

Supplemental Materials

Immediate Impact of Hurricane Lane on Microbiological Quality of Coastal Water in Hilo Bay,
Hawaii

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Table S1. qPCR assays targeting total bacteria and pathogens used in the study

Target bacterial species	Gene marker (target size)	Forward (F), reverse (R), and probe (P) sequences (5' - 3')	Conc. (μM)	Cycling condition	Ref.	
Total bacteria	16S rRNA (142 bp)	F: CGGTGAATACGTTCYCGG	0.2	95.0 °C for 2 min and followed with 40 cycles of 95.0 °C for 15 s, 60.0 °C for 30 s. and 72.0 °C for 30	[1]	
		R: GGWTACCTGTTACGACTT	0.2			
		P: FAM/CTTGTACAC/ZEN/ACCGCCCGTC/3IABkFQ	0.1			
Enteropathogenic <i>E. coli</i> (EPEC)	<i>eaeA</i> (102 bp)	F: CATTGATCAGGATTTCTGGTGATA	0.2	95.0 °C for 2 min and followed with 45 cycles of 95.0 °C for 15 s and 60.0 °C for 30 s.	[2]	
<i>Salmonella</i> spp.		R: CTCATGCGAAATAGCCGTTA	0.2			
		P: FAM/ATAGTCTGCCAGTATCGCCACCAATACC/TAMSp	0.05			
<i>invA</i> (116 bp)	F: AGCGTACTGGAAAGGGAAAG	0.2	[3]			
	<i>Campylobacter jejuni</i>				R: ATACCGCCAATAAGTTCACAAAG	0.2
					Probe: FAM/CGTCACCTTGATAAACTTCATCGCA/BHQ1	0.05
<i>hipO</i> (124 bp)	F: TGCACCACTGACTATGAATAACGA	0.2	[4, 5]			
	<i>L. pneumophila</i>				R: TCCAAAATCCTCACTTGCATT	0.2
					P: FAM/TGCAACCTC/ZEN/ACTAGCAAAATCCACAGCT/IABkFQ	0.05
<i>mip</i> (115 bp)	F: TTGTCTTATAGCATTGGTCCG	0.2	[6]			
					R: CCAATTGAGCGCCACTCATAG	0.2
					P: FAM/CGGAAGCAA/ZEN/TGGCTAAAGGCATGCA/IABkFQ	0.05

Table S2. qPCR assays targeting ARGs and class 1 integrase used in the study

Gene marker (target size)	Class of antibiotics or Integron	Forward (F), reverse (R), and probe (P) sequences (5' - 3')*	Conc. (μM)	Cycling condition	Ref.	
<i>ermB</i> (91 bp)	macrolide	F: GGATTCTACAAGCGTACCTTGGAA	0.2	95.0 °C for 2 min and followed with 40 cycles of 95.0 °C for 15 s, 60.0 °C (69.9 °C <i>sulI</i>) for 30 s. and 72.0 °C for 30 s	[7]	
		R: GCTGGCAGCTTAAGCAATTGCT	0.2			
		P: FAM/CACTAGGGT/ZEN/TGCTCTGCACACTCAAGTC/IABkFQ	0.1			
<i>qnrS</i> (118 bp)	quinolone	F: CGACGTGCTAACTTGCGTGA	0.2	95.0 °C for 2 min and followed with 40 cycles of 95.0 °C for 15 s, 60.0 °C (69.9 °C <i>sulI</i>) for 30 s. and 72.0 °C for 30 s	[8]	
		R: GGCATTGTTGGAAACTTGCA	0.2			
		P: FAM/AGTCATTG/ZEN/AACAGGGTGA/IABkFQ	0.1			
<i>tetO</i> (171 bp)	tetracycline	F: ACGGARAGTTATTGTATACC	0.2	95.0 °C for 2 min and followed with 40 cycles of 95.0 °C for 15 s, 60.0 °C (69.9 °C <i>sulI</i>) for 30 s. and 72.0 °C for 30 s	[9]	
		R: TGGCGTATCTATAATGTTGAC	0.2			
		P: FAM/CGTAGATGA/ZEN/AGGCACAAACAAGGAC/IABkFQ	0.1			
<i>tetM</i> (88 bp)		F: GGTTTCTCTGGATACTTAAATCAATCR	0.2		[10]	
		R: CCAACCATAYAATCCTTGTTCRC	0.2			
		P: FAM/ATGCAGTTA/ZEN/TGGARGGGATACGCTATGGY/IABkFQ	0.1			
<i>vanA</i> (65 bp)	vancomycin	F: CTGTGAGGTCGGTTGTGCG	0.2	95.0 °C for 2 min and followed with 40 cycles of 95.0 °C for 15 s, 60.0 °C (69.9 °C <i>sulI</i>) for 30 s. and 72.0 °C for 30 s	[11]	
		R: TTTGGTCCACCTCGCCA	0.2			
		P: FAM/CAACTAACG/ZEN/CGGCACTGTTCCAAT/IABkFQ	0.1			
<i>bla_{TEM}</i> (85 bp)	β-lactam	F: CACTATTCTCAGAACATGACTTGGT	0.1	95.0 °C for 2 min and followed with 40 cycles of 95.0 °C for 15 s, 60.0 °C (69.9 °C <i>sulI</i>) for 30 s. and 72.0 °C for 30 s	[12]	
		R: TGCATAATTCTCTTACTGTCATG	0.1			
		P: FAM/CCAGTCACA/ZEN/GAAAAGCATCTTACGG/IABkFQ	0.05			
<i>sulI</i> (163 bp)	sulfonamide	F: CGCACCGGAAACATCGCTGCAC	0.2	95.0 °C for 2 min and followed with 40 cycles of 95.0 °C for 15 s, 60.0 °C (69.9 °C <i>sulI</i>) for 30 s. and 72.0 °C for 30 s	[13]	
		R: TGAAGTTCCGCCGCAAGGCTCG	0.2			
		P: FAM/TTCTTGGGC/ZEN/GCCACCGTTGGCCTT/IABkFQ	0.1			
<i>intI1</i> (60 bp)	integrase	F: GCCTTGATGTTACCCGAGAG	0.2	95.0 °C for 2 min and followed with 40 cycles of 95.0 °C for 15 s, 60.0 °C (69.9 °C <i>sulI</i>) for 30 s. and 72.0 °C for 30 s	[14]	
		R: GATCGGTGCGAATGCGTGT	0.2			
		P: FAM/ATT CCTGGC/ZEN/CGTGGTTCTGGGTTTT/IABkFQ	0.1			

References

1. Suzuki, M. T.; Taylor, L. T.; DeLong, E. F., Quantitative analysis of small-subunit rRNA genes in mixed microbial populations via 5'-nuclease assays. *Applied and environmental microbiology* 2000, 66, (11), 4605-4614.
2. Nielsen, E. M.; Andersen, M. T., Detection and characterization of verocytotoxin-producing *Escherichia coli* by automated 5' nuclease PCR assay. *Journal of clinical microbiology* 2003, 41, (7), 2884-2893.
3. Kasturi, K. N.; Drgon, T., Real-time PCR method for detection of *Salmonella* spp. in environmental samples. *Applied and environmental microbiology* 2017, 83, (14).
4. LaGier, M. J.; Joseph, L. A.; Passaretti, T. V.; Musser, K. A.; Cirino, N. M., A real-time multiplexed PCR assay for rapid detection and differentiation of *Campylobacter jejuni* and *Campylobacter coli*. *Molecular and cellular probes* 2004, 18, (4), 275-282.
5. Vondrakova, L.; Pazlarova, J.; Demnerova, K., Detection, identification and quantification of *Campylobacter jejuni*, *coli* and *lari* in food matrices all at once using multiplex qPCR. *Gut pathogens* 2014, 6, (1), 1-9.
6. Benitez, A. J.; Winchell, J. M., Clinical application of a multiplex real-time PCR assay for simultaneous detection of *Legionella* species, *Legionella pneumophila*, and *Legionella pneumophila* serogroup 1. *Journal of clinical microbiology* 2013, 51, (1), 348-351.
7. Böckelmann, U.; Dörries, H.-H.; Ayuso-Gabella, M. N.; de Marçay, M. S.; Tandoi, V.; Levantesi, C.; Masciopinto, C.; Van Houtte, E.; Szewzyk, U.; Wintgens, T., Quantitative PCR monitoring of antibiotic resistance genes and bacterial pathogens in three European artificial groundwater recharge systems. *Appl. Environ. Microbiol.* 2009, 75, (1), 154-163.
8. Colomer-Lluch, M.; Jofre, J.; Muniesa, M., Quinolone resistance genes (*qnrA* and *qnrS*) in bacteriophage particles from wastewater samples and the effect of inducing agents on packaged antibiotic resistance genes. *Journal of Antimicrobial Chemotherapy* 2014, 69, (5), 1265-1274.
9. Aminov, R.; Garrigues-Jeanjean, N.; Mackie, R. I., Molecular ecology of tetracycline resistance: development and validation of primers for detection of tetracycline resistance genes encoding ribosomal protection proteins. *Appl. Environ. Microbiol.* 2001, 67, (1), 22-32.
10. Peak, N.; Knapp, C. W.; Yang, R. K.; Hanfelt, M. M.; Smith, M. S.; Aga, D. S.; Graham, D. W., Abundance of six tetracycline resistance genes in wastewater lagoons at cattle feedlots with different antibiotic use strategies. *Environmental Microbiology* 2007, 9, (1), 143-151.
11. Volkmann, H.; Schwartz, T.; Bischoff, P.; Kirchen, S.; Obst, U., Detection of clinically relevant antibiotic-resistance genes in municipal wastewater using real-time PCR (TaqMan). *Journal of microbiological methods* 2004, 56, (2), 277-286.
12. Lachmayr, K. L.; Kerkhof, L. J.; DiRienzo, A. G.; Cavanaugh, C. M.; Ford, T. E., Quantifying Nonspecific TEM β-Lactamase (β-bla_{TEM}</math>) Genes in a Wastewater Stream. *Applied and Environmental Microbiology* 2009, 75, (1), 203.
13. Pei, R.; Kim, S.-C.; Carlson, K. H.; Pruden, A., Effect of river landscape on the sediment concentrations of antibiotics and corresponding antibiotic resistance genes (ARG). *Water research* 2006, 40, (12), 2427-2435.
14. Barraud, O.; Baclet, M.-C.; Denis, F.; Ploy, M.-C., Quantitative multiplex real-time PCR for detecting class 1, 2 and 3 integrons. *Journal of antimicrobial chemotherapy* 2010, 65, (8), 1642-1645.