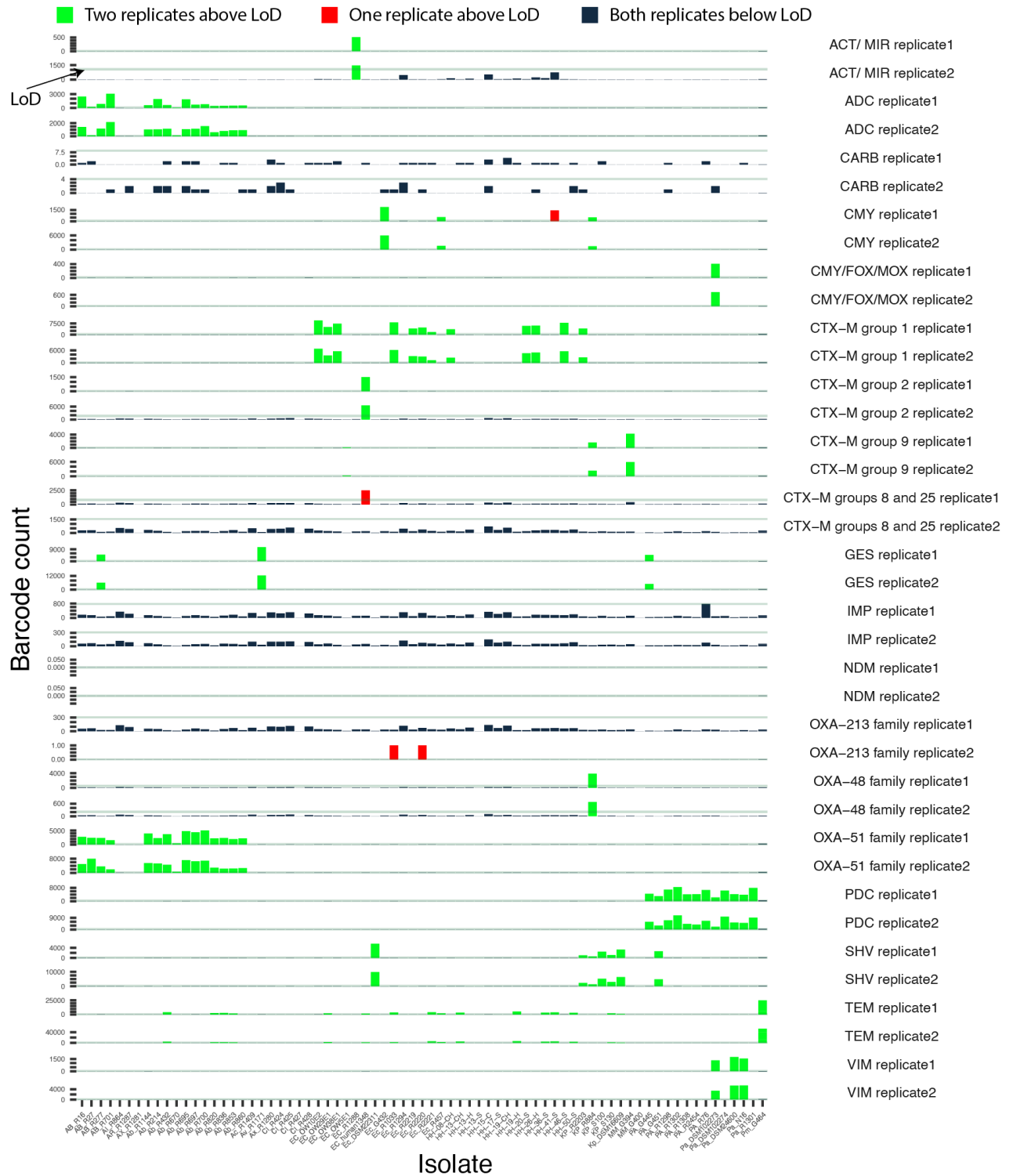


Supplementary Material: Digital Multiplex Ligation Assay for Highly Multiplexed Screening of β -Lactamase-encoding Genes in Bacterial Isolates

Supplementary Figure 1: Barchart visualization of the molecular counts for each resistance gene type (y-axis) within each bacterial isolate (x-axis). Limits of detection for each resistance gene type are marked with grey horizontal lines. Colors indicate molecular counts above the detection limit – green if both replicates were above the detection limit and red if only one replicate was above the detection limit.



Supplementary Table 1: Probe pair sequences for the 18 clusters covered by the dMLA method, the genes targeted by each probe pair sequence, and associated enzyme family, enzyme class, and distinctive substrates. The sequences cover 1187 out of 1557 (76%) of the β -lactamase genes in the Lahey database. The included targets were chosen based on a combination of clinical relevance and the number of ESBL sequences they target.

Cluster	No. of genes	Enzyme family	Class	Genes targeted	Distinctive substrates	Probe Set	Oligo Name	Sequence(5'-3')	Length (bp)	5' Mod
0	164	TEM	A	TEM-1-6/8/10-12/15-17/19-22/24/ 26/28-30/32/35/40/43/45/47-49/52-55/57/60-63/67/68/70-72/76-79/80-88/90-99/10/102/104/116/120-139/ 141-160/162-164/166-169/171/176-178/181-190/193/195/197/198/ 201/205-209-217/219/220/224	Penicillins, early cephalosporins, extended-spectrum cephalosporins	0_A_L	C0_1b_oh2_L	TGGGCCCAATTTTCCGTGACAATTAATCCCTTWTCCCTTYYTTGCG	48	
						0_A_R	C0_1b_oh2_R	GCWTTTTGCVTTCCTGTTTTNNNNNNNNNNGAATGAGTGTGCGTGCCTC	50	Phos.
						0_B_L	C0_3b_oh2_L	TGGGCCCAATTTTCCGTGACAATTAATTAAGCCCTCCCGTATCGTAK	48	
						0_B_R	C0_3b_oh2_R	TTATBTACAYGACGGKGRGTNNNNNNNNNNGAATGAGTGTGCGTGCCTC	50	Phos.
1	159	OXA (OXA-51 family)	D	OXA-51/64-71/75-80/82-84/86-95/98-100/106-113/115/117/120-128/130-132/138/144/148-150/172-180/194-197/200-203/206/208/216/ 217/219/223/241/242/248/249/250/254/259-263/312-317/336-346/365/ 371/374-391/400-404/406-409/411-414/424-426/429-433/441/442/480/ 497/507-509/516	Carbapenems	1_A_L	C1_2b_oh2_L	TGGGCCCAATTTTCCGTGACAATTAATTATCARGATTTASCTCGTCT	48	
						1_A_R	C1_2b_oh2_R	ATTGGRCCTTGARCTYATGCNNNNNNNNNNGAATGAGTGTGCGTGCCTC	50	Phos.
						1_B_L	C1_3b_oh2_L	TGGGCCCAATTTTCCGTGACAATTAATCCVACAAGTRGGYTGTTAA	48	
						1_B_R	C1_3b_oh2_R	CTGRATSGRTTGTMRGCCTNNNNNNNNNNGAATGAGTGTGCGTGCCTC	50	Phos.
2	142	SHV	A	SHV-1/2/2A/3/5/7-9/11/13-16/18/24-29/30-38/40-46/48/49-52/55-57/59-67/69-82/85/86/89/92-111/119-121/128/129/132-135/137/ 141-165/168/172/173/178-180/182/ 183/185-191/193	Penicillins, early cephalosporins, extended-spectrum cephalosporins	2_A_L	C2_2_oh2_L	TGGGCCCAATTTTCCGTGACAATTAATGCCGYCATTACCRGAGCGA	48	
						2_A_R	C2_2_oh2_R	TAACAGCGYCGCCAAATYGCNNNNNNNNNNGAATGAGTGTGCGTGCCTC	50	Phos.
						2_B_L	C2_3_oh2_L	TGGGCCCAATTTTCCGTGACAATTAATCTGYCGGCGGCTGGTTAT	48	
						2_B_R	C2_3_oh2_R	CGCCGATARGRCCGGAGCTRNNNNNNNNNNGAATGAGTGTGCGTGCCTC	50	Phos.
3	124	CMY	C	CFE-1, CMY-2/4-7/12-18/20-51/53-58/60-90/93-97/99-119/121/122/124-135/138/139 and LAT-1/3	Cephalosporins (CMY-30/37/42 have extended-spectrum)	3_A_L	C3_1b_oh2_L	TGGGCCCAATTTTCCGTGACAATTAATTAGTCACKCARCAWACKCTGT	48	
						3_A_R	C3_1b_oh2_R	TTGARITYTMGNTCCGGTYAGTNNNNNNNNNNGAATGAGTGTGCGTGCCTC	50	Phos.
						3_B_L	C3_2b_oh2_L	TGGGCCCAATTTTCCGTGACAATTAATGCRCTAARMGWYTTTAYKCT	48	
						3_B_R	C3_2b_oh2_R	AACTCCAGCATTGGCTKTTNNNNNNNNNNGAATGAGTGTGCGTGCCTC	50	Phos.
4	72	PDC	C	PDC-1-18/19a/19b/20/21a/21b/22-26/28/30-32/34-40/43-61/63-72/94-97	Cephalosporins (PDC-1-5 have extended-spectrum)	4_A_L	C4_1_oh2_L	TGGGCCCAATTTTCCGTGACAATTAATCCATCAGCCTGAAAGGARA	48	
						4_A_R	C4_1_oh2_R	CCGCATTTACTCAGCTAGTNNNNNNNNNNGAATGAGTGTGCGTGCCTC	50	Phos.
						4_B_L	C4_2_oh2_L	TGGGCCCAATTTTCCGTGACAATTAATTCARCCTGCTCGACCTCGCGA	48	
						4_B_R	C4_2_oh2_R	CCATACCAGCCGCGGCTGNNNNNNNNNNGAATGAGTGTGCGTGCCTC	50	Phos.
5	68	CTX-M group 1	A	CTX-M-1/3/10/12/15/22/23/28-30/32-37/42/52-55/58/60-62/64/ 66/68-72/79/80/82/88/96/101/103/ 114/116/117/123/132/136/139/142/144/150/1 55-158/162-164/166/167/	Extended-spectrum cephalosporins	5_A_L	C5_1_oh2_L	TGGGCCCAATTTTCCGTGACAATTAATTAGTTCACGCTSATGGCGACG	48	
						5_A_R	C5_1_oh2_R	GCARCCGTCACGCTGTRTTNNNNNNNNNNGAATGAGTGTGCGTGCCTC	50	Phos.
						5_B_L	C5_3_oh2_L	TGGGCCCAATTTTCCGTGACAATTAATCCACCAAYGATATCGCGGTG	48	
						5_B_R	C5_3_oh2_R	ATYTGCCAAAGATCGTGCNNNNNNNNNNGAATGAGTGTGCGTGCCTC	50	Phos.
6	54	ADC	C	ADC-1-7/10-23/25/26/30/38/39/41-44/50-54/56/58-64/67/68/73-81	Cephalosporins, extended-spectrum cephalosporins	6_A_L	C6_1_oh2_L	TGGGCCCAATTTTCCGTGACAATTAATTATRATGTRCCNGGTATGGC	48	
						6_A_R	C6_1_oh2_R	YGTGGGBGYATTCCARAATANNNNNNNNNNGAATGAGTGTGCGTGCCTC	50	Phos.
						6_B_L	C6_2_oh2_L	TGGGCCCAATTTTCCGTGACAATTAATTCAGARCARATYGTGATGAA	48	
						6_B_R	C6_2_oh2_R	RCCTAATAAAGTGACYGCVANNNNNNNNNNGAATGAGTGTGCGTGCCTC	50	Phos.
7	53	ACT/ MIR	C	ACT-1-10/12-15/17-25/27-39 and MIR-1-18	Cephalosporins	7_A_L	C7_2_oh2_L	TGGGCCCAATTTTCCGTGACAATTAATTARCCCHCTYARNCTGRACCAT	48	
						7_A_R	C7_2_oh2_R	ACHTGGATTAACGTCBCCSAANNNNNNNNNNGAATGAGTGTGCGTGCCTC	50	Phos.
						7_B_L	C7_3_oh2_L	TGGGCCCAATTTTCCGTGACAATTAATTAAGMRBMRCATWCGCCWGG	48	
						7_B_R	C7_3_oh2_R	GGVTAYGCGYAGBGTAARGCANNNNNNNNNNGAATGAGTGTGCGTGCCTC	50	Phos.
8	53	CTX-M group 9	A	CTX-M-9/13/14/16/17/19/21/24/27/ 38/46-51/64/65/67/73/81/83-87/90/ 93/98/ 99/102/104/105/110-113/ 121-123/125/126/129/130/132/ 134/137/147/148/159/161/168/174	Extended-spectrum cephalosporins	8_A_L	C8_2_oh2_L	TGGGCCCAATTTTCCGTGACAATTAATTGRAGGCGTGACGGCTTTGCG	48	
						8_A_R	C8_2_oh2_R	CCGCKMGATCGCGATGAGANNNNNNNNNNGAATGAGTGTGCGTGCCTC	50	Phos.
						8_B_L	C8_3_oh2_L	TGGGCCCAATTTTCCGTGACAATTAATTCGTCTGGAATCGCACTGAABV	48	
						8_B_R	C8_3_oh2_R	TACGCWGAATACCGCATTCCNNNNNNNNNNGAATGAGTGTGCGTGCCTC	50	Phos.
9	52	IMP	B	IMP-1-35/37/38/40-45/48/49/51-56/58	Carbapenems	9_A_L	C9_2b_oh2_L	TGGGCCCAATTTTCCGTGACAATTAATTTTTYCATRGYAYAGYDCRG	48	
						9_A_R	C9_2b_oh2_R	SNGGAAATWGRRTGGCTAAYNNNNNNNNNNGAATGAGTGTGCGTGCCTC	50	Phos.
						9_B_L	C9_3b_oh2_L	TGGGCCCAATTTTCCGTGACAATTAATGGAATWGRRTGGCTTAAATC	48	
						9_B_R	C9_3b_oh2_R	TCRRTCVATYYCMACRATGNNNNNNNNNNGAATGAGTGTGCGTGCCTC	50	Phos.

Cluster	No. of genes	Enzyme family	Class	Genes targeted	Distinctive substrates	Probe Set	Oligo Name	Sequence(5'-3')	Length (bp)	5' Mod
10	48	VIM	B	VIM-1-20/23/47/49-51	Carbapenems	10_A_L	C10_1_oh2_L	TGGGCCCAATTTCCGTGACAATTAATTCGAARAACACRGYRGCMTT	48	
						10_A_R	C10_1_oh2_R	CTCGCGGAGATTGARAAGCANNNNNNNNNNGAATGAGTGTGCGTGCACTC	50	Phos.
						10_B_L	C10_2_oh2_L	TGGGCCCAATTTCCGTGACAATTAATTCGCACTTYCATGACGAYCGM	48	
						10_B_R	C10_2_oh2_R	GTCCGGYGGMGTGATGYCCTNNNNNNNNNNGAATGAGTGTGCGTGCACTC	50	Phos.
12	38	OXA (OXA-213 family)	D	OXA-213/267-273/304/305/322-332/348-354/357-359/417/421/500-503/506	Carbapenems	12_A_L	C12_2b_oh2_L	TGGGCCCAATTTCCGTGACAATTAATTAYGGHCARAARCGYTRTTT	48	
						12_A_R	C12_2b_oh2_R	CCYGAYTGGRAAAARGAYATNNNNNNNNNNGAATGAGTGTGCGTGCACTC	50	Phos.
						12_B_L	C12_5b_oh2_L	TGGGCCCAATTTCCGTGACAATTAATTTWACVGGYTGRRNRTTCAA	48	
						12_B_R	C12_5b_oh2_R	YMDCARGGARAATTRTHGCNNNNNNNNNNGAATGAGTGTGCGTGCACTC	50	Phos.
13	30	GES	A	GES-1-22/24-31	Cephalosporins, extended-spectrum cephalosporins, carbapenems	13_A_L	C13_1_oh2_L	TGGGCCCAATTTCCGTGACAATTAATTAGCAGCTSAGATCGGTGTTG	48	
						13_A_R	C13_1_oh2_R	CGATCGTCGATCCCAAGGANNNNNNNNNNGAATGAGTGTGCGTGCACTC	50	Phos.
						13_B_L	C13_3_oh2_L	TGGGCCCAATTTCCGTGACAATTAATTCCTCTGTCCGGTCAAGTTAT	48	
						13_B_R	C13_3_oh2_R	TACACAACATCCTGAGCANNNNNNNNNNGAATGAGTGTGCGTGCACTC	50	Phos.
14	30	CMY/FOX/MOX	C	CMY-1/8/8b/9-11/19, FOX-1-10/12/13 and MOX-1-12	Cephalosporins (CMY-8/9 have extended-spectrum)	14_A_L	C14_2_oh2_L	TGGGCCCAATTTCCGTGACAATTAATGTGCARYGACGACSCGTGTT	48	
						14_A_R	C14_2_oh2_R	CGAKATWGGVTCSTGTSAGCANNNNNNNNNNGAATGAGTGTGCGTGCACTC	50	Phos.
						14_B_L	C14_3_oh2_L	TGGGCCCAATTTCCGTGACAATTAATGATVGCRTCGTCATGCTGG	48	
						14_B_R	C14_3_oh2_R	CCAAAYCGCACTMYCCYAWCNNNNNNNNNNGAATGAGTGTGCGTGCACTC	50	Phos.
15	27	CARB	A	CARB-17-20/22/24-36/38/40/41/43-48	Carbencillin	15_A_L	C15_1_oh2_L	TGGGCCCAATTTCCGTGACAATTAATTAAAGTTATTCTGTTGGYTG	48	
						15_A_R	C15_1_oh2_R	GCTGATGGTTRTCTCAACTGNNNNNNNNNNGAATGAGTGTGCGTGCACTC	50	Phos.
						15_B_L	C15_2_oh2_L	TGGGCCCAATTTCCGTGACAATTAATTACCATGCTAAGCGAYATGGA	48	
						15_B_R	C15_2_oh2_R	BAGCGGCAAACTCAACAAAANNNNNNNNNNGAATGAGTGTGCGTGCACTC	50	Phos.
18	22	CTX-M group 2	A	CTX-M-2/4-7/20/21/35/43/44/56/59/76/77/92/95/97/115/124/131/141/165	Extended-spectrum cephalosporins	18_A_L	C26_18L	TGGGCCCAATTTCCGTGACAATTAATTCAATGTTAACGGTGTGGCG	48	
						18_A_R	C26_18R	ACGCTACCCCTGCTATTTAGNNNNNNNNNNGAATGAGTGTGCGTGCACTC	50	Phos.
						18_B_L	C105_18L	TGGGCCCAATTTCCGTGACAATTAATTGCAGCTGRAAGCCCTGGAGA	48	
						18_B_R	C105_18R	AAAGTTCGGGAGGTCGGCTTNNNNNNNNNNGAATGAGTGTGCGTGCACTC	50	Phos.
20	20	OXA (OXA-48 family)	D	OXA-48/54/162/163/181/199/204/232/244/245/247/252/370/405/416/436/438/439/484/505	Carbapenems	20_A_L	C1_20L	TGGGCCCAATTTCCGTGACAATTAATTATGCGTYRTTAGCCTTATC	48	
						20_A_R	C1_20R	GRCTGTRTTDDTGGTGRCANNNNNNNNNNGAATGAGTGTGCGTGCACTC	50	Phos.
						20_B_L	C146_20L	TGGGCCCAATTTCCGTGACAATTAATTAGAAYAMRCAGCARGGWTTT	48	
						20_B_R	C146_20R	ACCAAYRATCTTAARCGGGCNNNNNNNNNNGAATGAGTGTGCGTGCACTC	50	Phos.
27	14	CTX-M group 8 and group 25	A	CTX-M-8/25/26/39-41/63/78/89/91/94/100/152/160	Extended-spectrum cephalosporins	27_A_L	C393_27L	TGGGCCCAATTTCCGTGACAATTAATTGTACAGCGAYAATAACKGCCA	48	
						27_A_R	C393_27R	TGAAYAAGCTGATTGCCCATNNNNNNNNNNGAATGAGTGTGCGTGCACTC	50	Phos.
						27_B_L	C584_27L	TGGGCCCAATTTCCGTGACAATTAATTATCTSACRTTGGGCARTGCC	48	
						27_B_R	C584_27R	YTRGGTGAMACTCAGCGTGCNNNNNNNNNNGAATGAGTGTGCGTGCACTC	50	Phos.