



**Figure S1.** Technological scheme of Wastewater Treatment Plant

**Table S1.** Oligonucleotide sequences and product size of primers for identifying enterococci.

Name	Gene	Sequences (5'-3')	Lenght (bp)	References
<i>Enterococcus</i>	<i>tuf</i>	TACTGACAAACCATTTCATGATG AACTTCGTCACCAACGCGAAC	112	Ke et al., [1]
<i>Enterococcus</i>	<i>sodA</i>	TCA ACC GGG GAG GGT ATT ACT AGC GAT TCC GG	733	Deasy et al., [2]
<i>E. faecalis</i>	<i>ddl</i>	ATCAAGTACAGTTAGTCTTTATTAG ACGATTCAAAGCTAACTGAATCAGT	941	Kariyama et al., [3]
<i>E. faecium</i>	<i>ddl</i>	TTGAGGCAGACCAGATTGACG TATGACAGCGACTCCGATTCC	658	Cheng et al., [4]
<i>E. durans</i>	<i>ddl</i>	CCTACTGATATTAAGACAGCG TAATCCTAAGATAGGTGTTTG	295	Jackson et al., [5]
<i>E. hirae</i>	<i>ddl</i>	CTTTCTGATATGGATGCTGTC TAAATTCTTCCTTAAATGTTG	187	
<i>E. avium</i>	<i>ddl</i>	GCTGCGATTGAAAAATATCCG AAGCCAATGATCGGTGTTTT	368	
<i>E. gallinarum</i>	<i>vanC1</i>	GGTATCAAGGAAACCTC CTTCCGCCATCATAGCT	822	Dutka-Malen et al., [6]
<i>E. casseliflavus</i>	<i>vanC2/C3</i>	CGGGGAAGATGGCAGTAT CGCAGGGACGGTGATTTT	484	Kariyama et al., [3]
Control	<i>rrs</i> (16S rRNA)	GGATTAGATACCCTGGTAGTCC TCGTTGCGGGACTTAACCCAAC	320	Van de Klundert and Vliegenthart et al., [7]

**Table S2.** Sequences of oligonucleotides and primers for identifying vancomycin resistance genes in enterococci.

Gene	Sequences (5'-3')	Lenght (bp)	References
<i>vanA</i>	CATGAATAGAATAAAAAGTTGCAATA CCCCTTTAACGCTAATACGATCAA	1.030	
<i>vanB</i>	GTGACAAACCGGAGGCGAGGA CCGCCATCCTCTGCAAAAAA	433	Kariyama et al., [3]
<i>vanC1</i>	GGTATCAAGGAAACCTC CTTCCGCCATCATAGCT	822	
<i>vanC2/C3</i>	CGGGGAAGATGGCAGTAT CGCAGGGACGGTGATTTT	484	

**Table S3.** Sequences of oligonucleotides and primers for identifying virulence factors in enterococci

Virulence factor	Gene	Sequences (5'-3')	Length (bp)	References
Cytolysin	<i>cylA</i>	Cyl1- <b>ACTCGGGGATTGATAGGC</b> Cyl2- <b>GCTGCTAAAGCTGCGCTT</b>	688	Vankerckhoven et al., [8]
Hyaluronidase	<i>hyl</i>	Hyl1- <b>ACAGAAGAGCTGCAGGAAATG</b> Hyl2- <b>GACTGACGTCCAAGTTTCCAA</b>	276	
Adhesion colaagen	<i>ace</i>	Ace1- <b>AAAGTAGAATTAGATCCACAC</b> Ace2- <b>TCTATCACATTCGGTTGCG</b>	320	Mannu et al., [9]
Antigen A	<i>efaA</i>	efaA1- <b>CGTGAGAAAGAAAATGGAGGA</b> efaA2- <b>CTACTAACACGTCACGAATG</b>	499	
Gelatinase	<i>gelE</i>	gelE1- <b>AGTTCATGTCTATTTTCTTCAC</b> gelE2- <b>CTTCATTATTTACACGTTG</b>	402	
Aggregation substance	<i>as</i>	AS1- <b>CCAGTAATCAGTCCAGAAACAACC</b> AS2- <b>TAGCTTTTTTCATTCTTGTTGTT</b>	406	
Enterococcal surface protein	<i>esp</i>	Esp1- <b>TTACCAAGATGGTTCTGTAGGCAC</b> Esp2- <b>CCAAGTATACTTAGCATCTTTTGG</b>	913	Shankar et al., [10]
Sex pheromones	<i>cpd</i>	Cpd1- <b>TGGTGGGTTATTTTCAATTC</b> Cpd2- <b>TACGGCTCTGGCTACTA</b>	782	Eaton and Gasson [11]
Sex pheromones	<i>cob</i>	Cob1- <b>AACATTCAGCAAACAAAGC</b> Cob2- <b>TTGTCATAAAGAGTGGTCAT</b>	1405	
Sex pheromones	<i>ccf</i>	Ccf1- <b>GGGAATTGAGTAGTGAAGAAG</b> Ccf2- <b>AGCCGCTAAAATCGGTAAAAT</b>	543	

**Table S4–Part A.** Correlations between the analyzed physicochemical parameters, total enterococcus counts, different species and the number of MDR and virulent strains (Spearman’s rank correlation coefficient; marked in red are values with  $p < 0.05$ ).

Variable	Season	Sampling sites	Temperature	pH	Dissolved oxygen (mg O <sub>2</sub> L <sup>-1</sup> )	Enterococci (log cfu·100mL <sup>-1</sup> )
Sampling sites	0.000					
Temperature	0.374	-0.086				
pH	0.337	<b>-0.703</b>	<b>0.530</b>			
Dissolved oxygen (mg O <sub>2</sub> L <sup>-1</sup> )	-0.249	<b>0.632</b>	-0.200	<b>-0.580</b>		
Enterococci (log cfu·100mL <sup>-1</sup> )	0.064	<b>-0.886</b>	0.162	<b>0.792</b>	<b>-0.606</b>	
Other <i>Enterococcus</i> spp	0.035	-0.146	-0.023	0.220	0.294	0.199
<i>E. faecalis</i>	-0.304	<b>-0.613</b>	-0.183	<b>0.466</b>	-0.204	<b>0.530</b>
<i>E. faecium</i>	0.179	<b>-0.620</b>	0.058	<b>0.657</b>	<b>-0.495</b>	<b>0.633</b>
<i>E. durans</i>	-0.088	-0.139	0.047	0.268	-0.007	0.139
<i>E. avium</i>	-0.147	<b>-0.599</b>	-0.006	0.269	-0.123	<b>0.532</b>
<i>E. hiare</i>	-0.163	-0.184	-0.209	0.185	-0.043	0.179
<i>E. gallinarum</i>	0.046	0.181	-0.050	-0.084	-0.245	-0.063
<i>E. casseliflavus/flavescens</i>	0.308	-0.324	-0.118	0.227	<b>-0.575</b>	0.232
AMP	0.210	<b>-0.680</b>	-0.015	<b>0.456</b>	<b>-0.581</b>	<b>0.506</b>
IPM	0.158	<b>-0.715</b>	0.046	<b>0.569</b>	<b>-0.541</b>	<b>0.576</b>
GEN	0.095	<b>-0.681</b>	0.096	<b>0.572</b>	<b>-0.496</b>	<b>0.534</b>
S	-0.023	<b>-0.693</b>	-0.046	<b>0.605</b>	-0.317	<b>0.619</b>
TEC	-0.021	-0.034	0.054	0.235	0.006	0.229
VAN	-0.237	-0.292	-0.105	0.347	0.129	0.397
QD	-0.069	<b>-0.588</b>	-0.103	<b>0.572</b>	-0.286	<b>0.582</b>
TGC	0.282	<b>-0.678</b>	-0.085	<b>0.631</b>	<b>-0.507</b>	<b>0.673</b>
LZD	0.208	<b>-0.518</b>	-0.094	<b>0.550</b>	-0.295	<b>0.509</b>

NIT	0.344	-0.554	0.148	0.713	-0.388	0.591
W5	0.168	-0.639	0.014	0.670	-0.354	0.646
CIP	0.096	-0.671	-0.003	0.671	-0.377	0.633
DO	-0.129	-0.012	-0.205	0.092	0.381	0.053
<i>vanA</i>	0.088	0.092	-0.234	-0.324	-0.049	-0.131
<i>vanB</i>	-0.198	-0.256	-0.201	0.021	-0.136	0.198
<i>vanC1</i>	-0.178	-0.259	0.162	0.350	-0.046	0.331
<i>vanC2/VC3</i>	0.140	-0.497	0.343	0.555	-0.600	0.527
<i>cylA</i>	-0.081	-0.428	-0.099	0.320	-0.400	0.365
<i>hyl</i>	0.044	-0.267	0.233	0.486	0.051	0.304
<i>ace</i>	-0.342	-0.262	-0.047	0.337	0.058	0.338
<i>efaA</i>	-0.342	-0.436	-0.183	0.381	-0.130	0.422
<i>gelE</i>	-0.422	-0.306	-0.037	0.278	-0.043	0.254
<i>as</i>	-0.227	-0.556	-0.019	0.396	-0.237	0.446
<i>esp</i>	-0.064	-0.214	-0.116	0.287	-0.166	0.173
<i>cob</i>	-0.430	-0.291	-0.135	0.162	0.210	0.273
<i>cpd</i>	-0.356	-0.459	-0.088	0.374	-0.032	0.441
<i>ccf</i>	-0.347	-0.512	-0.135	0.372	-0.108	0.474

AMP–ampicillin; IPM–imipenem; GEN–gentamicin; S–streptomycin; TEC–teicoplanin; VAN–vancomycin; QD–quinupristin/dalfopristin; TGC–tigecycline; LZD–linezolid; NIT–nitrofurantoin; W5–trimethoprim; CIP–ciprofloxacin; DO–doxycycline; vancomycin resistance genes (*vanA*–*C2/C3*); virulence factors: cytolysin (*cylA*), aggregation substance (AS), gelatinase (*gel E*), hyaluronidase (*hyl*), endocarditis antigen (*efaA*), factor encoding surface protein (*esp*), and sex pheromones (*cob*, *cpd* and *ccf*)

Table S4–Part B

Variable	Other		<i>Enterococcus</i> :					<i>casseliflavus/</i> <i>flavescens</i>
	<i>Enterococcus</i> spp	<i>faecalis</i>	<i>faecium</i>	<i>durans</i>	<i>avium</i>	<i>hiare</i>	<i>gallinarum</i>	
<i>E. faecalis</i>	0.213							
<i>E. faecium</i>	-0.001	0.668						
<i>E. durans</i>	0.496	0.466	0.295					
<i>E. avium</i>	0.009	0.381	0.279	-0.148				
<i>E. hiare</i>	0.420	0.549	0.177	0.876	0.022			
<i>E. gallinarum</i>	0.066	-0.007	-0.020	0.101	-0.121	0.133		
<i>E. casseliflavus/flavescens</i>	-0.243	0.242	0.512	0.360	0.076	0.393	0.118	
AMP	-0.031	0.446	0.659	0.324	0.338	0.277	-0.046	0.703
IPM	0.029	0.596	0.711	0.305	0.403	0.301	-0.036	0.583
GEN	0.093	0.573	0.721	0.445	0.354	0.347	-0.047	0.598
S	0.243	0.863	0.805	0.591	0.429	0.604	-0.132	0.500
TEC	0.565	0.368	0.283	0.593	-0.064	0.532	0.465	-0.091
VAN	0.468	0.777	0.527	0.439	0.305	0.460	0.169	-0.129
QD	0.231	0.890	0.760	0.581	0.406	0.675	0.006	0.473
TGC	0.018	0.629	0.807	0.240	0.423	0.334	-0.092	0.636
LZD	0.069	0.712	0.826	0.395	0.338	0.450	-0.091	0.614
NIT	0.107	0.613	0.845	0.397	0.273	0.366	-0.091	0.580
W5	0.268	0.790	0.885	0.504	0.390	0.496	-0.026	0.501
CIP	0.231	0.772	0.819	0.534	0.314	0.530	-0.142	0.581
DO	0.239	0.584	0.355	0.212	0.041	0.236	-0.026	-0.128
<i>vanA</i>	0.025	-0.074	-0.031	-0.128	-0.077	-0.119	0.435	-0.075
<i>vanB</i>	-0.209	0.396	0.297	-0.331	0.505	-0.182	0.227	-0.194
<i>vanC1</i>	-0.007	0.537	0.581	-0.044	0.450	-0.115	0.221	-0.135
<i>vanC2/VC3</i>	0.028	0.181	0.211	-0.031	0.007	-0.008	0.080	0.251
<i>cylA</i>	0.127	0.625	0.755	0.490	0.210	0.384	0.211	0.478

<i>hyl</i>	0.446	0.595	0.599	0.559	0.080	0.376	0.036	-0.010
<i>ace</i>	0.260	0.737	0.560	0.291	0.240	0.257	0.110	-0.214
<i>efaA</i>	0.343	0.886	0.633	0.591	0.335	0.635	0.140	0.185
<i>gelE</i>	0.299	0.724	0.524	0.418	0.143	0.304	0.154	-0.150
<i>as</i>	0,134	0,813	0,770	0,465	0,422	0,351	0,087	0,343
<i>esp</i>	-0,138	0,714	0,735	0,187	0,168	0,194	0,171	0,310
<i>cob</i>	0,206	0,766	0,482	0,244	0,469	0,252	0,023	-0,183
<i>cpd</i>	0,259	0,886	0,644	0,395	0,506	0,404	0,142	0,063
<i>ccf</i>	0,256	0,906	0,664	0,420	0,511	0,461	0,128	0,133

Table S4–Part C

Variable	AMP	IPM	GEN	S	TEC	VAN	QD	TGC	LZD	NIT	W5	CIP	DO
IPM	0.944												
GEN	0.925	0.938											
S	0.730	0.818	0.817										
TEC	-0.005	0.097	0.133	0.360									
VAN	0.098	0.293	0.260	0.646	0.740								
QD	0.549	0.681	0.654	0.949	0.450	0.740							
TGC	0.785	0.863	0.786	0.842	0.131	0.385	0.801						
LZD	0.673	0.773	0.722	0.890	0.221	0.534	0.892	0.933					
NIT	0.678	0.783	0.770	0.846	0.255	0.463	0.819	0.923	0.952				
W5	0.667	0.776	0.770	0.948	0.459	0.682	0.930	0.885	0.924	0.920			
CIP	0.746	0.841	0.820	0.951	0.262	0.539	0.908	0.901	0.937	0.925	0.935		
DO	0.004	0.193	0.076	0.439	0.305	0.737	0.527	0.298	0.529	0.406	0.444	0.447	
<i>vanA</i>	0.211	0.200	0.069	-0.071	0.347	0.124	-0.138	-0.003	-0.092	-0.130	-0.020	-0.139	0.061
<i>vanB</i>	0.123	0.225	0.064	0.216	0.176	0.472	0.252	0.187	0.155	0.024	0.228	0.052	0.304
<i>vanC1</i>	0.003	0.167	0.163	0.372	0.334	0.691	0.462	0.236	0.354	0.329	0.467	0.288	0.491
<i>vanC2/VC3</i>	0.265	0.382	0.350	0.182	-0.062	-0.093	0.178	0.360	0.201	0.365	0.234	0.349	-0.156
<i>cylA</i>	0.597	0.592	0.656	0.714	0.506	0.520	0.662	0.531	0.556	0.517	0.733	0.611	0.119
<i>hyl</i>	0.316	0.471	0.559	0.675	0.658	0.724	0.630	0.450	0.580	0.655	0.723	0.614	0.520
<i>ace</i>	-0.028	0.156	0.204	0.535	0.618	0.882	0.623	0.257	0.401	0.362	0.581	0.396	0.572
<i>efaA</i>	0.342	0.480	0.505	0.811	0.644	0.860	0.869	0.508	0.621	0.527	0.782	0.678	0.481
<i>gelE</i>	0.185	0.312	0.372	0.561	0.609	0.776	0.555	0.189	0.298	0.256	0.528	0.396	0.427
<i>as</i>	0,576	0,622	0,652	0,797	0,359	0,680	0,750	0,522	0,620	0,545	0,746	0,689	0,438
<i>esp</i>	0,315	0,456	0,389	0,624	0,269	0,645	0,709	0,536	0,705	0,593	0,673	0,600	0,669
<i>cob</i>	0,065	0,232	0,219	0,568	0,464	0,883	0,627	0,252	0,420	0,300	0,544	0,398	0,686
<i>cpd</i>	0,267	0,428	0,450	0,741	0,510	0,886	0,800	0,456	0,589	0,500	0,728	0,605	0,589
<i>ccf</i>	0,340	0,494	0,493	0,792	0,535	0,876	0,842	0,512	0,621	0,520	0,770	0,649	0,546

Table S4–Part D

Variable	<i>van A</i>	<i>van B</i>	<i>van C1</i>	<i>van C2/C3</i>	<i>cylA</i>	<i>hyl</i>	<i>ace</i>	<i>efaA</i>	<i>gelE</i>	<i>as</i>	<i>esp</i>	<i>cob</i>	<i>cpd</i>
<i>vanB</i>	0,459												
<i>vanC1</i>	-0,047	0,643											
<i>vanC2/VC3</i>	-0,153	-0,270	-0,052										
<i>cylA</i>	0,243	0,363	0,446	-0,008									
<i>hyl</i>	0,096	0,122	0,470	0,068	0,537								
<i>ace</i>	-0,023	0,510	0,807	-0,082	0,553	0,709							
<i>efaA</i>	0,025	0,421	0,583	-0,016	0,786	0,672	0,829						
<i>gelE</i>	0,178	0,519	0,659	-0,099	0,721	0,692	0,877	0,851					
<i>as</i>	0,065	0,456	0,666	-0,022	0,822	0,557	0,653	0,811	0,764				
<i>esp</i>	0,047	0,540	0,732	-0,037	0,624	0,501	0,679	0,696	0,625	0,739			
<i>cob</i>	0,063	0,623	0,809	-0,230	0,494	0,585	0,884	0,795	0,800	0,762	0,686		

<i>cpd</i>	-0,026	0,533	0,805	-0,023	0,664	0,658	0,885	0,912	0,827	0,866	0,748	0,932	
<i>ccf</i>	0,042	0,556	0,741	0,001	0,734	0,641	0,856	0,950	0,834	0,877	0,747	0,905	0,984

**Table S5.** Number and percentage of antibiotic resistant enterococci in wastewater and river water

Antibiotic Sampling sites		Number (%) of resistant <i>Enterococci</i> :							Other <i>Enterococcus spp</i>
		<i>faecium</i>	<i>faecalis</i>	<i>durans</i>	<i>avium</i>	<i>hirae</i>	<i>gallinarum</i>	<i>casseliflavus/ flavescens</i>	
AMP	HWW	26(72.2)	7(26.9)	1(100)	0.0	1(50.0)	0	0.0	9(90)
	UWW	6(21.4)	0(0.0)	0.0	0.0	0.0	0.0	0.0	1(8.3)
	TWW	10(26.3)	1(3.4)	1(33.3)	0.0	1(25.0)	0.0	0.0	2(9.5)
	URW	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	DRW	1(25)	0.0	0.0	0.0	0.0	1(20.0)	0.0	1(16.6)
IPM	HWW	26(72.2)	8(30.8)	1(100)	1(14.3)	1(50)	0.0	0.0	8(80.0)
	UWW	7(25.0)	3(15.0)	0.0	0.0	0.0	0.0	0.0	3(25.0)
	TWW	10(26.3)	8(27.6)	1(33.3)	0.0	1(25.0)	0.0	0.0	2(9.5)
	URW	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	DRW	2(50)	1(100)	0.0	0.0	0.0	1 (20)	0.0	3(50)
GEN	HWW	22(61.1)	14(53.8)	1(100)	2(28.6)	0.0	0.0	1(100)	8(80)
	UWW	5(17.8)	3(15.0)	0.0	1(25.0)	0.0	0.0	0.0	1(8.3)
	TWW	9(23.7)	11(37.9)	1(33.3)	1(50)	1(25.0)	0.0	0.0	1(4.8)
	URW	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	DRW	3(75)	0.0	0.0	0.0	0.0	0.0	0.0	1(16.6)
S	HWW	36(100)	25(96.2)	1(100)	7(100)	2(100)	1(100)	1(100)	10(100)
	UWW	22(78.6)	17(85.0)	0.0	3(75.0)	1(100)	0.0	0.0	7(58.3)
	TWW	33(86.8)	27(93.1)	3(100)	2(100)	4(100)	1(100)	0.0	10(47.6)
	URW	0.0	4(80)	0.0	0.0	0.0	0.0	0.0	4(33.3)
	DRW	3 (75)	0.0	0.0	0.0	0.0	0.0	0.0	2(33.3)
TEC	HWW	2(5.6)	2(7.7)	0.0	1(14.3)	1(50)	0.0	1(100)	0.0
	UWW	6(21.4)	4(20.0)	0.0	1(25.0)	0.0	1(33.3)	0.0	9(75.0)
	TWW	4(10.5)	9(31)	0.0	0.0	0.0	0.0	0.0	11(52.4)
	URW	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2(16.6)
	DRW	4 (100)	1(100)	0.0	0.0	0.0	3(60)	0.0	5(83.3)
VAN	HWW	8(22.2)	9(34.6)	0.0	1(14.3)	0.0	1(100)	1(100)	1(10)
	UWW	15(53.6)	14(70.0)	0.0	0.0	0.0	3(100)	0.0	6(50.0)
	TWW	22(57.9)	18(62.1)	0.0	0.0	3(75.0)	1(100)	0.0	13(61.9)
	URW	0.0	1 (20)	0.0	0.0	0.0	0.0	0.0	2(16.6)
	DRW	3 (75)	1(100)	0.0	0.0	0.0	3(60)	0.0	5(83.3)
QD	HWW	23(63.9)	24(92.3)	1(100)	6(85.7)	2(100)	1(100)	1(100)	6(60)
	UWW	20(71.4)	17(85.0)	0.0	4(100)	1(100)	1(33.3)	0.0	11(91.7)
	TWW	27(71.1)	27(93.1)	2(66.7)	2(100)	4(100)	1(100)	0.0	13(61.9)
	URW	0.0	3(60)	0.0	0.0	0.0	0.0	0.0	5(41.6)
	DRW	3 (75)	1(100)	0.0	0.0	0.0	2(40)	0.0	4(66.6)
TGC	HWW	16(44.4)	19(73.1)	1(100)	3(42.9)	1(50)	0.0	0.0	5(50)
	UWW	12(42.9)	7(35.0)	0.0	2(50.0)	1(100)	0.0	0.0	4(33.3)
	TWW	13(34.2)	12(41.4)	0.0	1(50)	1(25.0)	0.0	0.0	3(14.3)
	URW	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	DRW	0.0	0.0	0.0	0.0	0.0	1(20)	0.0	5(83.3)
LZD	HWW	23(63.9)	18(69.2)	1(100)	3(42.9)	2(100)	1(100)	1(100)	5(50)
	UWW	16(57.1)	9(45.0)	0.0	2(50.0)	1(100)	0.0	0.0	4(33.3)
	TWW	25(65.8)	19(65.5)	2(66.7)	2(100)	2(50)	1(100)	0.0	6(28.6)
	URW	0.0	1(20)	0.0	0.0	0.0	0.0	0.0	4(33.3)
	DRW	4(100)	0.0	0.0	0.0	0.0	1(20)	0.0	4(66.6)
NIT	HWW	26(72.2)	11(42.3)	1(100)	3(42.9)	2(100)	1(100)	0.0	5(50)
	UWW	13(46.4)	9(45.0)	0.0	2(50.0)	1(100)	0.0	0.0	6(50.0)



	DRW	2(50.0)	1(10.0)	0.0	0.0	0.0	2(40.0)	0.0	1(16.6)
<i>as</i>	HWW	4(11.1)	10.0(38.5)	0.0	4(57.1)	1(10.0)	1(10.0)	1(10.0)	2(20.0)
	UWW	4(14.3)	6(30.0)	0.0	0.0	1(10.0)	0.0	0.0	0.0
	TWW	8(21.1)	13(44.8)	2(66.7)	2(10.0)	0.0	0.0	0.0	0.0
	URW	0.0	1(20.0)	0.0	0.0	0.0	0.0	0.0	1(8.3)
	DRW	2(50.0)	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>esp</i>	HWW	2(5.6)	9(34.6)	0.0	1(14.3)	0.0	1(10.0)	0.0	0.0
	UWW	6(21.4)	1(5.0)	0.0	1(25.0)	0.0	0.0	0.0	1(8.3)
	TWW	7(18.4)	12(41.4)	13(3.3)	1(50.0)	0.0	0.0	0.0	1(4.8)
	URW	0.0	2(40.0)	0.0	0.0	0.0	0.0	0.0	1(8.3)
	DRW	2(50.0)	1(10.0)	0.0	0.0	0.0	4(80.0)	0.0	0.0
<i>cob</i>	HWW	0.0	10.0(38.5)	0.0	5(71.4)	0.0	0.0	0.0	3(30.0)
	UWW	10.0(35.7)	9(45.0)	0.0	1(25.0)	0.0	0.0	0.0	1(8.3)
	TWW	10.0(26.0)3)	24(82.8)	2(66.7)	1(50.0)	0.0	0.0	0.0	2(9.5)
	URW	0.0	2(40.0)	0.0	0.0	0.0	0.0	0.0	1(8.3)
	DRW	3(75)	0.0	0.0	0.0	0.0	1(20.0)	0.0	1(16.6)
<i>cpd</i>	HWW	4(11.1)	20.0(76.9))	0.0	6(85.7)	0.0	0.0	0.0	3(30.0)
	UWW	13(46.6)	14(70.0)	0.0	1(25.0)	0.0	1(33.3)	0.0	2(16.7)
	TWW	14(36.8)	29(10.0)	2(66.7)	1(50.0)	1(25.0)	0.0	0.0	4(19.0)
	URW	0.0	3(60.0)	0.0	0.0	0.0	0.0	0.0	1(8.3)
	DRW	2(50.0)	0.0	0.0	0.0	0.0	4(80.0)	0.0	1(16.6)
<i>ccf</i>	HWW	7(19.4)	22(84.6)	0.0	7(10.0)	0.0	1(10.0)	0.0	3(30.0)
	UWW	16(57.1)	14(70.0)	0.0	2(50.0)	0.0	2(66.7)	0.0	2(16.7)
	TWW	15(39.5)	29(10.0)	2(66.7)	1(50.0)	3(75.0)	1(10.0)	0.0	3(14.3)
	URW	0.0	3(60.0)	0.0	0.0	0.0	0.0	0.0	2(16.6)
	DRW	3(75)	0.0	0.0	0.0	0.0	4.80.0)	0.0	1(16.6)

virulence factors: cytotoxin (cylA), aggregation substance (AS), gelatinase (gel E), hyaluronidase (hyl), endocarditis antigen (efaA), factor encoding surface protein (esp), and sex pheromones (cob, cpd and ccf); HWW – hospital waste water; UWW and TWW – untreated and treated wastewater; URW and DRW – upstream and downstream river water;

## References

1. Ke, D.; Picard, F.J.; Martineau, F.; Menard, C.; Roy, P.H.; Ouellette, M. Bergeron M.G. Development of a PCR assay for rapid detection of enterococci. *J. Clin. Microbiol.* **1999**, *37*, 3497 – 3503. <https://doi.org/10.1128/JCM.37.11.3497-3503.1999>.
2. Deasy, B.M.; Rea, M.C.; Fitzgerald, G.F.; Cogan, T.M.; Beresford, T.P. A rapid PCR based method to distinguish between *Lactococcus* and *Enterococcus*. *Syst. Appl Microbiol.* **2000**, *23*, 510 – 522. [https://doi.org/10.1016/S0723-2020\(00\)80025-9](https://doi.org/10.1016/S0723-2020(00)80025-9).
3. Kariyama, R.; Mitsuhata, R.; Chow, J.W.; Clewell, D.B.; Kumon, H. Simple and reliable multiplex PCR assay for surveillance isolates of vancomycin resistant enterococci. *J. Clin. Microbiol.* **2000**, *38*, 3092 – 3095. <https://doi.org/10.1128/JCM.38.8.3092-3095.2000>.
4. Cheng, S.; McCleskey, F.K.; Gress, M.J.; Petroziello, J.M.; Liu, R.; Namdari, H.; Beninga, K.; Salmen, A.; DelVecchio, V.G. A PCR assay for identification of *Enterococcus faecium*. *J. Clin. Microbiol.* **1997**, *35*, 1248 – 1250. <https://doi.org/10.1128/JCM.35.5.1248-1250.1997>.
5. Jackson, C.R.; Fedorka-Cray, P.J.; Barrett, J.B. Use of a genus- and species-specific multiplex PCR for identification of enterococci. *J. Clin. Microbiol.* **2004**, *42*, 3558 – 3565. <https://doi.org/10.1128/JCM.42.8.3558-3565.2004>.

6. Dutka-Malen, S.; Evers, S.; Courvalin, P. Detection of glycopeptide resistance genotypes and identification to the species level of clinically relevant enterococci by PCR. *J. Clin. Microbiol.* **1995**, *33*, 24 – 27. <https://doi.org/10.1128/JCM.33.1.24-27.1995>.
7. Van de Klundert, J.A.M.; Vliegthart, J.S. PCR detection of genes coding for aminoglycoside-modifying enzymes. In: Persing D.H., Smith T.F., Tenover F.C., White T.J., editors. Diagnostic molecular microbiology. Washington, D.C.: ASM, **1993**, 547 – 552.
8. Vankerckhoven, V.; Van Autgaerden, T.; Vael, C.; Lammens, C.; Chapelle, S.; Rossi, R.; Jabes, D.; Goossens, H. Development of a Multiplex PCR for the Detection of *asa1*, *gelE*, *cylA*, *esp*, and *hyl* Genes in Enterococci and Survey for Virulence Determinants among European Hospital Isolates of *Enterococcus faecium*. *J. Clin. Microbiol.* **2004**, *42*(10), 4473 – 4479. <https://doi.org/10.1128/JCM.42.10.4473-4479.2004>.
9. Mannu, L.; Paba, A.; Daga, E.; Comunian, R.; Zanetti, S.; Duprè, I.; Sechi, L.A. Comparison of the incidence of virulence determinants and antibiotic resistance between *Enterococcus faecium* strains of dairy, animal and clinical origin. *Int. J. Food Microbiol.* **2003**, *88*(2-3), 291 – 304. [https://doi.org/10.1016/S0168-1605\(03\)00191-0](https://doi.org/10.1016/S0168-1605(03)00191-0).
10. Shankar, V.; Baghdayan, A.S.; Huycke, M.M.; Lindahl, G.; Gilmore, M.S. Infection-derived *Enterococcus faecalis* strains are enriched in *esp*, a gene encoding a novel surface protein. *Infect. Immun.* **1999**, *67*, 193 – 200. <https://doi.org/10.1128/IAI.67.1.193-200.1999>.
11. Eaton, T.J.; Gasson, M.J. Molecular screening of *Enterococcus* virulence determinants and potential for genetic exchange between food and medical isolates. *Appl. Environ. Microbiol.* **2001**, *67*, 1628 – 1635. <https://dx.doi.org/10.1128/AEM.67.4.1628-1635.2001>.