Electronic Supplementary Material (ESI) for Environmental Science: Water Research & Technology.

SUPPORTING INFORMATION

MICROBIAL COMMUNITIES IN A SIMULATED DRINKING WATER DISTRIBUTION SYSTEM SUBJECTED TO DISINFECTANT SWITCHING PRACTICES: RESILIENCE OF ANTIBIOTIC RESISTANCE GENES

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SUPPLEMENTARY FIGURES

SUPPLEMENTARY TABLES

SUPPLEMENTARY FIGURES

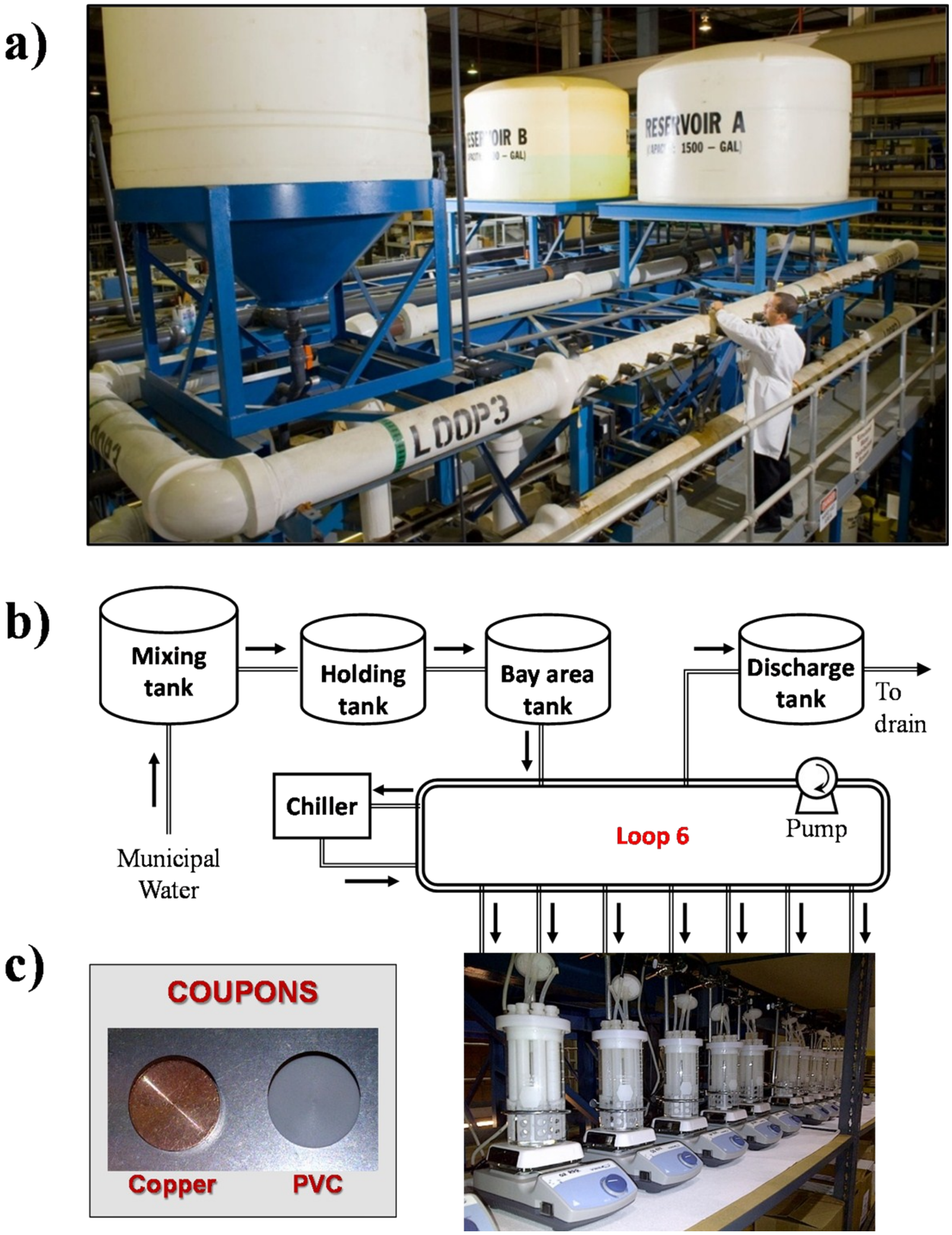


Figure S1. (A) **D**rinking **W**ater **D**istribution **S**ystem (DWDS) simulator currently in operation at the US EPA's Test and Evaluation Facility in Cincinnati, Ohio. (B) Experimental apparatus schematic of a distribution system simulator (PVC Pipe Loop #6). (C) Copper and PVC coupons were incubated in off-line CDC reactors.

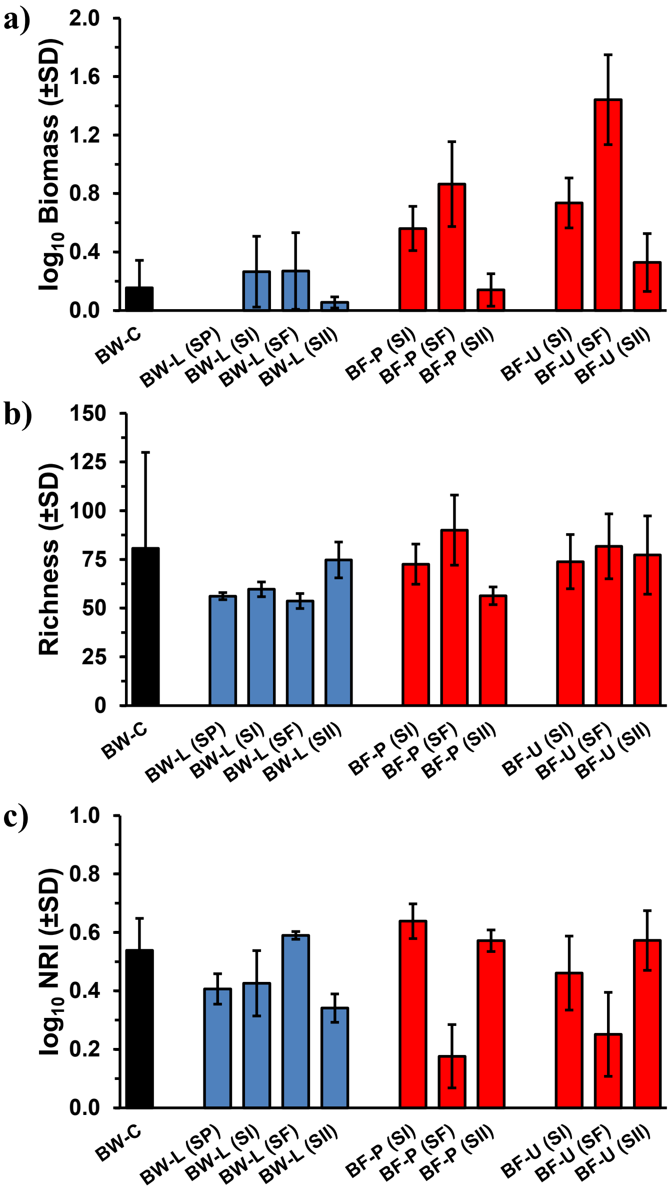


Figure S2. Bars representing mean of (A) biomass concentration (to $\log_{10}$: ATP, BCA protein, and DNA), (B) species richness (*S*) and (C) net relatedness index (to $\log_{10}$: NRI) for bulk water and biofilm communities. High and low NRI index values indicate phylogenetically clustered and overdispersed communities, respectively. Samples were identified by source: biofilm (PVC [BF-P]; Copper [BF-U]) and bulk water (municipal water [BW-C]; DWDS simulator [BW-L]). Operational schemes (in parenthesis): **SP + SS** = system stabilization (TP09: 11-15-2012); **SI** = stable chloramine residual (T07: 10-07-2013); **SF** = complete nitrification and minimal chloramine residual (T12: 12-12-2013); **SII** = stable chloramine residual (T13:03-05-2014).

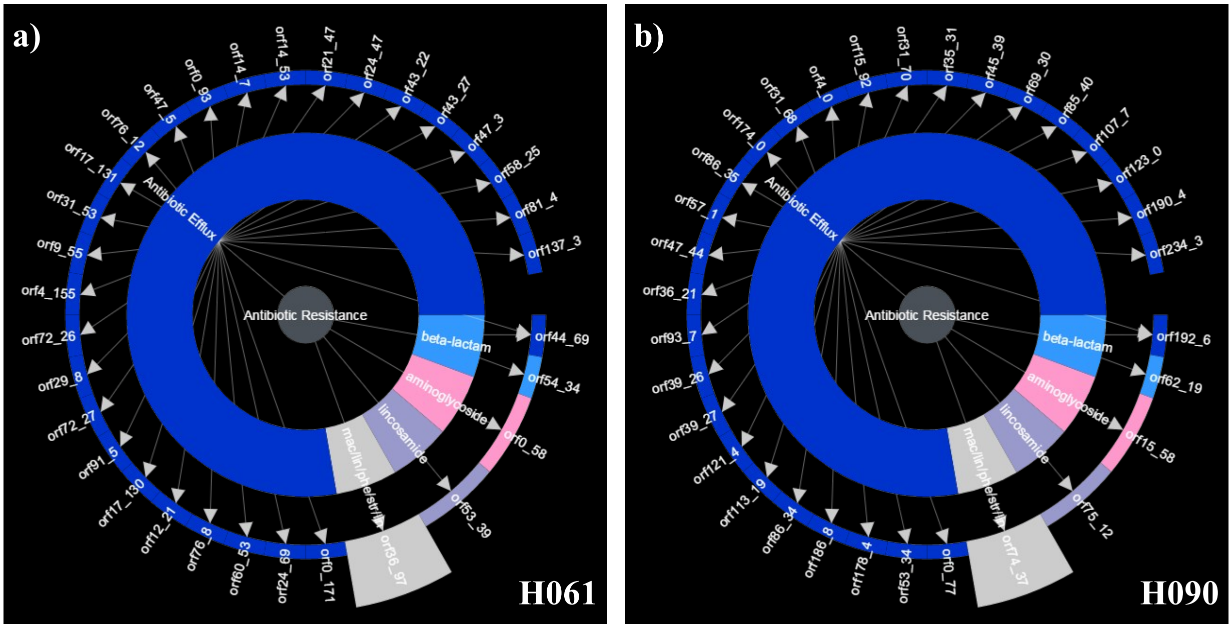


Figure S3. Analysis of the whole genome of *Variovorax paradoxus* strains by the Resistance Gene Identifier (RGI) with overall resistance in the center, resistance classes in the middle, and individual resistance genes on the outer (open reading frames). "Resistance wheel" for strains (A) H061 (SF; BF-P) and (B) H090 (SII; BF-U), predicting resistance to a broad range of antibiotic classes. Samples were identified by source: biofilm (PVC [BF-P]; Copper [BF-U]) and bulk water (municipal water [BW-C]; DWDS simulator [BW-L]). Operational schemes (in parenthesis): **SF** = complete nitrification and minimal chloramine residual; **SII** = stable chloramine residual.

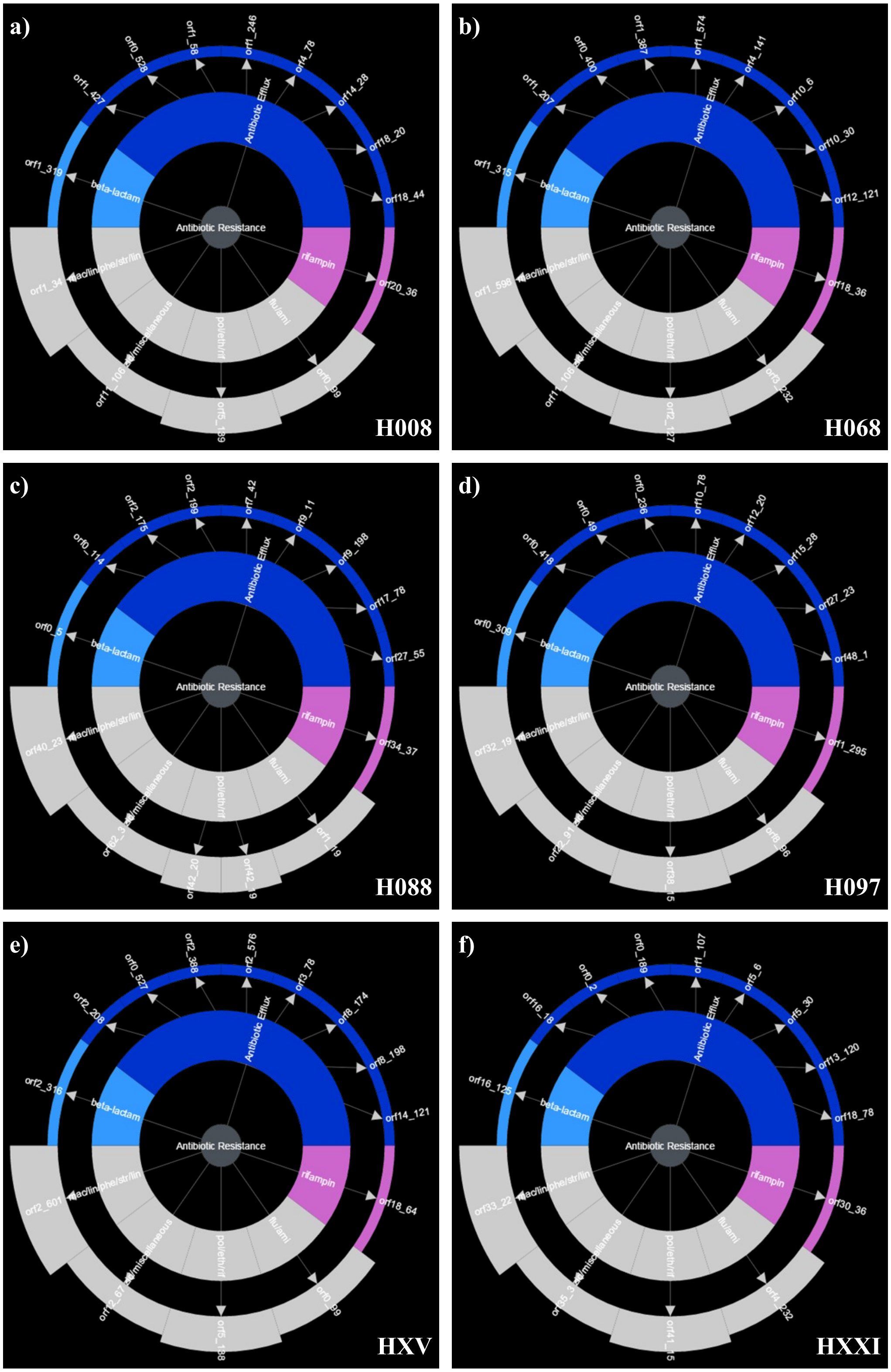

a)
orf1_427
orf0_528
orf1_58
orf1_246
orf4_78
orf14_28
orf18_20
orf18_44
orf1_319
beta-lactam
Antibiotic Efflux
Antibiotic Resistance
rifampin
orf20_36
orf1_34
mac/lin/phe/str/lin
flu/ami
pol/eth/rif
miscellaneous
orf0_99
orf5_139
H008
b)
orf1_207
orf0_400
orf1_387
orf1_574
orf4_141
orf10_6
orf10_30
orf12_121
orf1_315
orf18_36
orf1_598
orf3_232
orf2_127
H068
c)
orf0_114
orf2_175
orf2_199
orf7_42
orf9_11
orf9_198
orf17_78
orf27_55
orf0_5
orf34_37
orf40_23
orf1_19
orf42_20
orf42_19
H088
d)
orf0_418
orf0_49
orf0_236
orf10_78
orf12_20
orf15_28
orf27_23
orf48_1
orf0_309
orf1_295
orf32_19
orf8_98
orf38_15
H097
e)
orf2_208
orf0_527
orf2_388
orf2_576
orf3_78
orf8_174
orf8_198
orf14_121
orf2_316
orf18_64
orf2_601
orf0_99
orf5_138
HXV
f)
orf16_18
orf0_2
orf0_189
orf1_107
orf5_6
orf5_30
orf13_120
orf18_78
orf16_125
orf30_36
orf33_22
orf4_232
orf41_15
HXXI

Figure S4. Analysis of the whole genome of *Mycobacterium immunogenum* strains by the Resistance Gene Identifier (RGI) with overall resistance in the center, resistance classes in the middle, and individual resistance genes on the outer (open reading frames). "Resistance wheel" for strains (A) H008 (SI; BF-U), (B) H068 (SF; BF-U), (C) H088 (SII; BF-P), (D) H097 (SII; BF-U), (E) HXV (SF; BW-L) and (F) HXXI (SII; BW-L), predicting resistance to a broad range of antibiotic classes. Samples were identified by source: biofilm (PVC [BF-P]; Copper [BF-U]) and bulk water (municipal water [BW-C]; DWDS simulator [BW-L]). Operational schemes (in parenthesis): **SI** = stable chloramine residual; **SF** = complete nitrification and minimal chloramine residual; **SII** = stable chloramine residual.

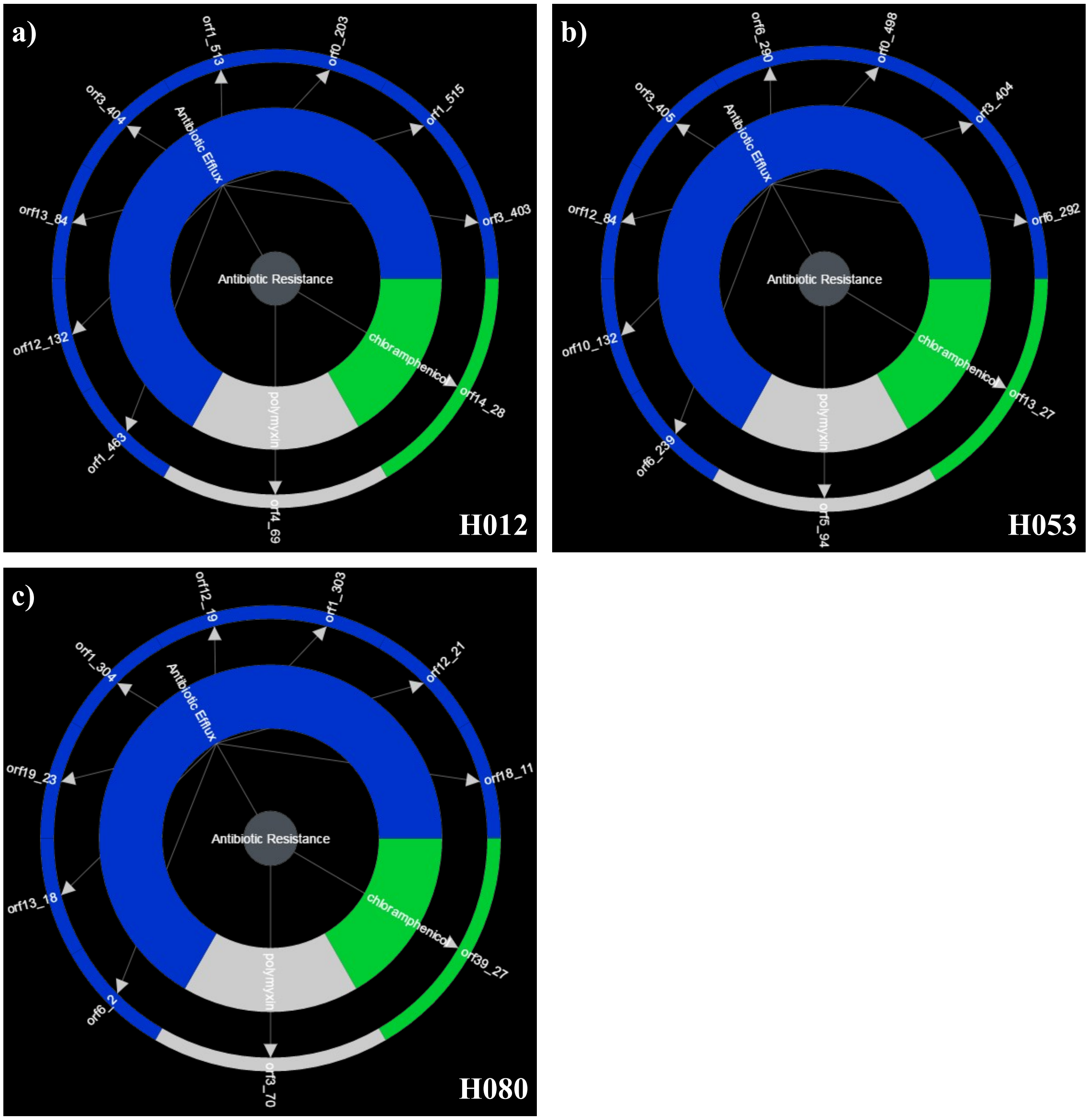
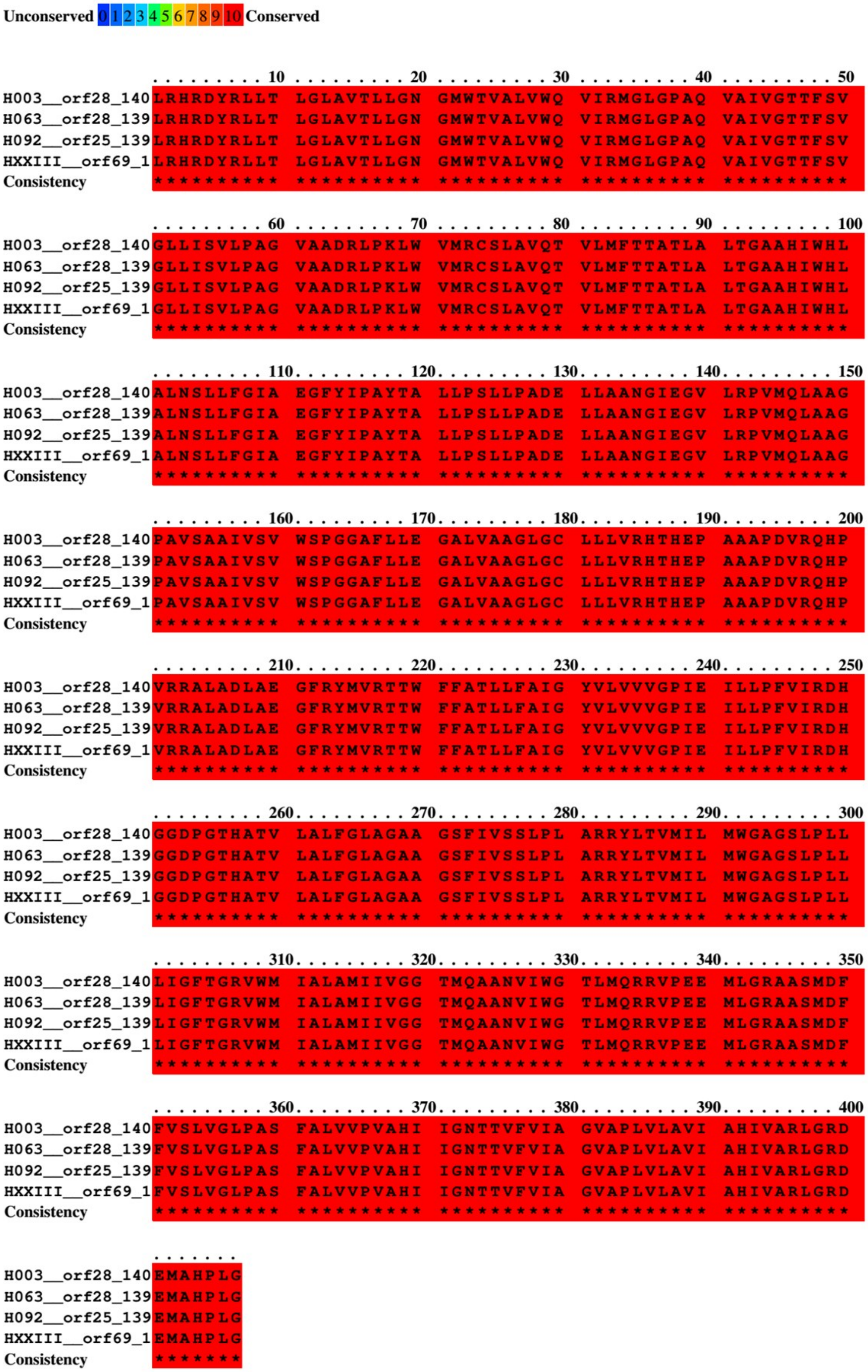


Figure S5. Analysis of the whole genome of *Sphingopyxis spp.* by the Resistance Gene Identifier (RGI) with overall resistance in the center, resistance classes in the middle, and individual resistance genes on the outer (open reading frames). "Resistance wheel" for strains (A) H012 (SI; BF-P), (B) H053 (SF; BF-P) and (C) H080 (SII; BF-P), predicting resistance to a broad range of antibiotic classes. Samples were identified by source: biofilm (PVC [BF-P]; Copper [BF-U]) and bulk water (municipal water [BW-C]; DWDS simulator [BW-L]). Operational schemes (in parenthesis): **SI** = stable chloramine residual; **SF** = complete nitrification and minimal chloramine residual; **SII** = stable chloramine residual.



Unconserved 0 1 2 3 4 5 6 7 8 9 10 Conserved

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                 .........10.........20.........30.........40.........50
H003__orf28_140  LRHRDYRLLT LGLAVTLLGN GMWTVALVWQ VIRMGLGPAQ VAIVGTTFSV
H063__orf28_139  LRHRDYRLLT LGLAVTLLGN GMWTVALVWQ VIRMGLGPAQ VAIVGTTFSV
H092__orf25_139  LRHRDYRLLT LGLAVTLLGN GMWTVALVWQ VIRMGLGPAQ VAIVGTTFSV
HXXIII__orf69_1  LRHRDYRLLT LGLAVTLLGN GMWTVALVWQ VIRMGLGPAQ VAIVGTTFSV
Consistency      ********** ********** ********** ********** **********

                 .........60.........70.........80.........90.........100
H003__orf28_140  GLLISVLPAG VAADRLPKLW VMRCSLAVQT VLMFTTATLA LTGAAHIWHL
H063__orf28_139  GLLISVLPAG VAADRLPKLW VMRCSLAVQT VLMFTTATLA LTGAAHIWHL
H092__orf25_139  GLLISVLPAG VAADRLPKLW VMRCSLAVQT VLMFTTATLA LTGAAHIWHL
HXXIII__orf69_1  GLLISVLPAG VAADRLPKLW VMRCSLAVQT VLMFTTATLA LTGAAHIWHL
Consistency      ********** ********** ********** ********** **********

                 .........110.........120.........130.........140.........150
H003__orf28_140  ALNSLLFGIA EGFYIPAYTA LLPSLLPADE LLAANGIEGV LRPVMQLAAG
H063__orf28_139  ALNSLLFGIA EGFYIPAYTA LLPSLLPADE LLAANGIEGV LRPVMQLAAG
H092__orf25_139  ALNSLLFGIA EGFYIPAYTA LLPSLLPADE LLAANGIEGV LRPVMQLAAG
HXXIII__orf69_1  ALNSLLFGIA EGFYIPAYTA LLPSLLPADE LLAANGIEGV LRPVMQLAAG
Consistency      ********** ********** ********** ********** **********

                 .........160.........170.........180.........190.........200
H003__orf28_140  PAVSAAIVSV WSPGGAFLLE GALVAAGLGC LLLVRHTHEP AAAPDVRQHP
H063__orf28_139  PAVSAAIVSV WSPGGAFLLE GALVAAGLGC LLLVRHTHEP AAAPDVRQHP
H092__orf25_139  PAVSAAIVSV WSPGGAFLLE GALVAAGLGC LLLVRHTHEP AAAPDVRQHP
HXXIII__orf69_1  PAVSAAIVSV WSPGGAFLLE GALVAAGLGC LLLVRHTHEP AAAPDVRQHP
Consistency      ********** ********** ********** ********** **********

                 .........210.........220.........230.........240.........250
H003__orf28_140  VRRALADLAE GFRYMVRTTW FFATLLFAIG YVLVVVGPIE ILLPFVIRDH
H063__orf28_139  VRRALADLAE GFRYMVRTTW FFATLLFAIG YVLVVVGPIE ILLPFVIRDH
H092__orf25_139  VRRALADLAE GFRYMVRTTW FFATLLFAIG YVLVVVGPIE ILLPFVIRDH
HXXIII__orf69_1  VRRALADLAE GFRYMVRTTW FFATLLFAIG YVLVVVGPIE ILLPFVIRDH
Consistency      ********** ********** ********** ********** **********

                 .........260.........270.........280.........290.........300
H003__orf28_140  GGDPGTHATV LALFGLAGAA GSFIVSSLPL ARRYLTVMIL MWGAGSLPLL
H063__orf28_139  GGDPGTHATV LALFGLAGAA GSFIVSSLPL ARRYLTVMIL MWGAGSLPLL
H092__orf25_139  GGDPGTHATV LALFGLAGAA GSFIVSSLPL ARRYLTVMIL MWGAGSLPLL
HXXIII__orf69_1  GGDPGTHATV LALFGLAGAA GSFIVSSLPL ARRYLTVMIL MWGAGSLPLL
Consistency      ********** ********** ********** ********** **********

                 .........310.........320.........330.........340.........350
H003__orf28_140  LIGFTGRVWM IALAMIIVGG TMQAANVIWG TLMQRRVPEE MLGRAASMDF
H063__orf28_139  LIGFTGRVWM IALAMIIVGG TMQAANVIWG TLMQRRVPEE MLGRAASMDF
H092__orf25_139  LIGFTGRVWM IALAMIIVGG TMQAANVIWG TLMQRRVPEE MLGRAASMDF
HXXIII__orf69_1  LIGFTGRVWM IALAMIIVGG TMQAANVIWG TLMQRRVPEE MLGRAASMDF
Consistency      ********** ********** ********** ********** **********

                 .........360.........370.........380.........390.........400
H003__orf28_140  FVSLVGLPAS FALVVPVAHI IGNTTVFVIA GVAPLVLAVI AHIVARLGRD
H063__orf28_139  FVSLVGLPAS FALVVPVAHI IGNTTVFVIA GVAPLVLAVI AHIVARLGRD
H092__orf25_139  FVSLVGLPAS FALVVPVAHI IGNTTVFVIA GVAPLVLAVI AHIVARLGRD
HXXIII__orf69_1  FVSLVGLPAS FALVVPVAHI IGNTTVFVIA GVAPLVLAVI AHIVARLGRD
Consistency      ********** ********** ********** ********** **********

                 .......
H003__orf28_140  EMAHPLG
H063__orf28_139  EMAHPLG
H092__orf25_139  EMAHPLG
HXXIII__orf69_1  EMAHPLG
Consistency      *******
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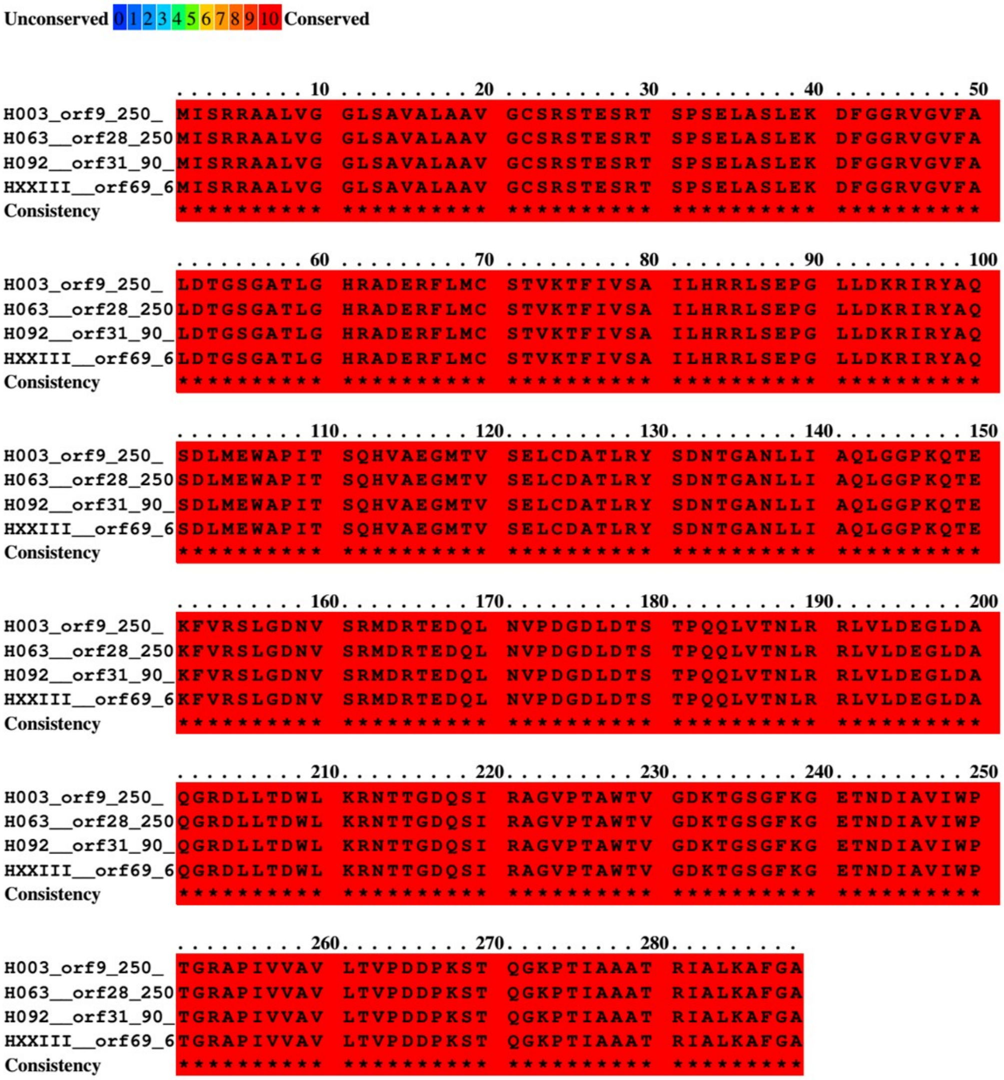
Figure S6. Alignment of the MFS transporter (TetV) amino acid sequences from *Mycobacterium chelonae* strains, colored according to relative sequence conservation at each position. The scoring scheme ranges from 0 for the least conserved alignment position up to 10 (indicated by an asterisk) for the most conserved alignment position. *M. chelonae* strains H003 (SI; BF-P; accession number: KRQ26162.), H063 (SF; BF-P; KRQ48799), H092 (SII; BF-P; KRQ36340) and HXXIII (SII; BW-L; KRQ78148). Samples were identified by source: biofilm (PVC [BF-P]; Copper [BF-U]) and bulk water (municipal water [BW-C]; DWDS simulator [BW-L]). Operational schemes (in parenthesis): **SI** = stable chloramine residual; **SF** = complete nitrification and minimal chloramine residual; **SII** = stable chloramine residual.



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Unconserved 0 1 2 3 4 5 6 7 8 9 10 Conserved

                    . . . . . . . . . 10 . . . . . . . . . 20 . . . . . . . . . 30 . . . . . . . . . 40 . . . . . . . . . 50
H003_orf9_250_      MISRRAALVG GLSAVALAAV GCSRSTESRT SPSELASLEK DFGGRVGVFA
H063__orf28_250     MISRRAALVG GLSAVALAAV GCSRSTESRT SPSELASLEK DFGGRVGVFA
H092__orf31_90_     MISRRAALVG GLSAVALAAV GCSRSTESRT SPSELASLEK DFGGRVGVFA
HXXIII__orf69_6     MISRRAALVG GLSAVALAAV GCSRSTESRT SPSELASLEK DFGGRVGVFA
Consistency         ********** ********** ********** ********** **********

                    . . . . . . . . . 60 . . . . . . . . . 70 . . . . . . . . . 80 . . . . . . . . . 90 . . . . . . . . . 100
H003_orf9_250_      LDTGSGATLG HRADERFLMC STVKTFIVSA ILHRRLSEPG LLDKRIRYAQ
H063__orf28_250     LDTGSGATLG HRADERFLMC STVKTFIVSA ILHRRLSEPG LLDKRIRYAQ
H092__orf31_90_     LDTGSGATLG HRADERFLMC STVKTFIVSA ILHRRLSEPG LLDKRIRYAQ
HXXIII__orf69_6     LDTGSGATLG HRADERFLMC STVKTFIVSA ILHRRLSEPG LLDKRIRYAQ
Consistency         ********** ********** ********** ********** **********

                    . . . . . . . . . 110. . . . . . . . . 120. . . . . . . . . 130. . . . . . . . . 140. . . . . . . . . 150
H003_orf9_250_      SDLMEWAPIT SQHVAEGMTV SELCDATLRY SDNTGANLLI AQLGGPKQTE
H063__orf28_250     SDLMEWAPIT SQHVAEGMTV SELCDATLRY SDNTGANLLI AQLGGPKQTE
H092__orf31_90_     SDLMEWAPIT SQHVAEGMTV SELCDATLRY SDNTGANLLI AQLGGPKQTE
HXXIII__orf69_6     SDLMEWAPIT SQHVAEGMTV SELCDATLRY SDNTGANLLI AQLGGPKQTE
Consistency         ********** ********** ********** ********** **********

                    . . . . . . . . . 160. . . . . . . . . 170. . . . . . . . . 180. . . . . . . . . 190. . . . . . . . . 200
H003_orf9_250_      KFVRSLGDNV SRMDRTEDQL NVPDGDLDTS TPQQLVTNLR RLVLDEGLDA
H063__orf28_250     KFVRSLGDNV SRMDRTEDQL NVPDGDLDTS TPQQLVTNLR RLVLDEGLDA
H092__orf31_90_     KFVRSLGDNV SRMDRTEDQL NVPDGDLDTS TPQQLVTNLR RLVLDEGLDA
HXXIII__orf69_6     KFVRSLGDNV SRMDRTEDQL NVPDGDLDTS TPQQLVTNLR RLVLDEGLDA
Consistency         ********** ********** ********** ********** **********

                    . . . . . . . . . 210. . . . . . . . . 220. . . . . . . . . 230. . . . . . . . . 240. . . . . . . . . 250
H003_orf9_250_      QGRDLLTDWL KRNTTGDQSI RAGVPTAWTV GDKTGSGFKG ETNDIAVIWP
H063__orf28_250     QGRDLLTDWL KRNTTGDQSI RAGVPTAWTV GDKTGSGFKG ETNDIAVIWP
H092__orf31_90_     QGRDLLTDWL KRNTTGDQSI RAGVPTAWTV GDKTGSGFKG ETNDIAVIWP
HXXIII__orf69_6     QGRDLLTDWL KRNTTGDQSI RAGVPTAWTV GDKTGSGFKG ETNDIAVIWP
Consistency         ********** ********** ********** ********** **********

                    . . . . . . . . . 260. . . . . . . . . 270. . . . . . . . . 280. . . . . . . . .
H003_orf9_250_      TGRAPIVVAV LTVPDDPKST QGKPTIAAAT RIALKAFGA
H063__orf28_250     TGRAPIVVAV LTVPDDPKST QGKPTIAAAT RIALKAFGA
H092__orf31_90_     TGRAPIVVAV LTVPDDPKST QGKPTIAAAT RIALKAFGA
HXXIII__orf69_6     TGRAPIVVAV LTVPDDPKST QGKPTIAAAT RIALKAFGA
Consistency         ********** ********** ********** *********
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Figure S7. Alignment of the β-lactamase (class A) amino acid sequences from *Mycobacterium chelonae* strains, colored according to relative sequence conservation at each position. The scoring scheme ranges from 0 for the least conserved alignment position up to 10 (indicated by an asterisk) for the most conserved alignment position. *M. chelonae* strains H003 (SI; BF-P; accession number: KRQ25928), H063 (SF; BF-P; KRQ48633), H092 (SII; BF-P; KRQ35053) and HXXIII (SII; BW-L; KRQ77943). Samples were identified by source: biofilm (PVC [BF-P]; Copper [BF-U]) and bulk water (municipal water [BW-C]; DWDS simulator [BW-L]). Operational schemes (in parenthesis): **SI** = stable chloramine residual; **SF** = complete nitrification and minimal chloramine residual; **SII** = stable chloramine residual.

SUPPLEMENTARY TABLES

Table S1. Water quality values for operational schemes in the pipe-loop system*.

Characteristics§	Operational schemes‡				
	SP + SS	**SI**	**SF**	**SR**	**SII**
Parameters					
temperature [°C]	18.4 ± 0.0	17.9 ± 0.6	23.7 ± 0.8	24.0 ± 0.0	19.3 ± 2.1
pH	8.19 ± 0.14	7.88 ± 0.07	7.86 ± 0.10	7.78 ± 0.03	7.85 ± 0.17
turbidity [NTU]	0.27 ± 0.36	0.09 ± 0.03	0.11 ± 0.04	0.29 ± 0.28	0.62 ± 0.49
ORP† [mV]	582.3 ± 17.9	586.6 ± 25.8	486.3 ± 47.4	839.2 ± 123.5	501.7 ± 89.1
pump flow rate [g min^{-1}]	80.9 ± 5.7	86.4 ± 0.6	88.0 ± 0.6	88.5 ± 0.9	86.7 ± 1.1
Disinfectant					
NH_2Cl [Cl_2 mg L^{-1}]	1.71 ± 0.42	0.91 ± 0.36	0.12 ± 0.12	0.02 ± 0.01	1.17 ± 0.42
Free Chlorine [Cl_2 mg L^{-1}]	ND	ND	0.01 ± 0.00	2.72 ± 0.82	0.03 ± 0.30
N & P compounds					
Ammonia-Nitrogen [mg L^{-1}]	0.20 ± 0.08	0.23 ± 0.05	0.05 ± 0.05	0.01 ± 0.01	0.18 ± 0.06
Nitrite-Nitrogen [mg L^{-1}]	0.009 ± 0.002	0.006 ± 0.002	0.198 ± 0.065	0.003 ± 0.002	0.005 ± 0.004
Nitrate-Nitrogen [mg L^{-1}]	0.74 ± 0.12	1.02 ± 0.14	0.91 ± 0.18	0.92 ± 0.04	0.99 ± 0.23
Phosphate [mg L^{-1}]	0.14 ± 0.03	0.15 ± 0.04	0.21 ± 0.06	0.07 ± 0.03	0.14 ± 0.03

*Pipe-loop properties: 150 mm gray schedule 80 PVC; 0.30 m s^{-1} recirculation flow rate; 27 m length.

§Average (±SD) results from continuous measurements.

‡Operational schemes: **SP + SS =** system stabilization; **SI =** stable chloramine residual; **SF =** complete nitrification and minimal chloramine residual; **SR =** chlorine burn; **SII =** stable chloramine residual.

†ORP, oxidation reduction potential.

ND = not determined.

Table S2. Results of ANOSIM test based on Jensen-Shannon dissimilarity matrix derived from the distribution of microbial communities*. Operational schemes (in parenthesis): **SI** = stable chloramine residual; **SF** = complete nitrification and minimal chloramine residual; **SII** = stable chloramine residual.

Source of variation[§]	**Global *R***[‡]	***p***	**Permutations**
One-way ANOSIM			
Global tests			
disinfectant	0.851	<0.001	9999
disturbance	0.794	<0.001	9999
coupons/operational scheme	0.685	<0.001	9999
source/operational scheme	0.728	<0.001	9999
Pairwise tests[†]			
disturbance			
BF (S) vs. BF (SF)	0.713	<0.001	9999
BL (S) vs. BL (SF)	0.875	<0.001	9999
coupons/operational scheme			
BF-P (SI) vs BF-U (SI)	0.152	0.083	9999
BF-P (SF) vs BF-U (SF)	0.227	0.072	9999
BF-P (SII) vs BF-U (SII)	0.000	1	9999
source/operational scheme			
BW-L (SI) vs BF (SI)	0.793	0.003	9999
BW-L (SF) vs BF (SF)	0.911	0.003	9999
BW-L (SII) vs BF (SII)	0.960	0.003	9999

*Samples were identified by source: biofilm (PVC [BF-P]; Copper [BF-U]) and bulk water (municipal water [BW-C]; DWDS simulator [BW-L]).

[‡] Global tests on the effect of biofilm devices: disinfectant (chlorine [*n* = 36] vs chloramine [*n* = 177]); disturbance (S [*n* = 131] vs SF [*n* = 46]); coupons (PVC [*n* = 48] vs copper [*n* = 51]); source (BW-L [*n* = 79] vs BF [*n* = 99]).

[§] *R* values greater than 0 (up to 1) indicate dissimilarities between groups, whereas those values near 0 indicate a true null hypothesis of no difference between groups (Clarke, 1993).

[†] Significance set at $\alpha = 0.05$.