

Table S1. Bacteria and ARG Primer List.

Macrolide (Erythromycin) Resistant Genes					
	Primer	Primer Sequence (5'-3') †	Tm ‡	PCR	Reference
Gene			(°C)	Product (bp) §	
<i>Enterococcus spp.</i>					
<i>ermB</i>	F	GAT ACC GTT TAC GAA ATT GG	58.0	364	Chen et al. 2007
	R	GAA TCG AGA CTT GAG TGT GC			
<i>msrA</i>	F	CAAATGGCACAAGCATCATC	58.0	158	Szczepanowski et al. 2009
	R	TGTGGTTTTTCAACTTCTTCCA			
<i>msrC</i>	F	TCGTTTTGTCATGAGACAAACAG	58.0	191	Beukers et al. 2015
	R	AAATTAGTCGGTTCATCTAACAG			
<i>mefA</i>	F	GGAGCTACCTGTCTGGATGG	58.0	199	Szczepanowski et al. 2009
	R	CAACCGCCGGACTAACAATA			
<i>ermQ</i>	F	CACCAACTGATATGTGGCTAG	68,60	154	Koike et al. 2009
	R	CTAGGCATGGGATGGAAGTC			
<i>ermT</i>	F	CATATAAATGAAATTTTGAG	51.0	369	Chen et al. 2007
	R	ACGATTTGTATTTAGCAACC			
Tetracycline Resistant Genes					
Target	Primer	Primer Sequence (5'-3') †	Tm‡	PCR	Reference
<i>Enterococcus spp.</i>					
<i>tetM</i>	F	GTG GAC AAA GGT ACA ACG AG	55.0	406	Ng et al. 2001
	R	CGG TAA AGT TCG TCA CAC AC			
<i>tetL</i>	F	TCG TTA GCG TGC TGT CAT TC	55.0	267	Ng et al. 2001
	R	GTA TCC CAC CAA TGT AGC CG			
<i>tetO</i>	F	AAC TTA GGC ATT CTG GCT CAC	55.0	515	Ng et al. 2001
	R	TCC CAC TGT TCC ATA TCG TCA			
<i>tetS</i>	F	CAT AGA CAA GCC GTT GAC C	55.0	667	Ng et al. 2001
	R	ATG TTT TTG GAA CGC CAG AG			

Gene			(°C)	Product (bp) [§]	
<i>E. coli</i>					
<i>tetA</i>	F	GCT ACA TCC TGC TTG CCT TC	55.0	210	Ng et al. 2001
	R	CAT AGA TCG CCG TGA AGA GG			
<i>tetB</i>	F	TTG GTT AGG GGC AAG TTT TG	55.0	659	Ng et al. 2001
	R	GTA ATG GGC CAA TAA CAC CG			
B-Lactam Resistant Genes					
Target	Primer	Primer Sequence (5'-3') [†]	Tm [‡]	PCR	Reference
Gene			(°C)	Product (bp) [§]	
<i>E. coli</i>					
<i>bla</i> CMY-2	F	GACAGCCTCTTTCTCCACA	55.0	1015	Gray et al. 2004
	R	TGGAACGAAGGCTACGTA			
<i>bla</i> CTX-M	F	ATGTGCAGYACCAGTAA	55.0	512	Cottell et al. 2013
	R	CCGCTGCCGGTYTTATC			
<i>bla</i> DHA	F	TTATCTCACACCTTTATTACTG	55.0	139	Geyer et al. 2012
	R	TATCTTTTGAGGCGGATT			
<i>bla</i> OXA-1	F	CACTTACAGGAAACTTGGGGTC G	55.0	~65	Knapp et al. 2010
	R	AGTGTGTTTAGAATGGTGATC			
Enterococcus spp. and E.coli Genes					
Target	Primer	Primer Sequence (5'-3') [†]	Tm [‡]	PCR	Reference
Gene			(°C)	Product (bp) [§]	
<i>E. coli</i>					
<i>uidA</i>	F	ATCGGCGAAATTCCATACCTG	58.0	319	Horakova 2006
	R	GTTCTGCGACGCTCACACC			

lacZ	F	ATGAAAGCTGGCTACAGGAAGG CC	58.0	264	Bej et al. 1991
	R	GGTTTATGCAGCAACGAGACGT CA			
lacY	F	ACCAGACCCAGCACCAGATAAG	58.0	463	Horakova 2008
	R	GCACCTACGATGTTTTTGACCA			
cyd	F	CCGTATCATGGTGGCGTGTGG	58.0	393	Horakova 2006
	R	GCCGGCTGAGTAGTCGTGGAAG			
<i>Enterococcus</i> spp.					
ENT	F	AGAAATTCCAAACGAACTTG	60.0	106	Farhm et al. 2003
	R	CAGTGCTCTACCTCCATCATT			

Table S2. Number of resistant isolates for Bowling Green, KY and Tampa Bay Metropolitan Area, FL urban karst groundwater systems defined by antibiotic group and resistance phenotypes.

Antibiotic Resistant Genes Associated with Resistance Phenotypes					
Enterococcus spp. Resistant Genes	Macrolide Antibiotic Resistant Gene	Bowling Green, KY number of resistant isolates for antibiotic group (n)	Bowling Green, KY number of resistant isolates for ARG (n)	Tampa Bay Metro, FL number of resistant isolates for antibiotic group (n)	Tampa Bay Metro, FL number of resistant isolates for ARG (n)
	<i>ermB</i>	492	409	11	11
	<i>msrA</i>		5		-
	<i>msrC</i>		42		-
	<i>ermQ</i>		16		-
	<i>ermT</i>		8		-
	<i>mefA</i>		12		-
	Tetracycline Antibiotic Resistant Gene				
	<i>tetM</i>	836	730	37	26
	<i>tetL</i>		85		7
	<i>tetO</i>		12		2
	<i>tetS</i>		9		2
E.coli Resistant Genes	Tetracycline Antibiotic Resistant Gene				
	<i>tetA</i>	865	475	28	12
	<i>tetB</i>		390		16
	Beta-Lactam (including 3rd generation cephalosporin) Antibiotic Resistant Gene				
	<i>bla</i> CMY-2	884	211	17	10
	<i>bla</i> CTX-M		673		5

	<i>bla</i> DHA		6		0
	<i>bla</i> OXA-1		5		2

Table S3. Number of *Enterococcus* spp. resistant isolates for Bowling Green, KY urban karst groundwater systems defined by sample site, season, antibiotic group, and resistance phenotypes.

Antibiotic Resistant Genes Associated with Resistant Phenotypes												
Season	Sample Site	<i>Enterococcus</i> spp. Resistant Genes										
		Macrolide Antibiotic Resistant Gene						Tetracycline Antibiotic Resistant Gene				
		Number of Resistant Isolates (n)	<i>erm</i> B Resistant Isolates (n)	<i>msr</i> A Resistant Isolates (n)	<i>msr</i> C Resistant Isolates (n)	<i>erm</i> Q Resistant Isolates (n)	<i>erm</i> T Resistant Isolates (n)	Number of Resistant Isolates (n)	<i>tet</i> M Resistant Isolates	<i>tet</i> L Resistant Isolates	<i>tet</i> O Resistant Isolates	<i>tet</i> S Resistant Isolates
Spring	ByPass Cave	58	5	0	4	4	0	89	28	1	0	0
	Durbin Well	58	14	0	2	2	0	90	30	0	0	0
	Barren River	53	14	0	5	0	0	89	27	1	1	2
	Carver Well Cave	33	6	2	0	0	0	83	26	0	0	2
	Lost River Rise	63	13	0	6	0	0	88	24	1	2	0
	New Spring	54	12	0	2	1	0	86	26	1	0	1
	Whiskey Run Spring	50	15	0	2	1	0	84	24	2	0	0
	Lost River Spring	51	11	0	3	0	0	92	26	2	0	0
	Payne Well	36	5	0	1	0	0	81	21	1	0	0

	Vine Well	36	16	0	4	0	0	56	23	2	1	0
Summer	ByPass Cave	58	17	2	2	4	0	89	21	3	0	0
	Durbin Well	58	14	0	0	0	0	90	17	6	1	0
	Barren River	53	10	0	0	0	0	89	21	3	0	0
	Carver Well Cave	33	12	0	0	0	0	83	19	5	0	0
	Lost River Rise	63	15	0	1	0	0	88	19	3	1	1
	New Spring	54	11	0	0	1	0	86	18	6	0	0
	Whiskey Run Spring	50	9	0	2	1	0	84	23	1	0	0
	Lost River Spring	51	10	0	1	0	2	92	26	0	0	0
	Payne Well	36	4	0	0	2	4	81	20	3	0	2
	Vine Well	36	10	0	0	1	0	56	18	4	0	0
Fall	ByPass Cave	58	25	0	0	0	0	89	21	4	1	0
	Durbin Well	58	16	0	0	0	0	90	19	5	2	0
	Barren River	53	16	0	0	0	0	89	23	3	0	0
	Carver Well Cave	33	10	0	0	0	0	83	19	5	0	0
	Lost River Rise	63	22	0	0	0	2	88	19	6	0	0
	New Spring	54	23	1	0	0	0	86	22	4	0	0
	Whiskey Run Spring	50	12	0	2	0	0	84	25	0	1	0
	Lost River Spring	51	20	0	0	0	0	92	19	7	0	0
	Payne Well	36	9	0	3	0	0	81	22	2	0	0
	Vine Well	36	-	-	-	-	-	56	2	-	-	-

Winter	ByPass Cave	58	4	0	0	0	0	89	10	0	0	0
	Durbin Well	58	2	0	1	1	0	90	10	0	0	0
	Barren River	53	14	0	0	0	0	89	6	0	0	1
	Carver Well Cave	33	3	0	0	0	0	83	6	0	0	0
	Lost River Rise	63	4	0	0	0	0	88	10	2	0	0
	New Spring	54	4	0	1	0	0	86	8	0	0	0
	Whiskey Run Spring	50	2	0	0	0	0	84	8	0	0	0
	Lost River Spring	51	2	0	1	0	0	92	8	2	2	0
	Payne Well	36	4	0	0	0	0	81	10	0	0	0
	Vine Well	36	4	0	0	0	0	56	6	0	0	0

Table S4. Number of *E. coli* resistant isolates for Bowling Green, KY urban karst groundwater systems defined by sample site, season, antibiotic group, and resistance phenotypes.

Antibiotic Resistant Genes Associated with Resistant Phenotypes									
Season	Sample Site	<i>E.coli</i> Antibiotic Resistant Genes							
		Tetracycline Antibiotic Resistant Gene			Beta-Lactam Antibiotic Resistant Gene				
		Number of Resistant Isolates (n)	<i>tetA</i> Resistant Isolates (n)	<i>tetB</i> Resistant Isolates (n)	Number of Resistant Isolates (n)	CMY-2 Resistant Isolates (n)	CTX-M Resistant Isolates (n)	<i>blaD</i> HA Resistant Isolates (n)	<i>blaOX</i> A-1 Resistant Isolates (n)
Spring	ByPass Cave	100	9	22	97	6	45	0	1
	Durbin Well	97	6	22	106	0	37	2	0

	Barren River	108	16	16	126	8	36	0	0
	Carver Well Cave	76	13	10	84	0	26	0	0
	Lost River Rise	94	11	17	127	7	35	0	0
	New Spring	92	9	13	145	3	45	0	0
	Whiskey Run Spring	93	21	8	64	6	16	0	0
	Lost River Spring	86	15	13	76	14	11	0	0
	Payne Well	66	9	8	18	-	-	0	-
	Vine Well	53	17	7	52	7	19	0	1
Summer	ByPass Cave	100	21	10	97	10	15	0	0
	Durbin Well	97	19	14	106	1	41	1	1
	Barren River	108	17	15	126	5	17	0	0
	Carver Well Cave	76	14	11	84	3	20	0	0
	Lost River Rise	94	15	16	127	8	21	0	0
	New Spring	92	15	16	145	4	39	0	1
	Whiskey Run Spring	93	14	14	64	14	3	2	0
	Lost River Spring	86	13	13	76	2	22	0	0
	Payne Well	66	15	9	18	2	9	0	0
	Vine Well	53	15	8	52	11	4	0	0
Fall	ByPass Cave	100	14	13	97	13	3	0	0
	Durbin Well	97	17	14	106	8	13	0	0
	Barren River	108	24	7	126	7	41	0	0
	Carver Well Cave	76	16	7	84	9	22	0	0
	Lost River Rise	94	14	12	127	11	34	0	1
	New Spring	92	18	11	145	10	30	0	0
	Whiskey Run Spring	93	20	10	64	12	11	0	0
	Lost River Spring	86	15	13	76	13	6	0	0

	Payne Well	66	9	12	18	2	5	0	0
	Vine Well	53	1	1	52	1	3	0	0
Winter	ByPass Cave	100	3	8	97	1	5	0	0
	Durbin Well	97	4	1	106	0	2	0	0
	Barren River	108	5	8	126	4	8	0	0
	Carver Well Cave	76	3	2	84	2	2	0	0
	Lost River Rise	94	8	1	127	0	8	0	0
	New Spring	92	6	4	145	2	11	0	0
	Whiskey Run Spring	93	5	1	64	-	-	-	-
	Lost River Spring	86	2	2	76	4	4	0	0
	Payne Well	66	3	1	18	-	-	-	-
	Vine Well	53	4	0	52	0	5	0	0