

Supplementary Materials

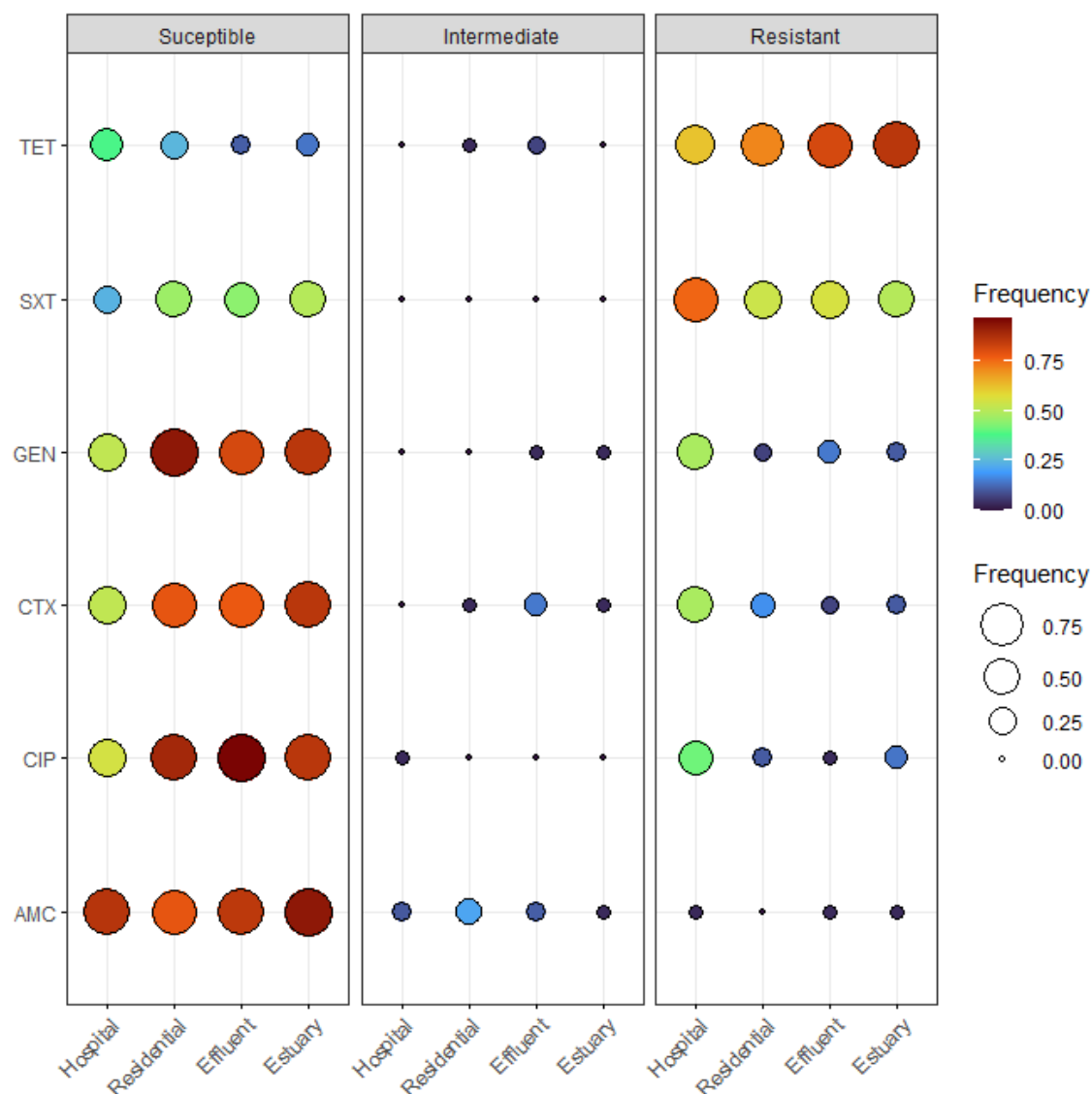


Figure S1. Frequency of resistance of ampicillin-resistant *E. coli* isolates at each site to the following antibiotics: cefotaxime (CTX), ciprofloxacin (CIP), amoxicillin + clavulanate (AMC), gentamicin (GEN), tetracycline (TET), and trimethoprim-sulfamethoxazole (SXT). Ampicillin is not shown in the plot as all isolates tested were fully resistant to ampicillin, the selective antibiotic in the isolation procedure. The size and color of the circle denotes frequency (larger circle and red color denotes higher frequency).

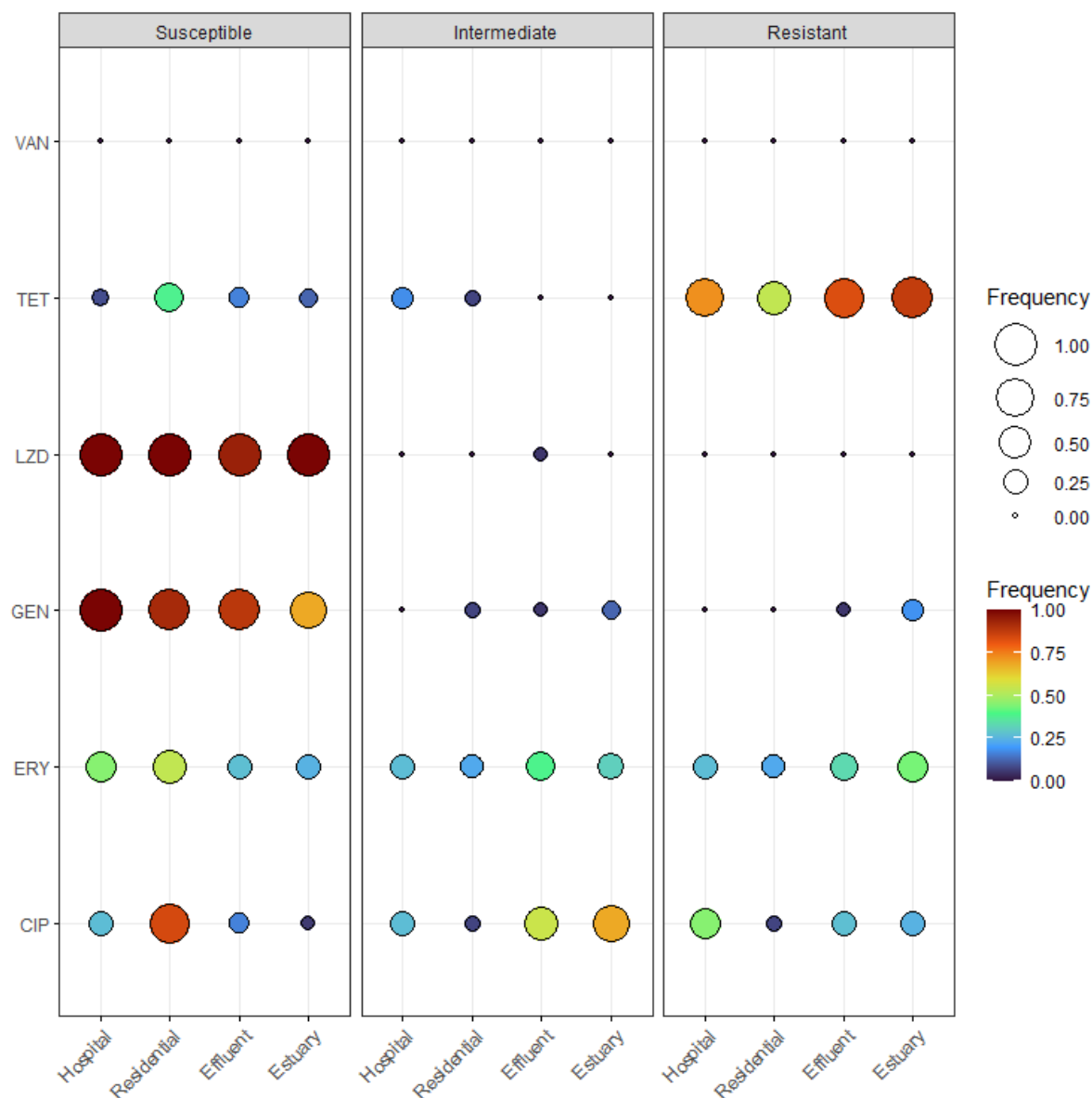


Figure S2. Frequency of resistance of ampicillin-resistant *Enterococcus* isolates to the following antibiotics: ciprofloxacin (CIP), erythromycin (ERY), gentamycin (GEN), linezolid (LZD), tetracycline (TET), and vancomycin (VAN). Ampicillin is not shown in the plot as all isolates tested were fully resistant to ampicillin, the selective antibiotic in the isolation procedure. The size and color of the circle denotes frequency (larger circle and red color denotes higher frequency).

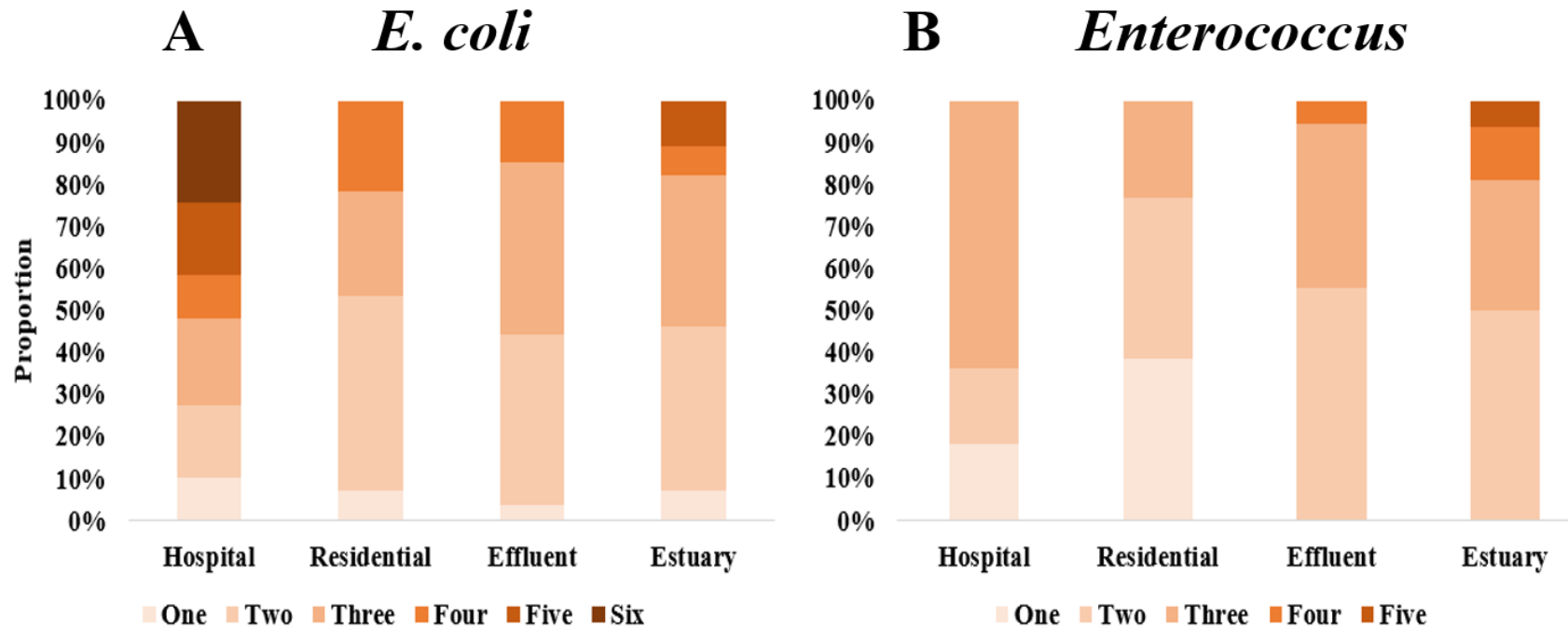


Figure S3. Proportion of isolates resistant to multiple classes of antibiotics among *E. coli* (panel A) and *Enterococcus* (panel B) isolates at each site. Sample size per site for *E. coli* and *Enterococcus* is as follows: Hospital influent (n = 27 and 11), residential influent (n=29 and 13), effluent (n = 28 and 18) and estuary (n=28 and 16). Color scale indicates number of resistance phenotypes: darker colors indicate more resistance phenotypes. The height of each block within a column indicates the proportion of isolates with a given number of resistance phenotypes.

Table S1. Statistical results for testing for differences in log₁₀ mean total and ampicillin-resistant *E. coli* and enterococci concentrations among sampling sites. Significant differences (p-values <0.05) are bolded. p-values associated with Tuckey HSD test are shown in the last column.

FIB	Total		Ampicillin-resistant	
	F statistic	P- value	F statistic	P- value
<i>E. coli</i>	23.13	<0.001	9.4	0.002
Enterococci	7.01	0.006	0.5	0.687

Table S2. Significance of pairwise comparisons of $\log_{10}$ mean concentrations of ampR *E. coli* by site. Significant differences between sites (p-values <0.05) are bolded. No significant difference among $\log_{10}$ mean concentrations of ampicillin-resistant enterococci by site were observed, therefore a post-hoc test was not necessary.

Pairwise comparison	<i>E. coli</i>
	Ampicillin-resistant
Residential-Hospital	0.047
Residential-Effluent	0.014
Residential-Estuary	0.001
Hospital-Effluent	0.892
Hospital-Estuary	0.196
Effluent-Estuary	0.505

Table S3. Frequency of susceptibility of ampicillin-resistant *E. coli* isolates at each site to the following antibiotics: cefotaxime (CTX), ciprofloxacin (CIP), amoxicillin + clavulanate (AMC), gentamicin (GEN), tetracycline (TET), and trimethoprim sulfamethoxazole (SXT). Ampicillin is not shown as all isolates tested were fully resistant to ampicillin, the selective antibiotic in the isolation procedure.

Antibiotic	Susceptible	Intermediate	Resistant
AMC			
Effluent	0.85	0.11	0.04
Estuary	0.92	0.04	0.04
Hospital	0.86	0.10	0.04
Residential	0.79	0.21	0.00
CIP			
Effluent	0.96	0.00	0.04
Estuary	0.86	0.00	0.14
Hospital	0.55	0.04	0.41
Residential	0.89	0.00	0.11
CTX			
Effluent	0.78	0.15	0.07
Estuary	0.85	0.04	0.11
Hospital	0.52	0.00	0.48
Residential	0.79	0.04	0.18
GEN			
Effluent	0.81	0.04	0.15
Estuary	0.85	0.04	0.11
Hospital	0.52	0.00	0.48
Residential	0.93	0.00	0.07
SXT			
Effluent	0.44	0.00	0.56
Estuary	0.50	0.00	0.50
Hospital	0.24	0.00	0.76
Residential	0.46	0.00	0.54
TET			
Effluent	0.11	0.07	0.81
Estuary	0.14	0.00	0.86
Hospital	0.38	0.00	0.62
Residential	0.25	0.04	0.71

Table S4. Frequency of susceptibility of ampicillin-resistant *Enterococcus* isolates to the following antibiotics: ciprofloxacin (CIP), erythromycin (ERY), gentamycin (GEN), linezolid (LZD), tetracycline (TET), and vancomycin (VAN). Ampicillin is not shown as all isolates tested were fully resistant to ampicillin, the selective antibiotic in the isolation procedure.

Antibiotic	Susceptible	Intermediate	Resistant
CIP			
Effluent	0.17	0.55	0.28
Estuary	0.06	0.69	0.25
Hospital	0.27	0.27	0.46
Residential	0.84	0.08	0.08
ERY			
Effluent	0.28	0.39	0.33
Estuary	0.25	0.31	0.44
Hospital	0.46	0.27	0.27
Residential	0.54	0.23	0.23
GEN			
Effluent	0.88	0.06	0.06
Estuary	0.69	0.12	0.19
Hospital	1.00	0.00	0.00
Residential	0.93	0.07	0.00
LZD			
Effluent	0.94	0.06	0.00
Estuary	1.00	0.00	0.00
Hospital	1.00	0.00	0.00
Residential	1.00	0.00	0.00
TET			
Effluent	0.17	0.00	0.83
Estuary	0.12	0.00	0.88
Hospital	0.09	0.18	0.73
Residential	0.38	0.08	0.54
VAN			
Effluent	1.00	0.00	0.00
Estuary	1.00	0.00	0.00
Hospital	1.00	0.00	0.00
Residential	1.00	0.00	0.00

Table S5. Akaike's information criterion (AIC) values for logistic regression models applied to *E. coli* and *Enterococcus* datasets for susceptibility to additional antibiotics. AIC for the preferred model is in bold.

Model AIC	<i>E. coli</i>	<i>Enterococcus</i>
Additive model (Site+Antibiotic)	599.79	233.33
Interaction model (Site*Antibiotic)	593.47	243.96
Interaction model with random effect (Site*Antibiotic + 1 SampleID)	958.70	479.90

Table S6. Influence of site and antibiotics on the log odds of *E. coli* resistance frequency to additional antibiotics. The model's intercept corresponds to site [residential wastewater], antibiotic [GEN] or their interaction [residential wastewater] * [GEN]. Significant relationships are bolded (p-values <0.05). The significant p-value for the intercept indicates that the predicted resistance is significantly different than 0.

Resistance			
Predictors	Log-Odds	CI	p
(Intercept: Residential*GEN)	-2.56 ***	-4.39 – -1.36	<0.001
Site [Hospital]	2.50 **	1.06 – 4.43	0.002
Site [Effluent]	0.82	-0.91 – 2.86	0.371
Site [Estuary]	0.44	-1.43 – 2.53	0.641
Antibiotic [TET]	3.48 ***	2.01 – 5.46	<0.001
Antibiotic [CTX]	1.04	-0.60 – 3.05	0.240
Antibiotic [SXT]	2.71 **	1.27 – 4.65	0.001
Antibiotic [CIP]	0.44	-1.43 – 2.53	0.641
Site [Hospital] *Antibiotic [TET]	-2.92 **	-5.11 – -1.08	0.003
Site [Effluent] *Antibiotic [TET]	-0.25	-2.61 – 1.91	0.823
Site [Estuary] *Antibiotic [TET]	0.43	-2.00 – 2.75	0.714
Site [Hospital] *Antibiotic [CTX]	-1.04	-3.26 – 0.90	0.313
Site [Effluent] *Antibiotic [CTX]	-1.82	-4.54 – 0.58	0.153
Site [Estuary] *Antibiotic [CTX]	-1.04	-3.62 – 1.36	0.401
Site [Hospital] *Antibiotic [SXT]	-1.49	-3.69 – 0.37	0.137
Site [Effluent] *Antibiotic [SXT]	-0.74	-3.00 – 1.29	0.488
Site [Estuary] *Antibiotic [SXT]	-0.59	-2.90 – 1.55	0.591
Site [Hospital] *Antibiotic [CIP]	-0.72	-3.03 – 1.41	0.507
Site [Effluent] *Antibiotic [CIP]	-1.95	-5.42 – 0.80	0.192
Site [Estuary] *Antibiotic [CIP]	-0.12	-2.69 – 2.37	0.926
Observations	560		
R ² Tjur	0.314		

* p<0.05 ** p<0.01 *** p<0.001

Table S7. Post-hoc comparisons of the logistic model on influence of site and antibiotics on the log odds of *E. coli* resistance frequency to additional antibiotics by (A) site and (B) antibiotic.

Significant relationships are bolded (p-values <0.05), marginally significant relationships (p-values = 0.05) are italicized.

(A)

Site	Estimate	Std. Error	z value	Pr(> z)
Hospital - Residential	2.4960	0.8225	3.034	0.013
Effluent - Residential	0.8157	0.8157	0.894	0.804
Estuary - Residential	0.4447	0.9549	0.466	0.966
Effluent - Hospital	-1.6802	0.6569	-2.558	0.050
Estuary - Hospital	-2.0513	0.7151	-2.868	0.021
Estuary - Effluent	-0.3711	0.8166	-0.454	0.968

(B)

Antibiotic	Estimate	Std. Error	z value	Pr(> z)
CTX - GEN	1.0389	0.8843	1.175	0.760
SXT - GEN	2.7081	0.8259	3.279	0.009
CIP - GEN	0.4447	0.9549	0.466	0.990
TET - GEN	3.4812	0.8447	4.121	< 0.001
SXT - CTX	1.6692	0.6221	2.683	<i>0.054</i>
CIP - CTX	-0.5942	0.7854	-0.757	0.941
TET - CTX	2.4423	0.6469	3.775	0.002
CIP - SXT	-2.2634	0.719	-3.148	0.014
TET - SXT	0.7732	0.5644	1.370	0.640

Table S8. Influence of site and antibiotic on the log odds of *Enterococcus* resistance frequency to additional antibiotics. The model's intercept corresponds to site [residential wastewater] and antibiotic [GEN]. Significant relationships are bolded (p-values <0.05). The significant p-value for the intercept indicates that the predicted resistance is significantly different than 0.

Resistance			
<i>Predictors</i>	<i>Log Odds</i>	<i>CI</i>	<i>p</i>
(Intercept: Residential+GEN)	-3.68***	-5.12 – -2.48	<0.001
Site [Hospital]	1.06	-0.00 – 2.17	0.052
Site [Effluent]	1.13*	0.18 – 2.15	0.022
Site [Estuary]	1.50**	0.53 – 2.54	0.002
Antibiotic [TET]	3.93***	2.81 – 5.29	<0.001
Antibiotic [CIP]	1.59	0.48 – 2.91	0.009
Antibiotic [ERY]	1.94**	0.86 – 3.24	0.001
Observations	232		
R ² Tjur	0.310		

* p<0.05 ** p<0.01 *** p<0.001

Table S9. Post-hoc comparisons of the logistic model on influence of site and antibiotics on the log odds of *Enterococcus* resistance frequency to additional antibiotics by (A) site and (B) antibiotic. Significant relationships are bolded (p-values <0.05).

(A)

Site	Estimate	Std. Error	z value	Pr(> z)
Hospital - Residential	1.06037	0.55176	1.922	0.207
Effluent - Residential	1.13013	0.49974	2.261	0.100
Estuary - Residential	1.50016	0.51083	2.937	0.009
Effluent - Hospital	0.06976	0.47453	0.147	0.999
Estuary - Hospital	0.43979	0.48133	0.914	0.680
Estuary - Effluent	0.37003	0.41909	0.883	0.681

(B)

Antibiotic	Estimate	Std. Error	z value	Pr(> z)
TET - GEN	3.9303	0.6207	6.332	< 0.001
CIP - GEN	1.5872	0.6048	2.624	0.0410
ERY - GEN	1.9369	0.5957	3.251	0.006
CIP - TET	-2.343	0.4508	-5.198	< 0.001
ERY - TET	-1.9934	0.4362	-4.57	< 0.001
ERY - CIP	0.3496	0.4196	0.833	0.834

Table S10. Antibiotic resistant profiles observed in *E. coli* isolates. Sample size (isolate number) per site is as follows: Hospital influent (n = 27), residential influent (n=29), effluent (n = 28) and estuary (n=28).

	Site				
Combination	Hospital	Residential	Effluent	Estuary	Total
AMP	3	2	1	2	8
AMP + GEN	0	0	1	0	1
AMP + CTX	1	0	0	1	2
AMP + TET	2	10	9	9	30
AMP + SXT	2	3	1	1	7
AMP + GEN + CTX	1	0	0	0	1
AMP + GEN + SXT	0	0	1	0	1
AMP + CTX + TET	0	1	0	1	2
AMP + CTX + SXT	2	1	1	0	4
AMP + TET + SXT	3	5	9	9	26
AMP + GEN + CTX + SXT	2	2	0	0	4
AMP + GEN + CIP + TET	0	0	1	0	1
AMP + CTX + AMC + TET	0	0	0	1	1
AMP + GEN + TET + STX	0	0	1	0	1
AMP + CIP + TET + SXT	1	3	0	1	5
AMP + CTX + TET + SXT	0	1	1	0	2
AMP + AMC + TET + SXT	0	0	1	0	1
AMP + GEN + CIP + TET + STX	4	0	0	3	7
AMP + CTX + AMC + TET + SXT	1	0	0	0	1
AMP + GEN + CIP + AMC + TET + STX	7	0	0	0	7

Table S11. Antibiotic resistant profiles observed in *Enterococcus* spp. isolates. Sample size (isolate number) is as follows: Hospital influent (n = 11), residential influent (n= 13), effluent (n =18) and estuary (n=16).

	Site				
Profile	Hospital	Residential	Effluent	Estuary	Total
AMP	2	5	0	0	7
AMP + CIP	1	0	0	0	1
AMP + TET	1	4	7	6	18
AMP + ERY	0	1	3	2	6
AMP + CIP + TET	4	1	3	2	10
AMP + TET + ERY	3	2	2	3	10
AMP + GEN + CIP + TET	0	0	0	1	1
AMP + GEN + TET + ERY	0	0	1	1	2
AMP + GEN + CIP + TET + ERY	0	0	0	1	1

Table S12. Antibiotics chosen for the Kirby-Bauer disc diffusion assay for *E. coli*.

Antibiotic	Class	Disk Content
Ampicillin	Penicillins	10 µg
Gentamycin	Aminoglycosides	10 µg
Amoxicillin with clavulanate	Beta-Lactamase inhibitor combinations	20/10 µg
Cefotaxime	Cephem	30 µg
Ciprofloxacin	Fluoroquinolones	5 µg
Trimethoprim-sulfamethoxazole	Folate pathway inhibitors	1.25/23.75 µg
Tetracycline	Tetracyclines	30 µg

Table S13. Antibiotics chosen for the Kirby-Bauer disc diffusion assay for *Enterococcus* spp.

Antibiotic	Class	Disk Content
Ampicillin	Penicillins	10 µg
Gentamycin	Aminoglycosides	120 µg
Linezolid	Oxazolidones	30 µg
Vancomycin	Glycopeptides	30 µg
Tetracycline	Tetracyclines	30 µg
Erythromycin	Macrolides	15 µg
Ciprofloxacin	Quinolones and Fluoroquinolones	5 µg