escherichia coli]	64.29	4	1.27	plasmid_unitig_3
ARA20442.1	DNA-binding protein (plasmid) [Escherichia coli]	29.50	5	1.22	plasmid_unitig_1
ARA20419.1	DNA-binding protein HU (plasmid) [Escherichia coli]	63.33	4	1.21	plasmid_unitig_1
ARA20591.1	hypothetical protein AM365_27555 (plasmid) [Escherichia coli]	59.09	8	1.18	plasmid_unitig_2
ARA20439.1	hypothetical protein AM365_26690 (plasmid) [Escherichia coli]	41.49	6	1.17	plasmid_unitig_1
ARA20722.1	recombinase (plasmid) [Escherichia coli]	12.26	3	1.14	plasmid_unitig_3
ARA20481.1	RmtC family 16S rRNA (guanine(1405)-N(7))-methyltransferase (plasmid) [Escherichia coli]	49.47	12	1.13	plasmid_unitig_1
ARA20673.1	hypothetical protein AM365_28055 (plasmid) [Escherichia coli]	25.00	2	1.12	plasmid_unitig_2
ARA20467.1	hypothetical protein AM365_26860 (plasmid) [Escherichia coli]	44.00	5	1.12	plasmid_unitig_1[b]
ARA20659.1	hypothetical protein AM365_27970 (plasmid) [Escherichia coli]	23.17	2	1.11	plasmid_unitig_2
ARA20622.1	hypothetical protein AM365_27730 (plasmid) [Escherichia coli]	17.31	3	1.07	plasmid_unitig_2
ARA20734.1	transcriptional regulator (plasmid) [Escherichia coli]	45.54	5	1.01	plasmid_unitig_3
ARA20623.1	thioesterase (plasmid) [Escherichia coli]	25.78	2	1.01	plasmid_unitig_2
ARA20739.1	hypothetical protein AM365_28415 (plasmid) [Escherichia coli]	27.39	5	1.00	plasmid_unitig_3
ARA20582.1	hypothetical protein AM365_27500 (plasmid) [Escherichia coli]	61.97	6	0.97	plasmid_unitig_2
ARA20562.1	ATPase (plasmid) [Escherichia coli]	35.32	12	0.96	plasmid_unitig_1
ARA20759.1	DNA polymerase III subunit theta (plasmid) [Escherichia coli]	24.10	2	0.95	plasmid_unitig_3
ARA20724.1	cobyrinic acid ac-diamide synthase (plasmid) [Escherichia coli]	31.92	6	0.90	plasmid_unitig_3
ARA20726.1	glutamine--tRNA ligase (plasmid) [Escherichia coli]	46.47	25	0.88	plasmid_unitig_3
ARA20778.1	olxA (plasmid) [Escherichia coli]	48.61	3	0.84	plasmid_unitig_3
ARA20584.1	transcriptional regulator (plasmid) [Escherichia coli]	57.41	5	0.83	plasmid_unitig_2
ARA20544.1	chromosome partitioning protein ParB (plasmid) [Escherichia coli]	36.13	8	0.83	plasmid_unitig_1
ARA20723.1	plasmid stabilization protein (plasmid) [Escherichia coli]	42.86	3	0.79	plasmid_unitig_3
ARA20579.1	CopG family transcriptional regulator (plasmid) [Escherichia coli]	31.97	4	0.75	plasmid_unitig_2
ARA20451.1	KfrA protein (plasmid) [Escherichia coli]	52.02	15	0.71	plasmid_unitig_1
ARA20733.1	hypothetical protein AM365_28380 (plasmid) [Escherichia coli]	59.00	6	0.71	plasmid_unitig_3
ARA20516.1	hypothetical protein AM365_27215 (plasmid) [Escherichia coli]	46.89	3	0.65	plasmid_unitig_1
ARA20417.1	transcriptional regulator (plasmid) [Escherichia coli]	30.00	2	0.63	plasmid_unitig_1
ARA20725.1	hypothetical protein AM365_28335 (plasmid) [Escherichia coli]	38.95	4	0.60	plasmid_unitig_3
ARA20574.1	CMY-2 family class C beta-lactamase (plasmid) [Escherichia coli]	75.79	20	0.58	plasmid_unitig_1[b]
ARA20653.1	arsenate reductase (glutaredoxin) (plasmid) [Escherichia coli]	53.90	3	0.46	plasmid_unitig_2
ARA20761.1	prevent-host-death family protein (plasmid) [Escherichia coli]	63.01	3	0.46	plasmid_unitig_3
ARA20581.1	recombinase (plasmid) [Escherichia coli]	39.26	8	0.38	plasmid_unitig_2
ARA20454.1	co-chaperone GroES (plasmid) [Escherichia coli]	48.96	5	0.00	plasmid_unitig_1
ARA20459.1	subclass B1 metallo-beta-lactamase NDM-1 (plasmid) [Escherichia coli]	37.78	6	0.00	plasmid_unitig_1

[a]plasmid_unitig_1 165 proteins
plasmid_unitig_2 114 proteins
plasmid_unitig_3 105 proteins

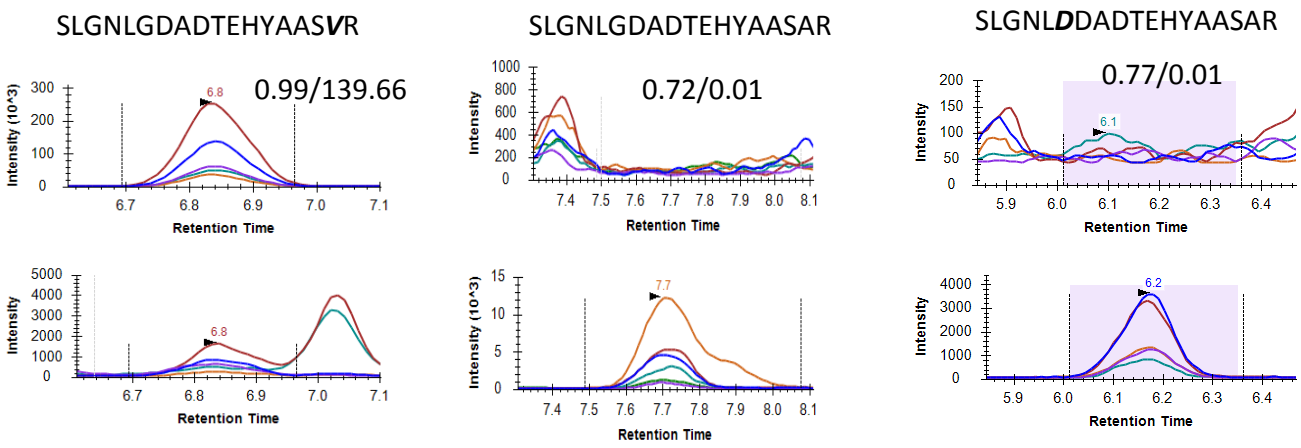
[b]slightly different sequences, not 100% sequence match

Supplemental Table 5b: Label-free quantification of detected plasmid proteins two preparations of L099

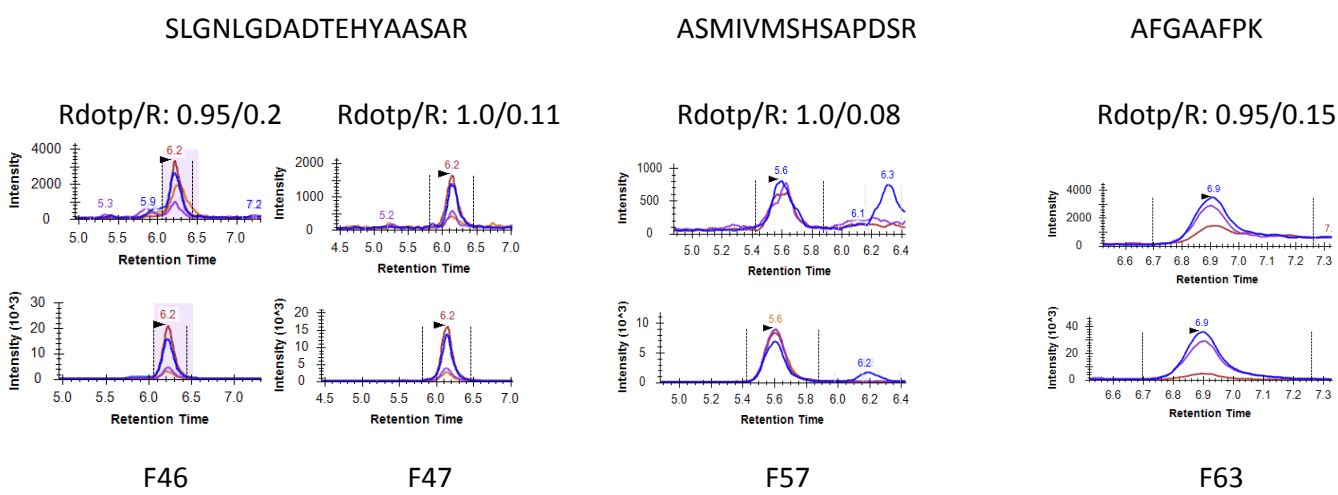
Accession	*Description*	*Coverage [%]*	*# Unique Peptides*	*ratio of first and second preparations of L099*	*plasmid*[a]
ARX53762.1	DNA-binding protein HU (plasmid) [Escherichia coli]	63.33	4	12.02	plasmid tig00000792
ARX53891.1	hypothetical protein AM375_00865 (plasmid) [Escherichia coli]	51.56	2	6.19	plasmid tig00000792
ARX53925.1	DUF86 domain-containing protein (plasmid) [Escherichia coli]	32.33	3	5.53	plasmid tig00000793
ARX54073.1	hypothetical protein AM375_01900 (plasmid) [Escherichia coli]	59.09	7	5.37	plasmid tig00000793
ARX53874.1	hypothetical protein AM375_00780 (plasmid) [Escherichia coli]	63.33	5	5.11	plasmid tig00000792
ARX53792.1	KfrA protein (plasmid) [Escherichia coli]	48.27	16	4.88	plasmid tig00000792
ARX53800.1	hypothetical protein AM375_00295 (plasmid) [Escherichia coli]	14.50	2	4.05	plasmid tig00000792
ARX53903.1	hypothetical protein AM375_00300 (plasmid) [Escherichia coli]	35.00	4	3.38	plasmid tig00000792
ARX54083.1	hypothetical protein AM375_01955 (plasmid) [Escherichia coli]	61.97	6	3.35	plasmid tig00000793
ARX53907.1	aminoglycoside N-acetyltransferase AAC(6')-Ib3 (plasmid) [Escherichia coli]	39.67	5	3.35	plasmid tig00000792[b]
ARX54111.1	hypothetical protein AM375_02125 (plasmid) [Escherichia coli]	23.17	2	3.02	plasmid tig00000793
ARX54081.1	transcriptional regulator (plasmid) [Escherichia coli]	64.81	6	2.79	plasmid tig00000793
ARX53848.1	hypothetical protein AM375_00645 (plasmid) [Escherichia coli]	51.41	3	2.78	plasmid tig00000792
ARX53779.1	hypothetical protein AM375_00185 (plasmid) [Escherichia coli]	31.38	6	2.73	plasmid tig00000792
ARX53875.1	chromosome partitioning protein ParB (plasmid) [Escherichia coli]	46.06	9	2.73	plasmid tig00000792
ARX53782.1	DNA-binding protein (plasmid) [Escherichia coli]	30.22	4	2.72	plasmid tig00000792
ARX54117.1	arsenate reductase (glutaredoxin) (plasmid) [Escherichia coli]	34.04	2	2.72	plasmid tig00000793
ARX53793.1	DNA replication terminus site-binding protein (plasmid) [Escherichia coli]	26.80	6	2.59	plasmid tig00000792
ARX53876.1	cobalamin biosynthesis protein CobQ (plasmid) [Escherichia coli]	55.94	10	2.37	plasmid tig00000792
ARX53816.1	sulfonamide-resistant dihydropteroate synthase Sul1 (plasmid) [Escherichia coli]	48.75	9	1.91	plasmid tig00000792
ARX53812.1	RmtC family 16S rRNA (guanine(1405)-N(7))-methyltransferase (plasmid) [Escherichia coli]	46.62	11	1.89	plasmid tig00000792
ARX53803.1	hypothetical protein AM375_00320 (plasmid) [Escherichia coli]	27.80	5	1.79	plasmid tig00000792
ARX53849.1	class C beta-lactamase CMY-6 (plasmid) [Escherichia coli]	67.98	17	1.65	plasmid tig00000792
ARX53760.1	transcriptional regulator (plasmid) [Escherichia coli]	30.00	2	1.63	plasmid tig00000792
ARX54097.1	cobyrinic acid ac-diamide synthase (plasmid) [Escherichia coli]	21.63	4	1.49	plasmid tig00000793
ARX53892.1	hypothetical protein AM375_00870 (plasmid) [Escherichia coli]	50.00	6	1.37	plasmid tig00000792
ARX54086.1	CopG family transcriptional regulator (plasmid) [Escherichia coli]	13.61	2	1.27	plasmid tig00000793
ARX53893.1	ATPase (plasmid) [Escherichia coli]	25.26	9	1.18	plasmid tig00000792
ARX54084.1	recombinase (plasmid) [Escherichia coli]	36.81	7	1.14	plasmid tig00000793
ARX54041.1	hypothetical protein AM375_01725 (plasmid) [Escherichia coli]	25.64	4	0.93	plasmid tig00000793
ARX53988.1	glutamine--tRNA ligase (plasmid) [Escherichia coli]	36.89	2	0.58	plasmid tig00000793
ARX54135.1	adenine specific DNA methylase (plasmid) [Escherichia coli]	9.53	5	0.42	plasmid tig00000856
ARX54026.1	toxin (plasmid) [Escherichia coli]	39.68	3	0.20	plasmid tig00000793
ARX53985.1	plasmid stabilization protein (plasmid) [Escherichia coli]	42.86	3	0.00	plasmid tig00000793
ARX53940.1	subclass B1 metallo-beta-lactamase NDM-1 (plasmid) [Escherichia coli]	37.78	6	0.00	plasmid tig00000793
ARX53997.1	transcriptional regulator (plasmid) [Escherichia coli]	28.57	2	0.00	plasmid tig00000793
ARX53935.1	co-chaperone GroES (plasmid) [Escherichia coli]	39.58	4	0.00	plasmid tig00000793
ARX54025.1	prevent-host-death family protein (plasmid) [Escherichia coli]	53.42	2	0.00	plasmid tig00000793

[a]plasmid tig00000792 156 proteins
plasmid tig00000793 222 proteins
plasmid tig00000856 7 proteins

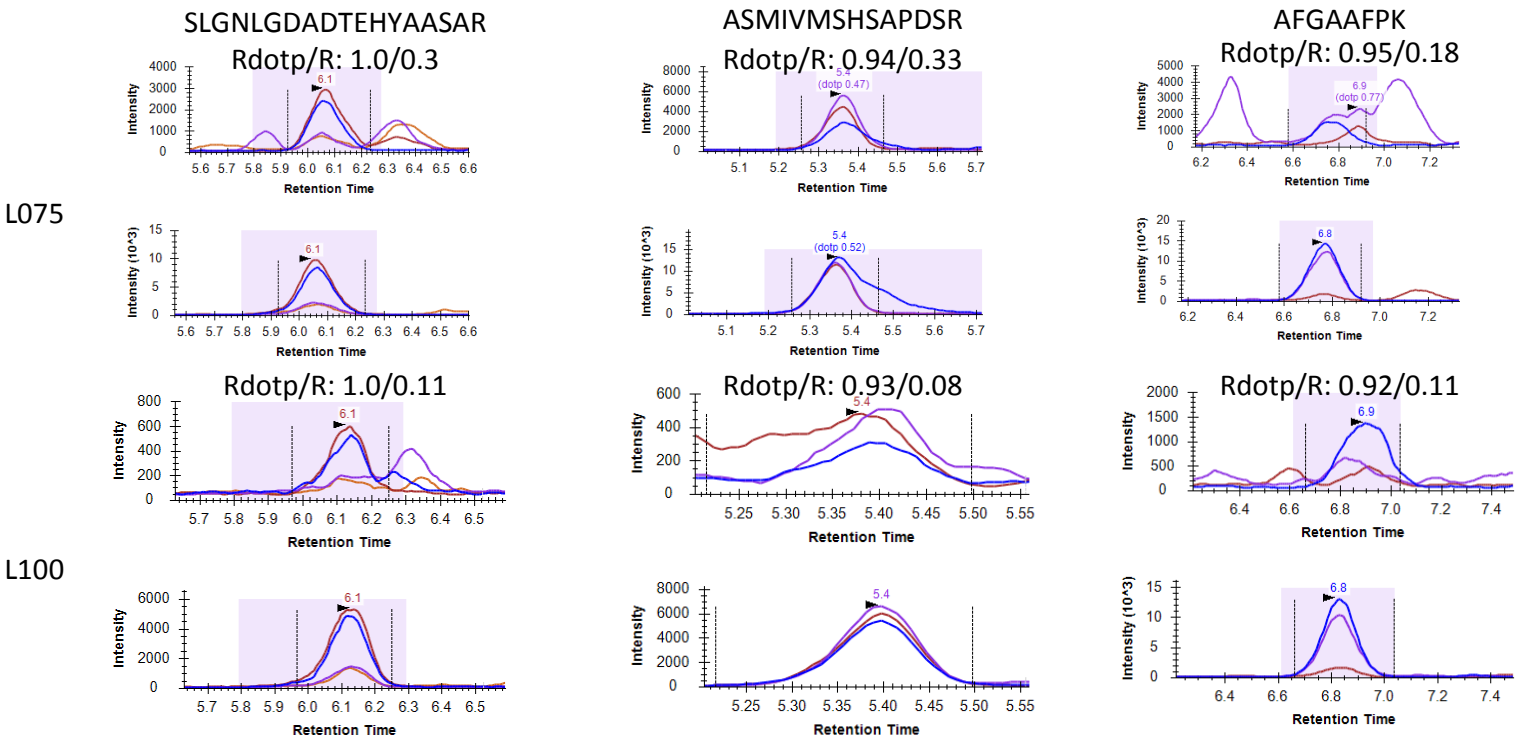
[b]slightly diffferent sequence or not 100% match



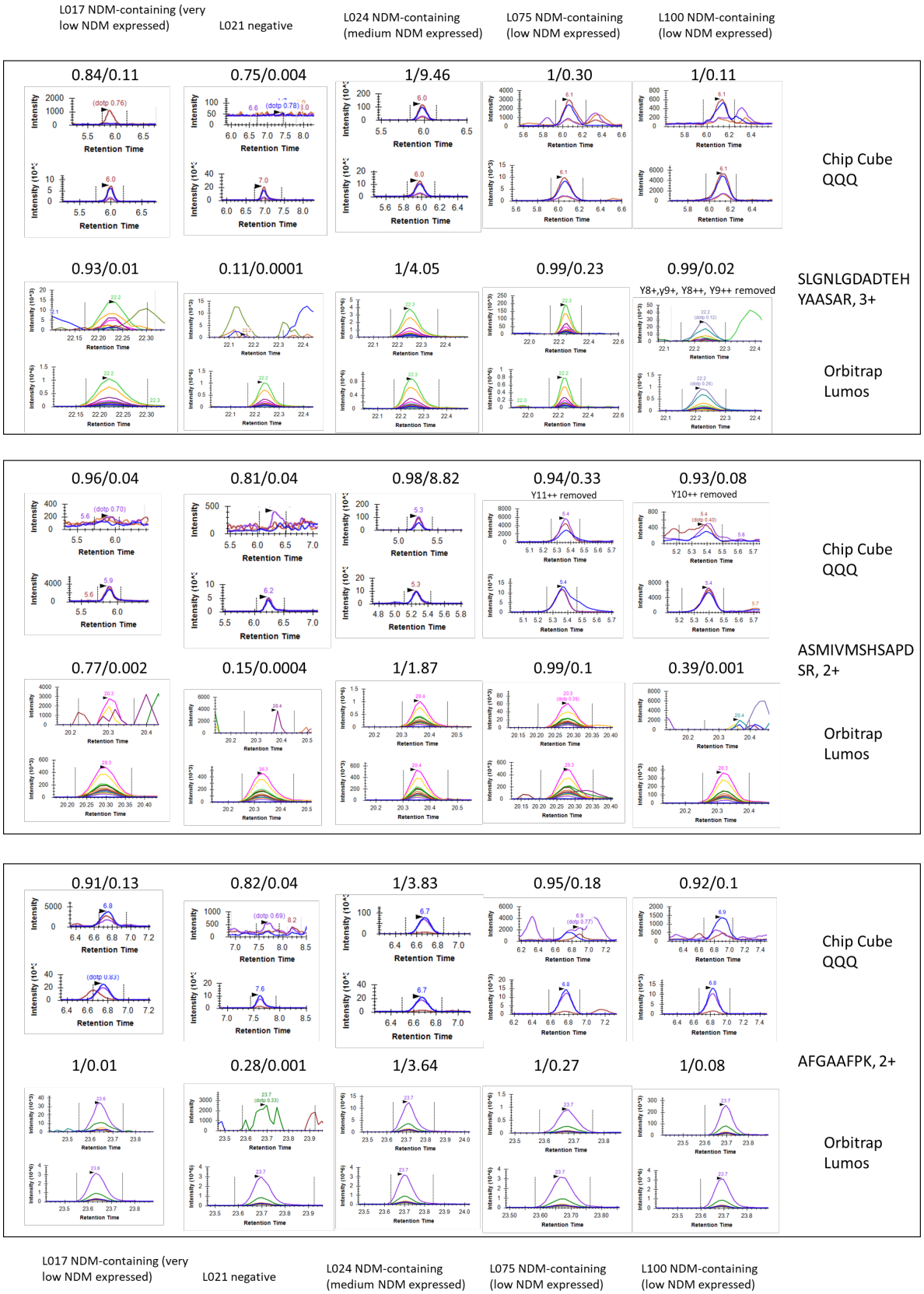
Supplemental Figure 1: Allelic variant of SLGNLGDADTEHYAAS***V***R was detected in validation isolate L063 with strong signal (R-ratio value was 139.66) indicating that L063 was either NDM-6 or NDM-15



Supplemental Figure 2: LC-MS chromatograms of three NDM peptides for isolate L017 after high pH fractionation with 10µg total protein. All three peptides were detected at the predicted fractions per the retention times of the labeled peptides. F46, F47, F57 and F63 in the figures are fraction number. Rdotp and R-ratio values for each peptide are shown in the figures.



Supplemental Figure 3: LC-MS chromatograms of three NDM peptides for isolates L075 and L100 which were manually identified as NDM positives. One strong interfering transition in ASMIVMSHSAPDSR had been removed. Rdotp and R-ratio values for each peptide are shown in the figures.



Supplemental Figure 4:
Comparison of the chromatograms acquired by the Agilent 6495 Chip Cube QQQ and Thermo Orbitrap Lumos on five isolate lysate samples. The numbers shown on the top of the chromatograms are rdotp/R-ratios. L021 was used a negative control and L024 as a positive control. L075 and L100 are two isolates that had low NDM-expression and were identified as NDM positives by manual inspection using the Agilent 6495 Chip Cube QQQ MRM assay. L017 had very low NDM expression and was not detected by the Agilent 6495 Chip Cube QQQ MRM assay. The higher mass solution and longer gradient in the Orbitrap Lumos reduced the interference, especially for the AFGAAFPK peptide and improved the detection of peptide markers for the isolates with low NDM abundance. For L017 and L100, SLGNLGDADTEHYAASAR and AFGAAFPK were detected by the Orbitrap Lumos while ASMIVMSHSAPDSR was not detected. Thus, these isolates could be identified as NDM-positive by the Orbitrap method. Interferences were observed for SLGNLGDADTEHYAASAR in sample L100. However, MS/MS spectra acquired by Orbitrap Lumos contains the full set of transitions, which allows for the removal of transitions due to interference. After removing the y8+, y8++, y9+ and y9++ transitions, the Orbitrap Lumos correctly identified this peptide.