Digital PCR assay for identification of critical β-lactamases associated with carbapenem- and ESBL-producing bacteria

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BACKGROUND

Post-pandemic monitoring of emerging infectious diseases has reemphasized the importance of surveillance for antimicrobial resistant (AMR) bacteria. Often labeled as the "silent pandemic," AMR bacteria are responsible for an estimated 700,000 deaths annually. To address the gaps in national laboratory testing capacity, including AMR mechanism testing, the CDC has organized the Antibiotic Resistance Laboratory Network, which released a technical appendix that establishes Streck ARM-D® kits as a method for AMR mechanism testing. Streck ARM-D kits are qPCR-based methods that test for 21 different β-lactamase gene families and over 1,000 different allelic variants. Recently the CDC has expanded the scope for AMR genetic mechanism surveillance to include wastewater through the National Wastewater Surveillance System. CDC guidance for wastewater testing defines prioritized AMR mechanisms and recommended methods for wastewater testing, including use of digital PCR, as the preferred testing technology. Consequently, this study describes the compatibility of Streck ARM-D kits with the QIAGEN QIAcuity dPCR system and expanded coverage of ARM-D Kit OXA gene families to support detection of new AMR mechanisms of interest. Advancement of the ARM-D kit technology to comply with new CDC surveillance strategies permits use of the same commercial product for the expanded applications, allowing public health labs to use the same test with multiple sample types and technologies for testing these medically relevant resistance mechanisms.

METHODS

Extracts from bacterial isolates and wastewater samples, previously characterized as AMR positive or negative by qPCR using the Streck ARM-D kits, were reevaluated using the Streck ARM-D Kits, B-Lactamase and OXA with the QIAGEN QIAcuity dPCR System. Reactions were prepared using the ARM-D Kit 10X PCR Mixes paired with the QIAGEN QIAcuity OneStep Advanced Probe Kit on 26k QIAGEN QIAcuity nanoplates. All samples were diluted 1:1000 in nuclease-free water prior to dPCR analysis. As external positive controls, extracts from 5 AR Bank Isolates were also evaluated for concordance to whole genome sequencing and ARM-D kit qPCR data.

ARM-D Kit Digital PCR Workflow

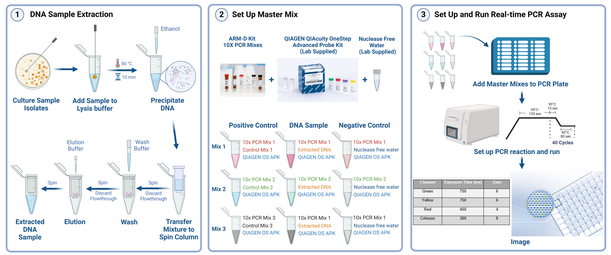


Figure 1. Digital PCR Workflow for Streck ARM-D Kits β-Lactamase and OXA. Created with BioRender.com

RESULTS

Following ARM-D kit protocol optimization for compatibility with QIAGEN's digital PCR system, positive and negative agreement for qualitative detection of AMR mechanisms, detected by qPCR and dPCR, were concordant across 40 total extracts obtained from either culture isolates or wastewater samples as well as the 5 AR isolate bank isolates.

Table 1. ARM-D Kit, β-Lactamase Gene Variants

10x PCR Mix	β-Lactamase-like Gene Family	Detected Variants
ARM-D Kit, β-Lactamase PCR Mix #1	CMY-2 (CIT)	CMY-2, 4-7, 12-18, 20-51, 53-63, 65-69, 71, 72, 75-81, 84-87, 89, 90, 94-97, 99, 102, 105-108, 110-116, 118, 119, 121, 122, 124-126, 128, 130-135, 138-141, 143-146
	CTX-M-14	CTX-M-9, 13, 14, 16, 17, 19, 21, 24, 27, 38, 46-51, 65, 67, 73, 81-87, 90, 93, 98, 99, 102, 104, 105, 110-113, 121, 122, 125, 126, 129, 130, 134, 147, 148, 159, 161, 168, 174
	CTX-M-15	CTX-M-1, 3, 10, 12, 15, 22, 23, 28, 29, 30, 32, 36, 37, 52, 55, 58, 60, 61, 64, 69, 71, 72, 79, 80, 82, 96, 103, 114, 116, 117, 123, 132, 136, 138, 139, 142, 144, 150, 155-158, 163, 164, 166, 167, 170, 172, 173, 175, 176, 179, 180, 181, 183, 184
ARM-D Kit, β-Lactamase PCR Mix #2	IMP	IMP-1-4, 6-8, 10, 13, 14, 17-20, 23-25, 27, 30, 32-34, 37, 40, 42, 43, 48, 49, 51, 52, 54-56, 60, 61, 63, 64, 67, 70-73, 75
	VIM	VIM-1-6, 8-12, 14-20, 23-46, 49-51
	OXA-48	OXA-48, 162, 163, 181, 199, 204, 232, 244, 245, 247, 370, 405, 416, 438, 439, 505, 514, 515, 517, 519, 538, 546, 547, 566, 567
ARM-D Kit, β-Lactamase PCR Mix #3	KPC	KPC-1-22
	NDM	NDM-1-21, 32
	DHA	DHA-1-7, 9, 10, 12-22

Table 2. ARM-D Kit, OXA Gene Variants

10x PCR Mix	OXA-like Gene Family	Detected Variants
ARM-D Kit, OXA PCR Mix #1	OXA-143	143, 182, 231, 253, 255, 499, 649, 825, 945, 1139, 1182
	OXA-48	48, 162, 163, 181, 199, 204, 232, 244, 245, 247, 252, 370, 405, 416, 438, 439, 484, 505, 514, 515, 517, 519, 546, 547, 566, 788, 793, 833, 894, 918, 920, 922, 923, 924, 929, 933, 934, 1038, 1039, 1055, 1119, 1146, 1181
	OXA-24/40	24, 25, 26, 72, 139, 160, 207, 437, 653, 897, 1040, 1081
ARM-D Kit, OXA PCR Mix #2	OXA-58	58, 96, 97, 164, 397, 420, 512, 1178
	OXA-51	51, 64, 65, 66, 67, 68, 69, 70, 71, 75, 76, 77, 78, 79, 80, 82, 83, 84, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 98, 99, 100, 104, 106, 107, 108, 109, 110, 111, 112, 113, 115, 117, 120, 121, 122, 123, 124, 125, 126, 127, 128, 130, 131, 132, 138, 144, 148, 149, 150, 172, 173, 174, 175, 176, 177, 178, 179, 180, 194, 195, 196, 197, 200, 201, 202, 203, 206, 208, 216, 217, 219, 223, 234, 241, 242, 248, 249, 250, 254, 259, 260, 261, 262, 263, 312, 313, 314, 315, 316, 317, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 365, 371, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 400, 401, 402, 403, 404, 406, 407, 408, 409, 411, 412, 413, 414, 424, 425, 426, 429, 430, 431, 432, 433, 441, 442, 480, 497, 507, 508, 509, 510, 511, 516, 521, 523, 524, 525, 528, 529, 530, 531, 532, 536, 545, 554, 555, 556, 557, 558, 559, 560, 561, 562, 654, 680, 682, 683, 684, 685, 686, 687, 688, 689, 690, 691, 692, 693, 694, 695, 696, 697, 698, 699, 700, 701, 702, 703, 704, 705, 706, 707, 708, 709, 710, 711, 712, 713, 714, 715, 716, 717, 718, 719, 720, 721, 722, 723, 733, 734, 735, 738, 739, 740, 741, 742, 743, 744, 745, 746, 747, 748, 749, 750, 751, 752, 753, 754, 755, 756, 757, 758, 759, 760, 761, 762, 763, 764, 765, 766, 767, 768, 769, 770, 771, 772, 773, 774, 775, 776, 781, 782, 797, 798, 799, 800, 828, 829, 831, 834, 852, 853, 854, 855, 856, 857, 858, 859, 860, 861, 862, 863, 864, 865, 866, 867, 868, 869, 870, 871, 872, 873, 874, 875, 876, 877, 878, 879, 880, 881, 882, 883, 884, 885, 886, 887, 888, 889, 890, 891, 892, 909, 910, 916, 927, 938, 939, 940, 954, 955, 970, 971, 972, 973, 974, 975, 976, 977, 1013, 1047, 1048, 1058, 1059, 1060, 1061, 1062, 1063, 1064, 1065, 1066, 1067, 1068, 1069, 1070, 1071, 1072, 1073, 1074, 1075, 1076, 1077, 1078, 1079, 1080, 1092, 1093, 1094, 1096, 1097, 1098, 1109, 1117, 1118, 1121, 1122, 1123
	OXA-23	23, 27, 49, 73, 103, 133, 146, 165, 166, 167, 168, 169, 170, 171, 225, 239, 366, 398, 422, 423, 435, 440, 482, 483, 565, 657, 806, 807, 808, 809, 810, 811, 812, 813, 814, 815, 816, 817, 818, 893, 911, 966, 967, 968, 969, 1095
NEW OXA PCR MIX (Not available for commercial use)	OXA-198	198, 1056, 1057, 1186
	OXA-134	134, **235, 236, 237**, 276, 277, 278, 282, 283, 284, 285, 335, 360, 361, 362, 363, 496, 537, 646, 647, 648, 915, 991, 1005, 1110, 1111, 1112, 1116
	OXA-372	372, 641, 1016

RESULTS (cont.)

Table 3. Concordance of qPCR and dPCR results for the ARM-D Kit, β-Lactamase using a panel of 40 previously characterized AMR-positive bacterial DNA extracts. For each 40 µL dPCR reaction, 5 µL of sample was added to the reaction mix with sample bacterial genomic DNA concentrations ranging from 10 to 300 µg/reaction. ARM-D Kit 10X PCR Mixes and the QIAGEN QIAcuity OneStep Advanced Probe Mix were added per manufacturer's instructions and no reverse transcriptase step was utilized in the cycling protocol. Digital PCR results were 100% concordance to qPCR data and 100% positive and negative agreement to expected results.

A. ARM-D Kit, β-Lactamase qPCR and dPCR Results

			ARM-D KIT, β-LACTAMASE								
			PCR Mix 1			PCR Mix 2			PCR Mix 3		
Isolate #	Organism	Expected Detection	Expected	qPCR Result	dPCR Result	Expected	qPCR Result	dPCR Result	Expected	qPCR Result	dPCR Result
1	*K. pneumoniae*	DHA	NEG	NEG	NEG	NEG	NEG	NEG	POS	POS	POS
2	*K. pneumoniae*	OXA-48, NDM, CTX-M-15	POS	POS	POS	POS	POS	POS	POS	POS	POS
3	*K. pneumoniae*	Neg	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
4	*E. cloacae*	Neg	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
5	*E. coli*	Neg	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
6	*E. cloacae*	VIM, CTX-M-15	POS	POS	POS	POS	POS	POS	NEG	NEG	NEG
7	*E. aerogenes*	Neg	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
8	*K. pneumoniae*	CMY-2, CTX-M-14, VIM, CTX-M-15	POS	POS	POS	POS	POS	POS	NEG	NEG	NEG
9	*C. koseri*	KPC	NEG	NEG	NEG	NEG	NEG	NEG	POS	POS	POS
10	*K. oxytoca*	Neg	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
11	*E. cloacae*	Neg	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
12	*E. aerogenes*	Neg	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
13	*P. mirabilis*	Neg	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
14	*C. freundii*	CMY-2, NDM	POS	POS	POS	NEG	NEG	NEG	POS	POS	POS
15	*C. freundii*	Neg	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
16	*K. pneumoniae*	DHA, CTX-M-14	POS	POS	POS	NEG	NEG	NEG	POS	POS	POS
17	*E. cloacae*	CTX-M-14	POS	POS	POS	NEG	NEG	NEG	NEG	NEG	NEG
18	*E. coli*	CMY-2	POS	POS	POS	NEG	NEG	NEG	NEG	NEG	NEG
19	*K. pneumoniae*	IMP	NEG	NEG	NEG	POS	POS	POS	NEG	NEG	NEG
20	*P. mirabilis*	Neg	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
21	*A. baumannii*	Neg	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
22	*E. coli*	Neg	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
23	*E. coli*	Neg	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
24	*S. marcescens*	OXA-48	POS	POS	POS	NEG	NEG	NEG	NEG	NEG	NEG
25	*Acinetobacter spp*	OXA-48	POS	POS	POS	NEG	NEG	NEG	NEG	NEG	NEG
26	*S. Typhimurium*	Neg	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
27	*P. rettgeri*	Neg	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
28	*A. baumannii*	OXA-23, OXA-51	NEG	NEG	NEG	POS	POS	POS	NEG	NEG	NEG
29	*K. pneumoniae*	Neg	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
30	*E. coli*	Neg	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
33	*E. coli*	Neg	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
34	*K. oxytoca*	Neg	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
35	*E. coli*	Neg	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
36	*P. putida*	Neg	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
37	*A. baumannii*	OXA-23, OXA-51	NEG	NEG	NEG	POS	POS	POS	NEG	NEG	NEG
38	*A. baumannii*	OXA-23, OXA-51	NEG	NEG	NEG	POS	POS	POS	NEG	NEG	NEG
39	*E. cloacae*	Neg	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
40	*K. pneumoniae*	Neg	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
44	*A. baumannii*	OXA-51	NEG	NEG	NEG	POS	POS	POS	NEG	NEG	NEG
45	*A. baumannii*	OXA-23, OXA-51	NEG	NEG	NEG	POS	POS	POS	NEG	NEG	NEG
46	*A. baumannii*	OXA-24/40, OXA-51	POS	POS	POS	POS	POS	POS	NEG	NEG	NEG
50	*A. baumannii*	OXA-24/40, OXA-51	POS	POS	POS	POS	POS	POS	NEG	NEG	NEG
51	*A. baumannii*	OXA-51, OXA-58	NEG	NEG	NEG	POS	POS	POS	NEG	NEG	NEG
56	*A. baumannii*	OXA-23, OXA-51	NEG	NEG	NEG	POS	POS	POS	NEG	NEG	NEG
58	*A. baumannii*	OXA-51	NEG	NEG	NEG	POS	POS	POS	NEG	NEG	NEG
64	*A. baumannii*	OXA-143, OXA-51	POS	POS	POS	POS	POS	POS	NEG	NEG	NEG
66	*A. baumannii*	OXA-24/40, OXA-23, OXA-51	POS	POS	POS	POS	POS	POS	NEG	NEG	NEG
201	*A. baumannii*	OXA-23, OXA-51	NEG	NEG	NEG	POS	POS	POS	NEG	NEG	NEG
202	*A. baumannii*	OXA-134, OXA-51	NEG	NEG	NEG	POS	POS	POS	POS	POS	POS
203	*A. baumannii*	OXA-134, OXA-51	NEG	NEG	NEG	POS	POS	POS	POS	POS	POS
204	*A. baumannii*	OXA-23, OXA-51	NEG	NEG	NEG	POS	POS	POS	NEG	NEG	NEG
205	Contrived Sample	OXA-198	NEG	NEG	NEG	NEG	NEG	NEG	POS	POS	POS
206	Contrived Sample	OXA-372	NEG	NEG	NEG	NEG	NEG	NEG	POS	POS	POS
Ctrl	ARM- D Control Mix 1	All Targets	POS	POS	POS	POS	POS	POS	POS	POS	POS
NTC	Nuclease Free Water	No Targets	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG

B. ARM-D Kit, β-Lactamase Results Summary

Target	Expected Positive Samples (n=3)	Expected Negative Samples (n=3)	Positive Percent Agreement	Negative Percent Agreement
CMY-2	5	35	100%	100%
CTX-M-15	7	31	100%	100%
CTX-M-14	4	36	100%	100%
OXA-48	4	36	100%	100%
IMP	2	38	100%	100%
VIM	3	37	100%	100%
DHA	4	36	100%	100%
KPC	3	37	100%	100%
NDM	4	36	100%	100%

Table 4. Concordance of qPCR and dPCR results for the ARM-D Kit, OXA using a panel of 40 previously characterized AMR positive bacterial DNA extracts. For each dPCR reaction, 5 µL of sample was added to the reaction mix with sample bacterial genomic DNA concentrations ranging from 10 to 300 µg/reaction. Digital PCR results were 100% concordance to qPCR data and 100% positive and negative agreement to expected results.

A. ARM-D Kit, OXA qPCR and dPCR Results

			ARM-D KIT, OXA								
			PCR Mix 1			PCR Mix 2			PCR Mix 3 (NEW)		
Isolate #	Organism	Expected Detection	Expected	qPCR Result	dPCR Result	Expected	qPCR Result	dPCR Result	Expected	qPCR Result	dPCR Result
1	*K. pneumoniae*	Neg	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
2	*K. pneumoniae*	OXA-48	POS	POS	POS	NEG	NEG	NEG	NEG	NEG	NEG
16	*K. pneumoniae*	Neg	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
17	*E. cloacae*	Neg	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
18	*E. coli*	Neg	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
19	*K. pneumoniae*	Neg	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
20	*P. mirabilis*	Neg	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
21	*A. baumannii*	OXA-23, OXA-51	NEG	NEG	NEG	POS	POS	POS	NEG	NEG	NEG
22	*E. coli*	Neg	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
23	*E. coli*	Neg	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
24	*S. marcescens*	OXA-48	POS	POS	POS	NEG	NEG	NEG	NEG	NEG	NEG
25	*Acinetobacter spp*	OXA-48	POS	POS	POS	NEG	NEG	NEG	NEG	NEG	NEG
26	*S. Typhimurium*	Neg	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
27	*P. rettgeri*	Neg	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
28	*A. baumannii*	OXA-23, OXA-51	NEG	NEG	NEG	POS	POS	POS	NEG	NEG	NEG
29	*K. pneumoniae*	Neg	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
30	*E. coli*	Neg	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
33	*E. coli*	Neg	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
34	*K. oxytoca*	Neg	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
35	*E. coli*	Neg	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
36	*P. putida*	Neg	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
37	*A. baumannii*	OXA-23, OXA-51	NEG	NEG	NEG	POS	POS	POS	NEG	NEG	NEG
38	*A. baumannii*	OXA-23, OXA-51	NEG	NEG	NEG	POS	POS	POS	NEG	NEG	NEG
39	*E. cloacae*	Neg	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
40	*K. pneumoniae*	Neg	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
44	*A. baumannii*	OXA-51	NEG	NEG	NEG	POS	POS	POS	NEG	NEG	NEG
45	*A. baumannii*	OXA-23, OXA-51	NEG	NEG	NEG	POS	POS	POS	NEG	NEG	NEG
46	*A. baumannii*	OXA-24/40, OXA-51	POS	POS	POS	POS	POS	POS	NEG	NEG	NEG
50	*A. baumannii*	OXA-24/40, OXA-51	POS	POS	POS	POS	POS	POS	NEG	NEG	NEG
51	*A. baumannii*	OXA-51, OXA-58	NEG	NEG	NEG	POS	POS	POS	NEG	NEG	NEG
56	*A. baumannii*	OXA-23, OXA-51	NEG	NEG	NEG	POS	POS	POS	NEG	NEG	NEG
58	*A. baumannii*	OXA-51	NEG	NEG	NEG	POS	POS	POS	NEG	NEG	NEG
64	*A. baumannii*	OXA-143, OXA-51	POS	POS	POS	POS	POS	POS	NEG	NEG	NEG
66	*A. baumannii*	OXA-24/40, OXA-23, OXA-51	POS	POS	POS	POS	POS	POS	NEG	NEG	NEG
201	*A. baumannii*	OXA-23, OXA-51	NEG	NEG	NEG	POS	POS	POS	NEG	NEG	NEG
202	*A. baumannii*	OXA-134, OXA-51	NEG	NEG	NEG	POS	POS	POS	POS	POS	POS
203	*A. baumannii*	OXA-134, OXA-51	NEG	NEG	NEG	POS	POS	POS	POS	POS	POS
204	*A. baumannii*	OXA-23, OXA-51	NEG	NEG	NEG	POS	POS	POS	NEG	NEG	NEG
205	Contrived Sample	OXA-198	NEG	NEG	NEG	NEG	NEG	NEG	POS	POS	POS
206	Contrived Sample	OXA-372	NEG	NEG	NEG	NEG	NEG	NEG	POS	POS	POS
Ctrl	ARM- D Control Mix 1	All Targets	POS	POS	POS	POS	POS	POS	POS	POS	POS
NTC	Nuclease Free Water	No Targets	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG

B. ARM-D Kit, OXA Results Summary

Target	Expected Positive Samples (n=3)	Expected Negative Samples (n=3)	Positive Percent Agreement	Negative Percent Agreement
OXA-143	1	39	100%	100%
OXA-48	3	37	100%	100%
OXA-24/40	3	37	100%	100%
OXA-58	1	39	100%	100%
OXA-51	15	25	100%	100%
OXA-23	9	31	100%	100%
OXA-198	1	39	100%	100%
OXA-134	2	38	100%	100%
OXA-372	1	39	100%	100%

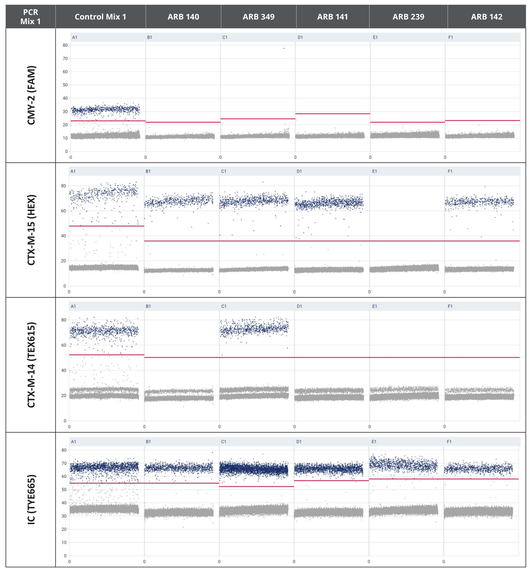
RESULTS (cont.)

Table 5. ARM-D Kit, β-Lactamase Results Summary for Antimicrobial Resistance Isolate Bank Samples

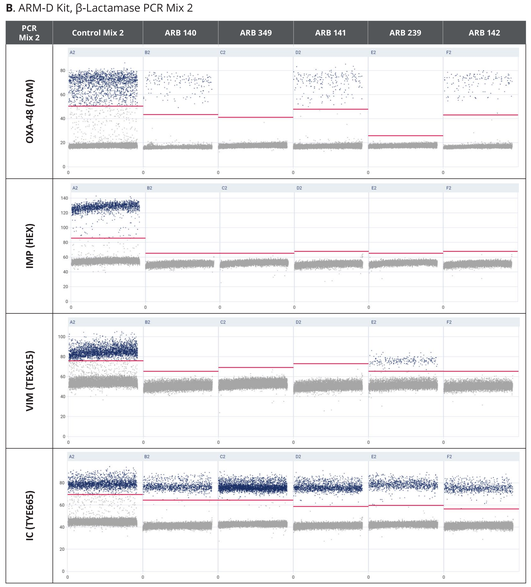
AR Bank Sample	ARM-D Kit dPCR Results	ARM-D Kit qPCR Results	Characterized Antibiotic Resistance Mechanisms from ARB (Identified families are highlighted)
ARB 140	CTX-M-15, OXA-48	CTX-M-15, OXA-48	aac(3)-IId, aadA2, armA, catA1, **CTX-M-15**, dfrA12, dfrA14, OmpK35, **OXA-181**, SHV-26, sul1, sul2, tet(A)
ARB 349	CTX-M-14, CTX-M-15	CTX-M-14, CTX-M-15	aph(4)-Ia, **CTX-M-14**, **CTX-M-55**, dfrA14, fosA, mcr-1, mph(A), strA, strB, TEM-1B, tet(A)
ARB 141	CTX-M-15, OXA-48	CTX-M-15, OXA-48	aac(3)-IId, aadA2, armA, **CTX-M-15**, dfrA12, dfrA14, OmpK35, **OXA-181**, SHV-26, sul1, sul2, tet(A)
ARB 249	VIM	VIM	aac(3)-Id, aadA2, cmlA1, dfrB5, OXA-4, OXA-50, PAO, tet(G), **VIM-2**
ARB 142	CTX-M-15, OXA-48	CTX-M-15, OXA-48	aac(3)-IId, aadA2, armA, **CTX-M-15**, dfrA12, dfrA14, OmpK35, **OXA-181**, SHV-26, sul1, sul2, tet(A)

Figure 2. 1D scatter plots showing AMR gene detection for representative AR Bank Isolate samples. For each dPCR reaction, 5 µg of bacterial genomic DNA extract was added to the reaction mix. Digital PCR results were 100% concordant to whole genome sequencing results provided for each characterized AR Bank isolate.

A. ARM-D Kit, β-Lactamase PCR Mix 1

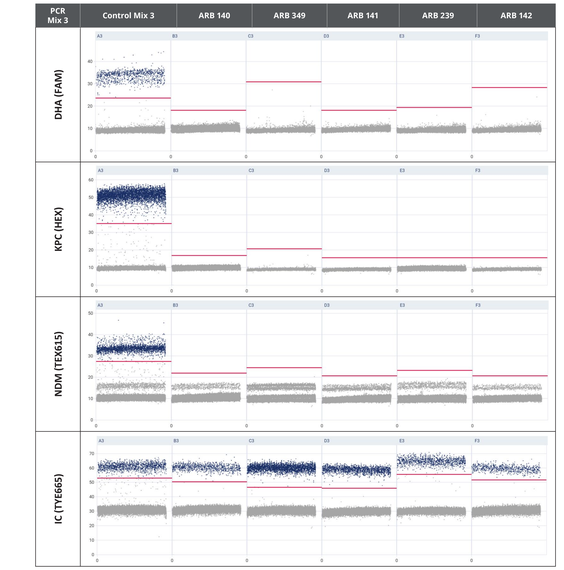


B. ARM-D Kit, β-Lactamase PCR Mix 2



RESULTS (cont.)

C. ARM-D Kit, β-Lactamase PCR Mix 3



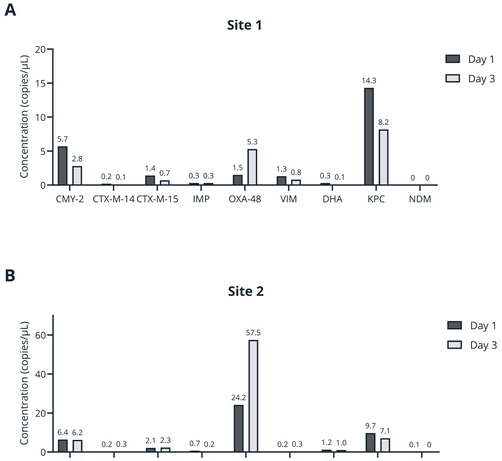


Figure 3. ARM-D Kit, β-Lactamase analysis of wastewater samples by dPCR. Wastewater samples were collected on Day 1 at Site 1 (**A**) and Site 2 (**B**) and resampled on Day 3. Samples were concentrated using Nanotrap® Microbiome A Particles, Microbiome B Particles, and Nanotrap Enhancement Reagent 3. Automated extractions were performed using the MagMAX™ Microbiome Ultra Nucleic Acid Isolation Kit on the KingFisher™ Apex. dPCR was performed on the Qiagen QIAcuity using 1µL of eluate and tested with the Streck ARM-D Kit, β-lactamase.

CONCLUSIONS

Data presented in this study demonstrate the ARM-D Kits can be optimized to support digital PCR testing when combined with manufacturer reagents. As AMR spreads, it's crucial to collaborate with the Public Health sector to promote continued monitoring of emerging antibiotic resistance mechanisms and to support expanded initiatives, such as wastewater testing for AMR using digital PCR, by ensuring that tests are updated to detect these critical mechanisms of interest.