

Supplementary Materials

Global diversity and commonality of the antimicrobial resistome in activated sludge at wastewater treatment plants

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Supplementary Figure 2. Profiles of mobile genetic elements (MGEs) in activated sludge and untreated wastewater. Heatmap on normalized abundances of MGEs per 16S-based microbial population in activated sludge and influent wastewater (a). Heatmap on correlation coefficients between proportions of ARGs and MGEs (b).

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Supplementary Table 3. Principal component loadings of ARGs in Figure 4b (PCA on ARG profiles of activated sludge only). (a) Top 30 positive loading on PC1, (b) top 30 negative loading on PC1, (c) top 30 positive loading on PC2, (d) top 30 negative loading on PC2.

Supplementary Table 4. Principal component loadings of ARGs in Figure 4c (PCA on ARG profiles of influent wastewater only). (a) Top 30 positive loading on PC1, (b) top 30 negative loading on PC1, (c) top 30 positive loading on PC2, (d) top 30 negative loading on PC2.

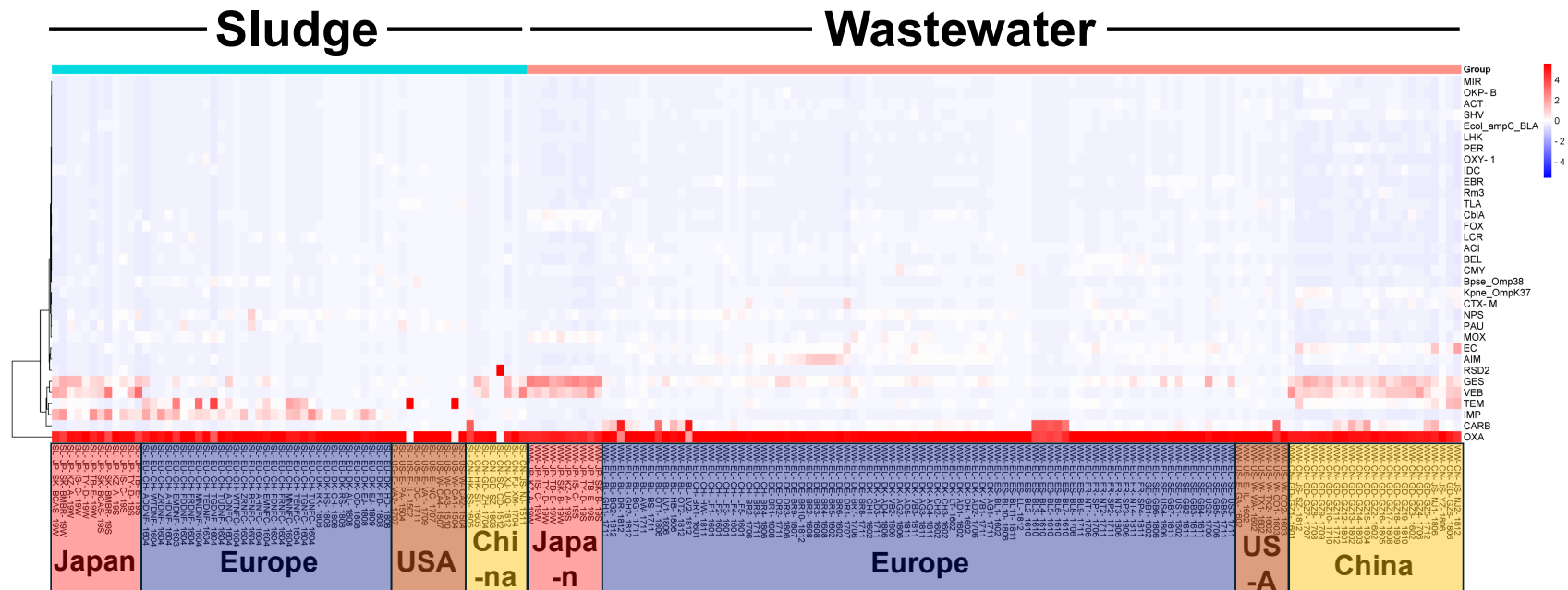
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Supplementary Table 6. Principal component loadings of ARGs in Figure 4e (PCA on 16S-based microbial community of activated sludge only). (a) Top 30 positive loading on PC1, (b) top 30 negative loading on PC1, (c) top 30 positive loading on PC4, (d) top 30 negative loading on PC4.

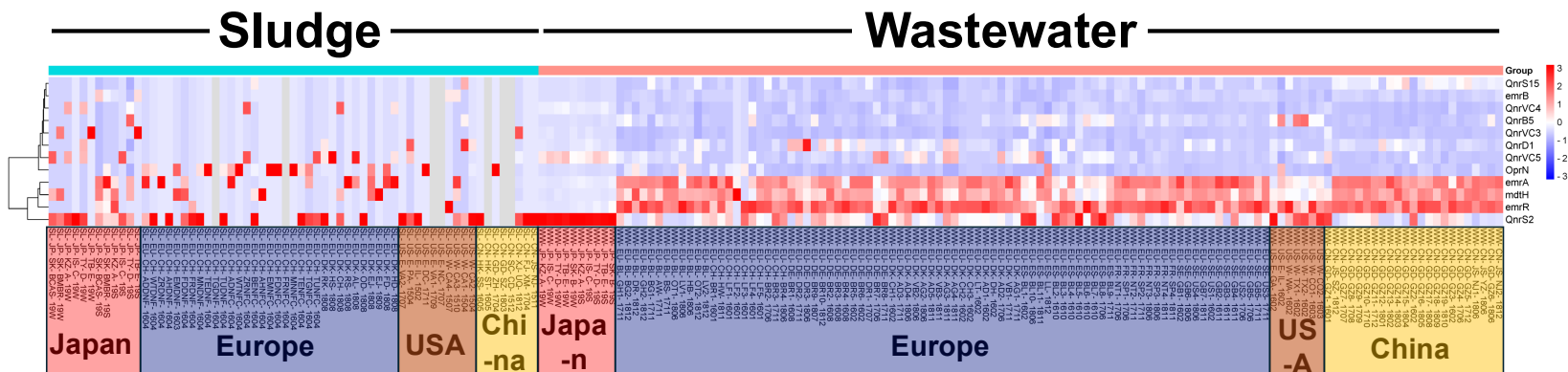
Supplementary Table 7. Principal component loadings of ARGs in Figure 4f (PCA on 16S-based microbial community of influent wastewater only). (a) Top 30 positive loading on PC1, (b) top 30 negative loading on PC1, (c) top 30 positive loading on PC4, (d) top 30 negative loading on PC4.

Supplementary Table 8. Frequency of horizontal gene transfer (HGT) reported in past studies. The maximum HGT rate was estimated from the highest frequency and contact time.

(a) Cephalosporins

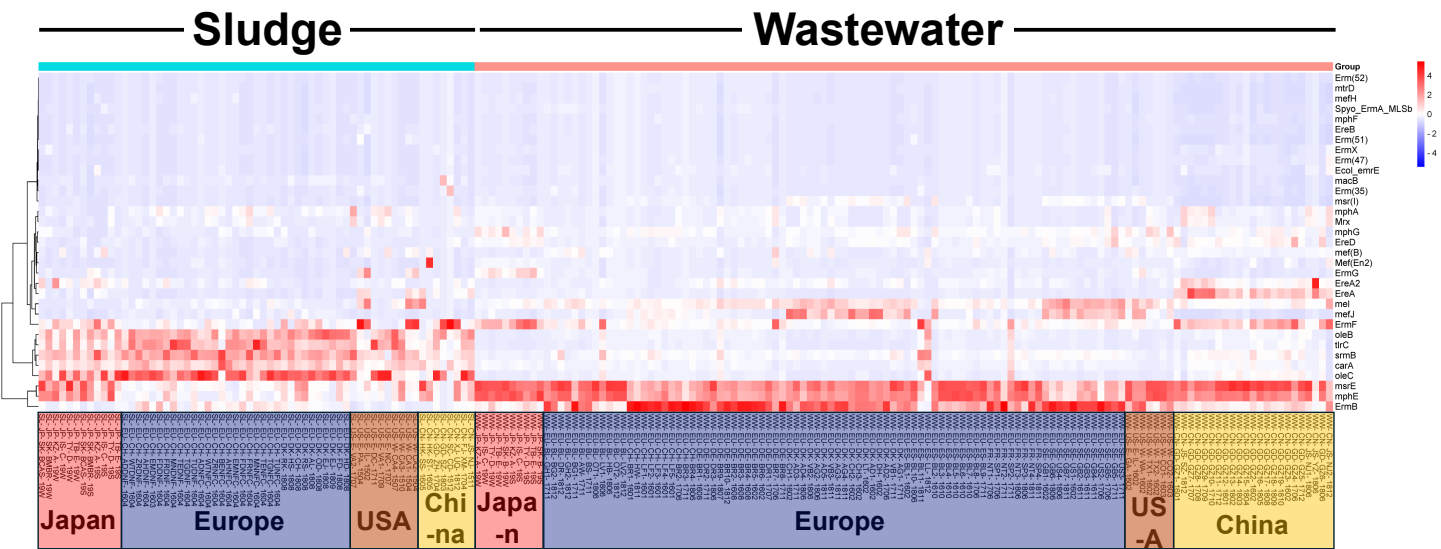


(b) Fluoroquinolones

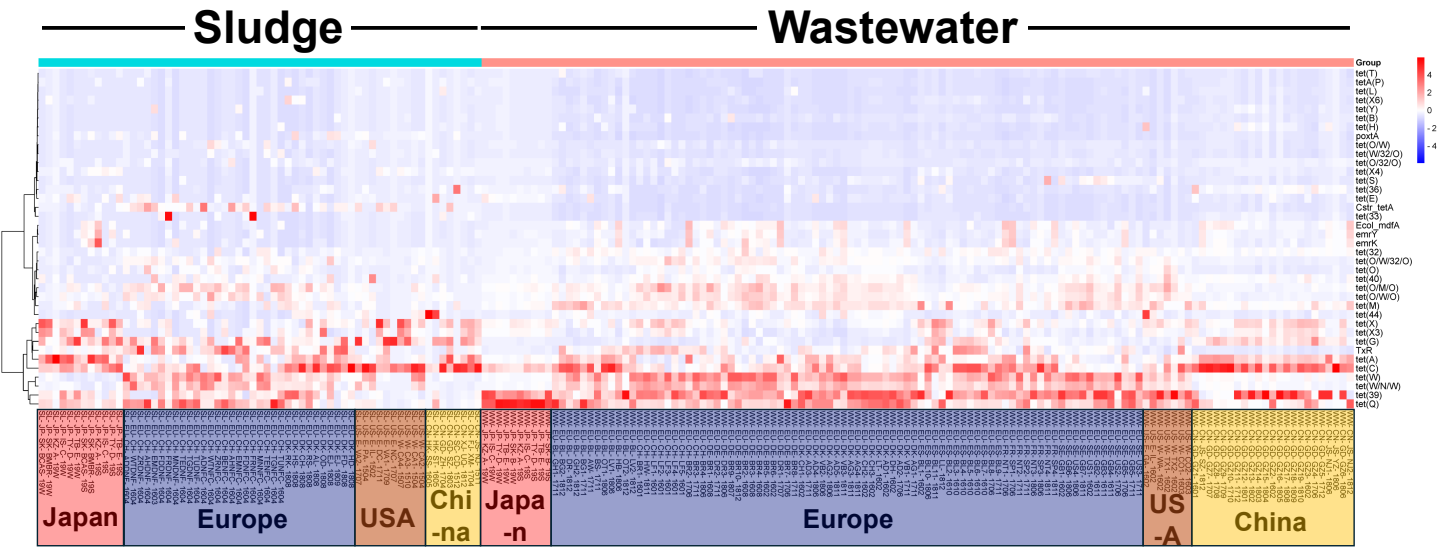


Supplementary Figure 1. Relative abundances of ARG in activated sludge and influent wastewater in all countries. cephalosporins (a), fluoroquinolones (b), macrolide (c), tetracyclines (d), aminoglycosides (e), sulfonamides (f), and multidrug efflux (g). The heatmap shows the normalized proportion within each sample among a set of ARGs on each drug group.

(c) Macrolide

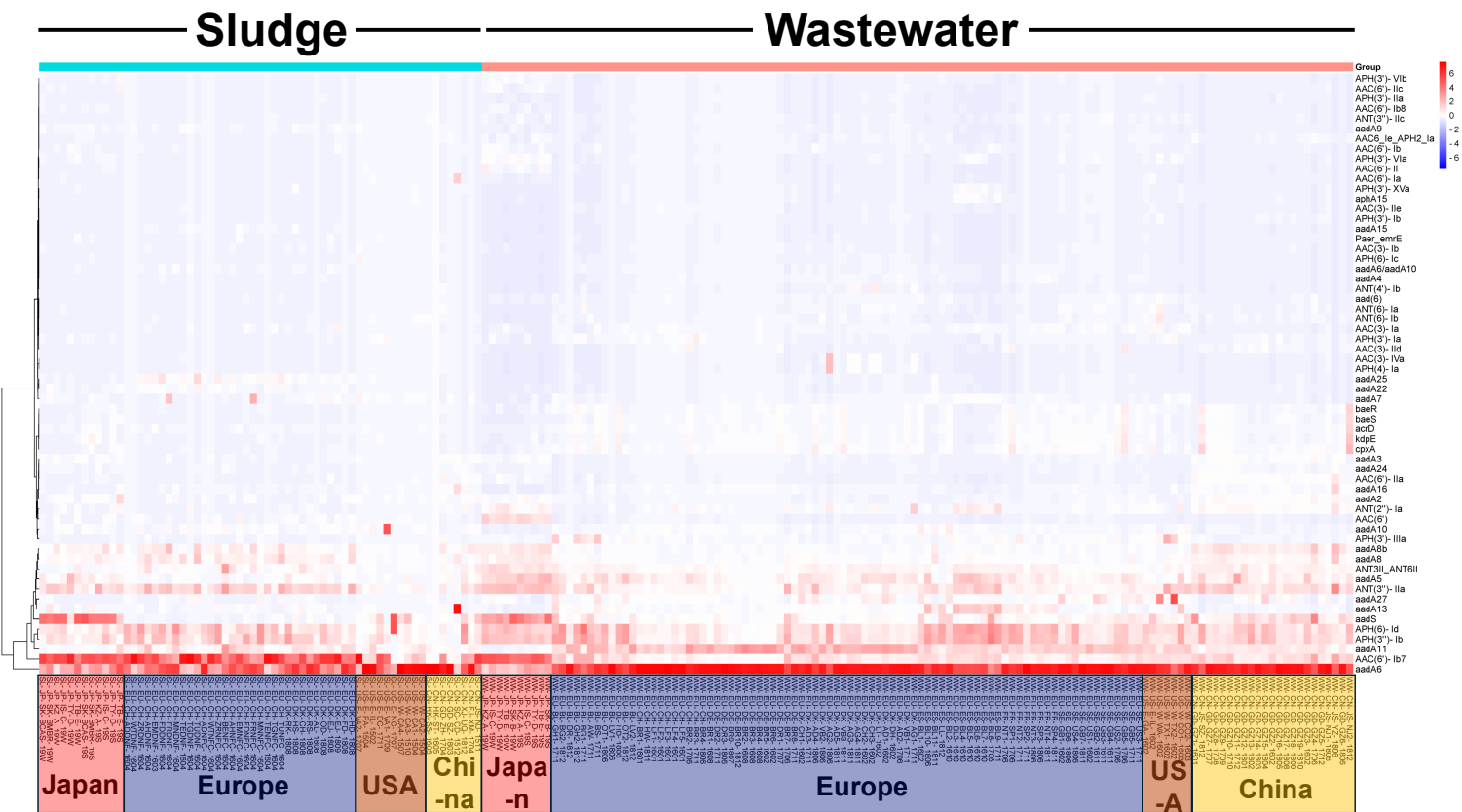


(d) Tetracyclines

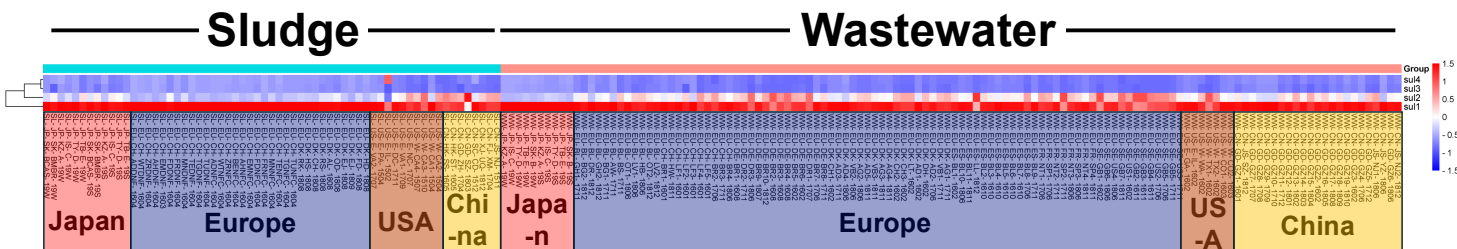


Supplementary Figure 1 (cont'd). Relative abundances of ARG in activated sludge and influent wastewater in all countries. cephalosporins (a), fluoroquinolones (b), macrolide (c), tetracyclines (d), aminoglycosides (e), sulfonamides (f), and multidrug efflux (g). The heatmap shows the normalized proportion within each sample among a set of ARGs on each drug group.

(e) Aminoglycosides

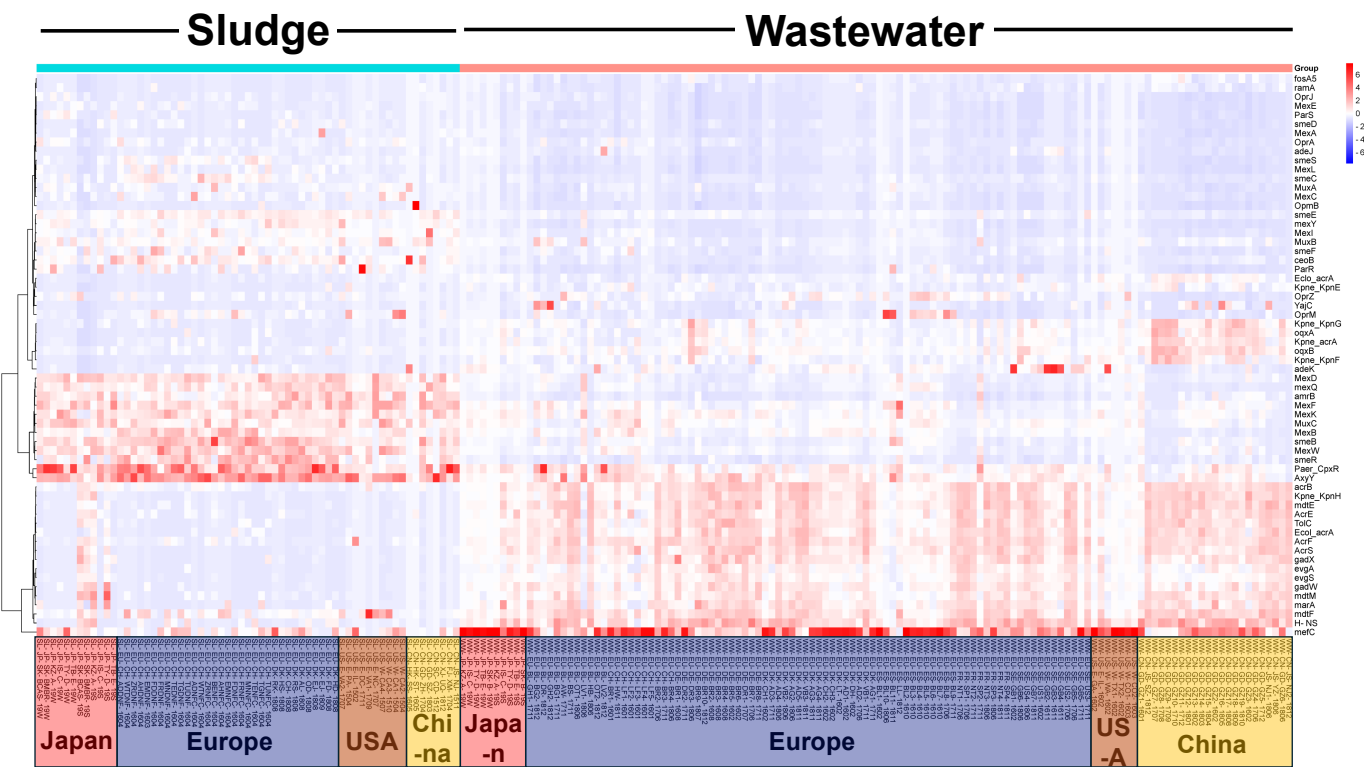


(f) Sulfonamides



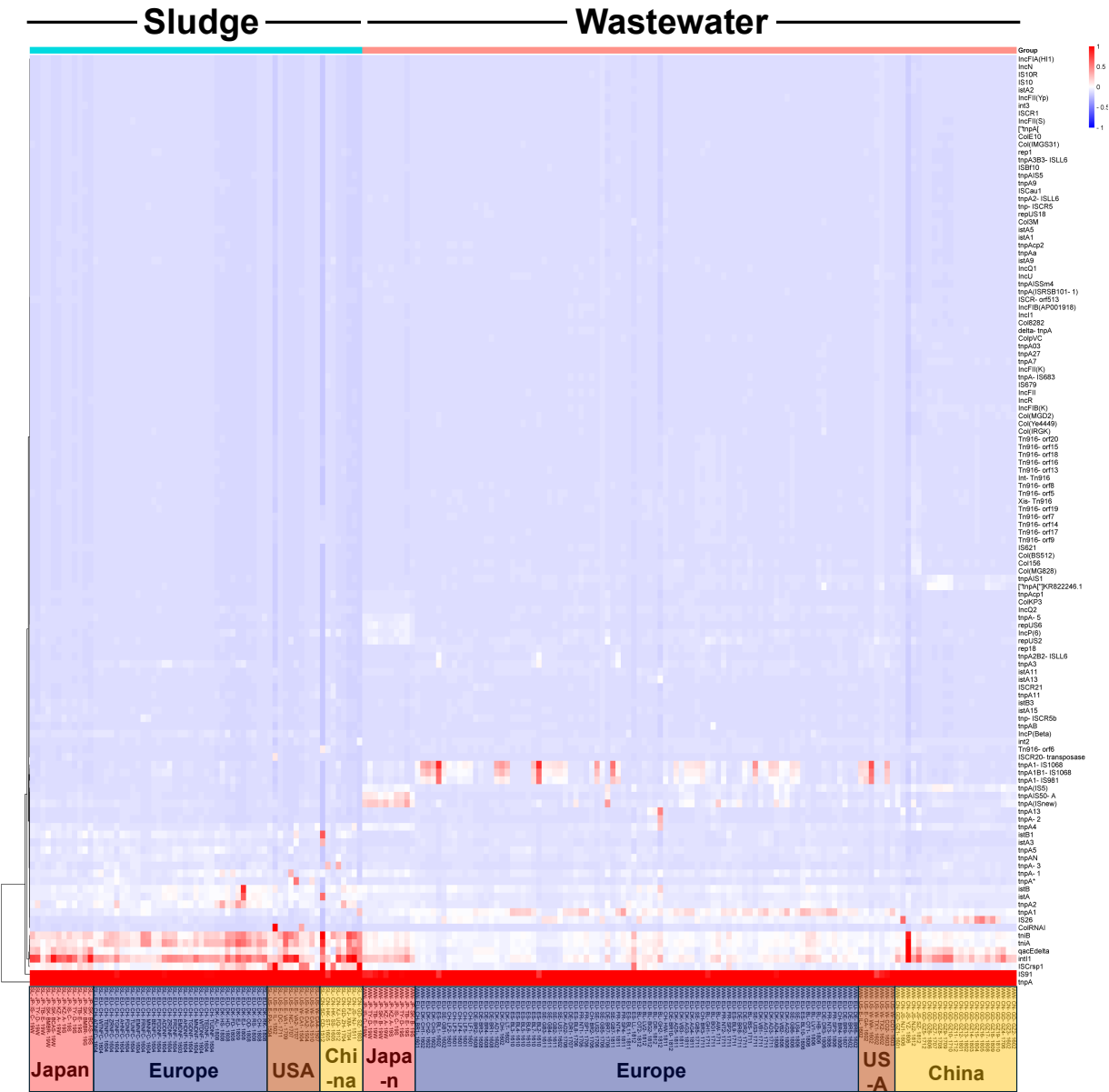
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(g) Multidrug efflux



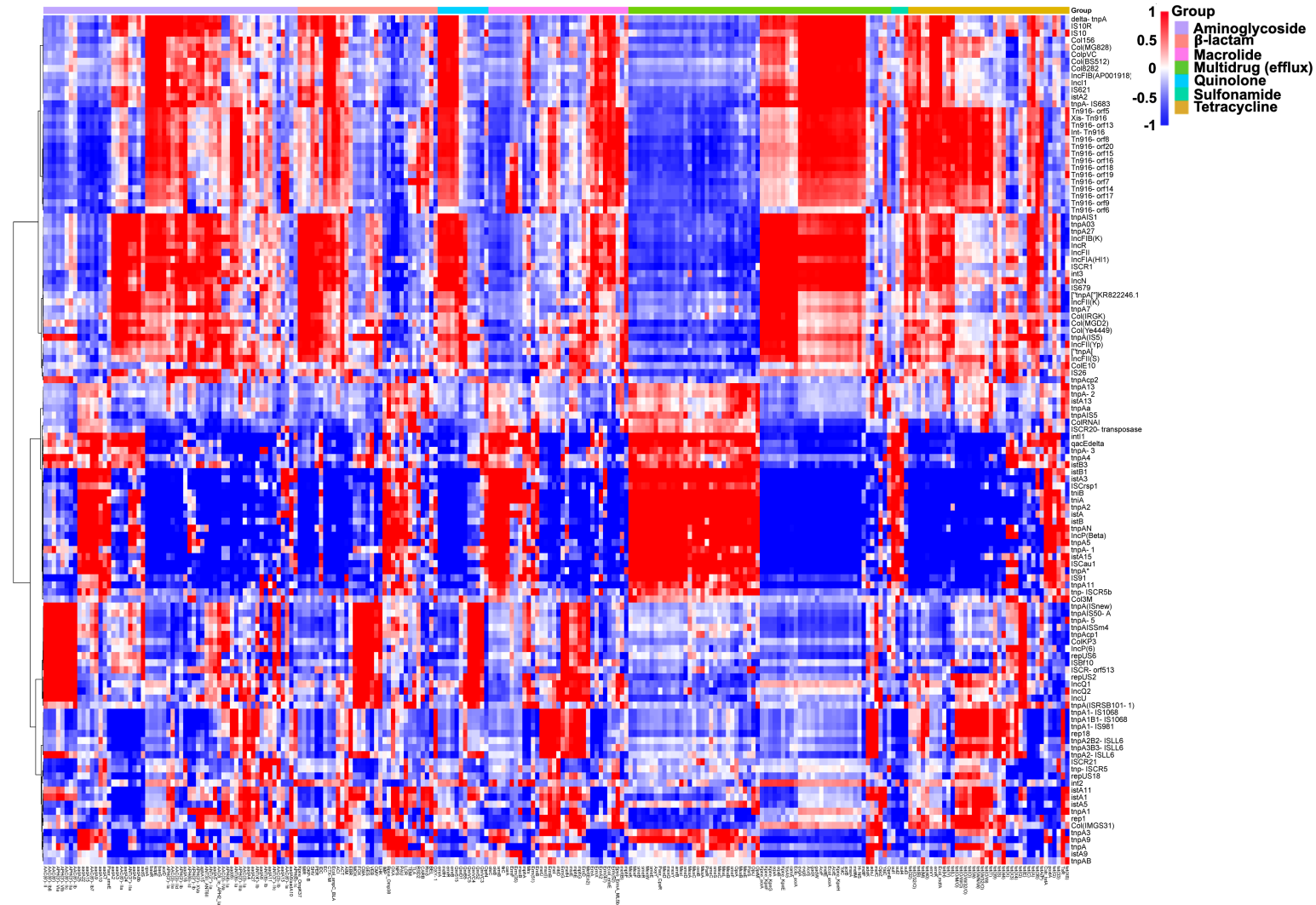
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(a)

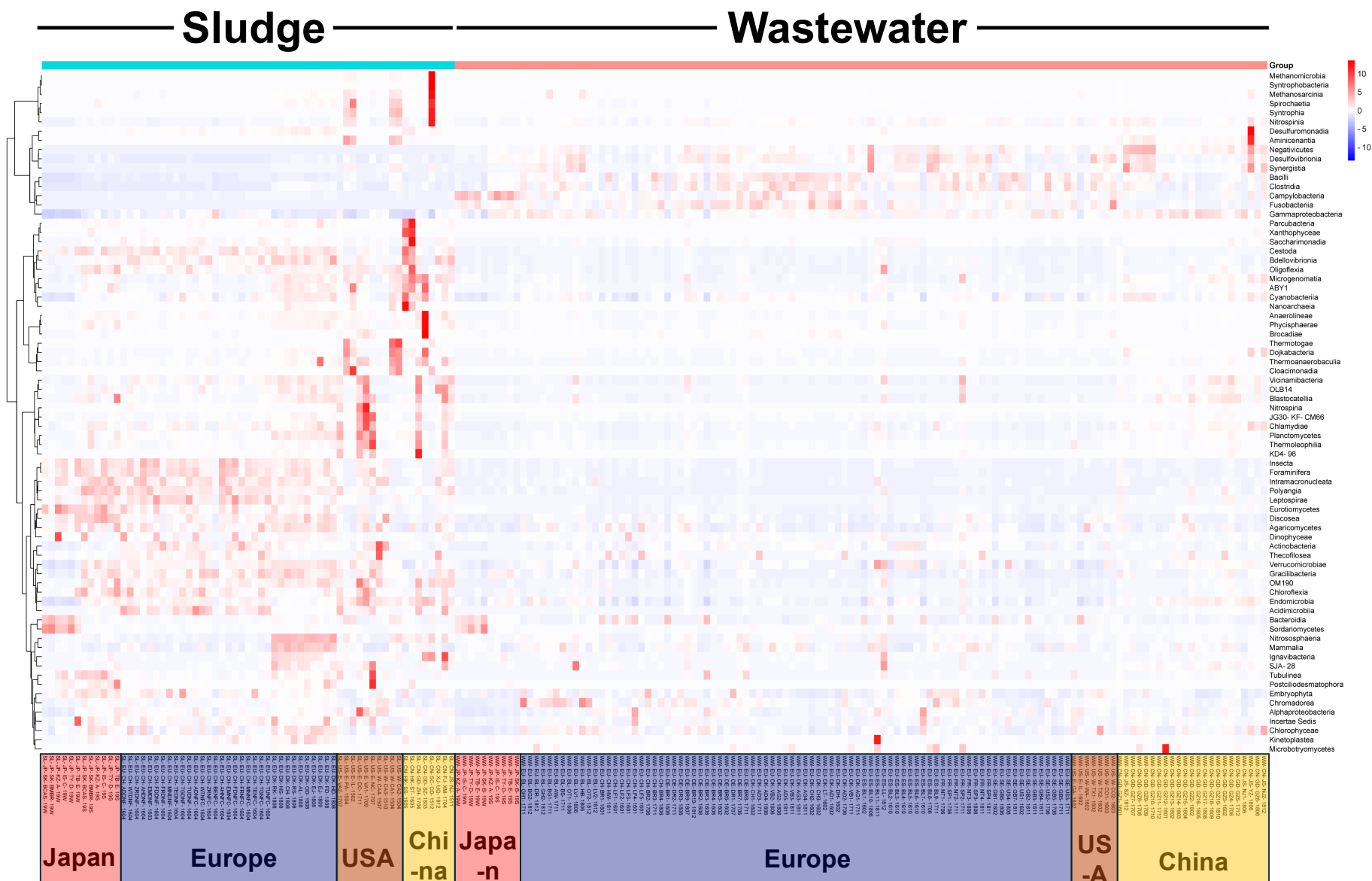


Supplementary Figure 2. Profiles of mobile genetic elements (MGEs) in activated sludge and untreated wastewater. Heatmap on normalized abundances of MGEs per 16S-based microbial population in activated sludge and influent wastewater (a). Heatmap on correlation coefficients between proportions of ARGs and MGEs (b).

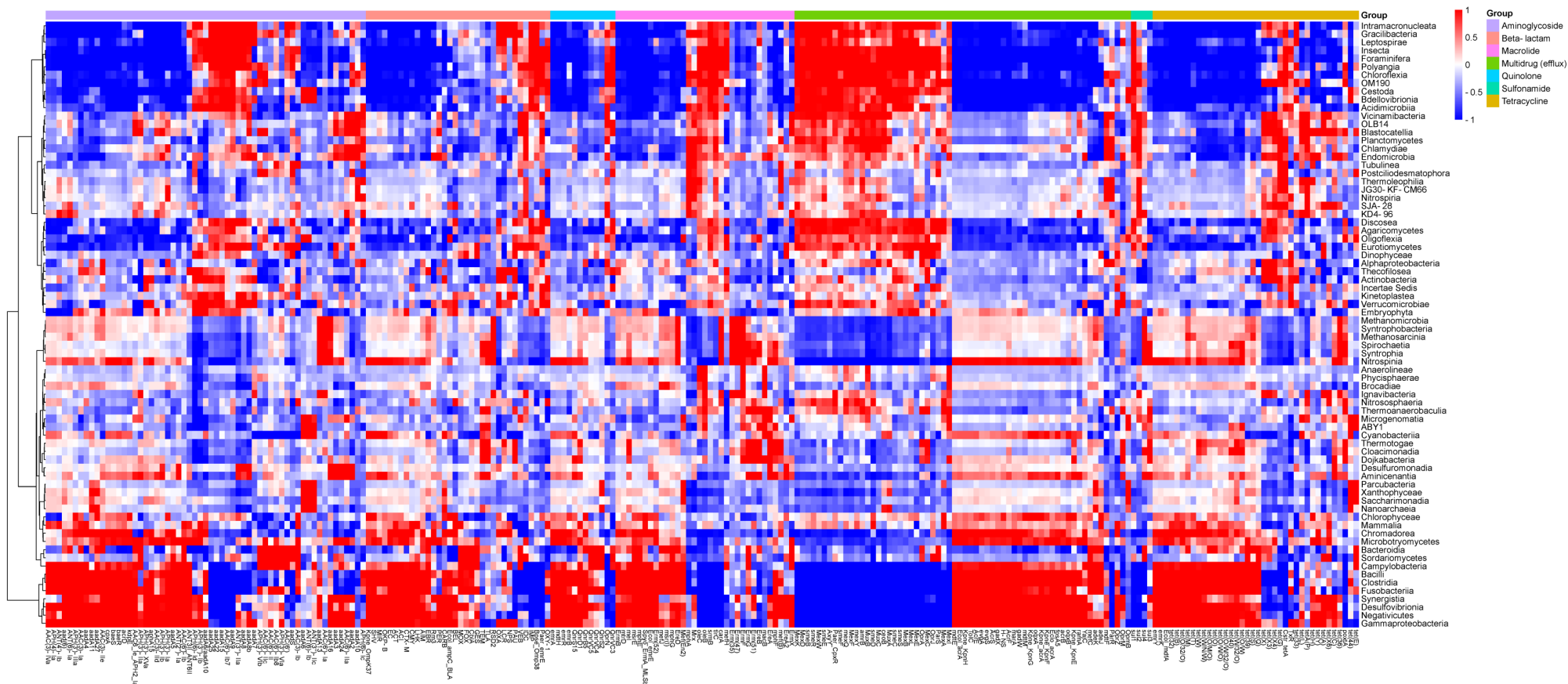
(b)



Supplementary Figure 2 (cont'd). Profiles of mobile genetic elements (MGEs) in activated sludge and untreated wastewater. Heatmap on normalized abundances of MGEs per 16S-based microbial population in activated sludge and influent wastewater (a). Heatmap on correlation coefficients between proportions of ARGs and MGEs (b).



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Supplementary Figure 4. Correlation coefficients between the relative abundance of ARGs and phylogenetic classes, which were present at >0.001% abundance in at least one of the samples.

Supplementary Table 1. Information of sample and sequence data.

Sample Name	Sample Type	INDSC Database	Accession ID	Continent	Country	City	Date of Sampling	Treatment Process	Raw sequence reads	Quality sequence reads	Total 16S reads
SL-CN-FJ-XM-1704	Activated sludge	NCBI	SRR9852197	Asia	China	Xiamen, Fujian	2017/4/16	NA	120,301,974	119,619,002	58,419
SL-CN-GD-ZH-1704	Activated sludge	NCBI	SRR9852172	Asia	China	Zhuhai, Guang Dong	2017/4/23	NA	108,945,344	108,187,482	51,961
SL-CN-JS-NJ-1511	Activated sludge	NCBI	SRR9852211	Asia	China	Nanjing, Jiangsu	2015/11/1	NA	65,908,236	65,399,388	33,817
SL-CN-SC-CD-1512	Activated sludge	NCBI	SRR5007460	Asia	China	Chengdu, Sichuan	2015/12/16	NA	75,426,040	73,776,194	51,019
SL-CN-XJ-UQ-1812	Activated sludge	NCBI	SRR9038830	Asia	China	Urumqi, Xinjiang	2018/12/9	NA	44,208,110	43,740,572	34,439
SL-EU-CH-ADDNF-1604	Activated sludge	ENA	ERR2814987, ERR2814994	Europe	Switzerland	Altdorf	2016/4/20	CND	39,857,890	38,125,082	29,348
SL-EU-CH-ADNFC-1604	Activated sludge	ENA	ERR2808645, ERR2808647	Europe	Switzerland	Altdorf	2016/4/20	CND	38,585,992	36,981,352	24,902
SL-EU-CH-AHDNF-1604	Activated sludge	ENA	ERR2815000, ERR2814993	Europe	Switzerland	Altenrhein	2016/4/29	CND	36,500,788	35,103,322	23,149
SL-EU-CH-AHNFC-1604	Activated sludge	ENA	ERR2808660, ERR2808651	Europe	Switzerland	Altenrhein	2016/4/29	CND	39,650,836	38,162,030	22,849
SL-EU-CH-BENFC-1604	Activated sludge	ENA	ERR2808668, ERR2808654	Europe	Switzerland	Bern	2016/4/21	CND	44,536,120	42,981,124	74,702
SL-EU-CH-EMDNF-1603	Activated sludge	ENA	ERR2814992, ERR2815006	Europe	Switzerland	Emmen	2016/3/17	CND	43,122,946	41,582,128	25,470
SL-EU-CH-EMNFC-1604	Activated sludge	ENA	ERR2808665, ERR2808663	Europe	Switzerland	Emmen	2016/3/17	CND	37,946,440	36,414,170	20,207
SL-EU-CH-FDDNF-1604	Activated sludge	ENA	ERR2814986, ERR2814996	Europe	Switzerland	Fallanden	2016/4/15	CND	39,242,066	37,764,648	32,399
SL-EU-CH-FDNFC-1604	Activated sludge	ENA	ERR2808662, ERR2808656	Europe	Switzerland	Fallanden	2016/4/15	CND	41,059,582	39,069,402	30,587
SL-EU-CH-FRDNF-1604	Activated sludge	ENA	ERR2814991, ERR2815003	Europe	Switzerland	Fribourg	2016/4/28	CND	37,682,742	36,310,308	30,506
SL-EU-CH-FRNFC-1604	Activated sludge	ENA	ERR2808666, ERR2808659	Europe	Switzerland	Fribourg	2016/4/28	CND	43,078,428	41,268,504	34,441
SL-EU-CH-MNDNF-1604	Activated sludge	ENA	ERR2815002, ERR2814998	Europe	Switzerland	Menznau	2016/4/4	CND	40,822,464	39,587,928	34,231
SL-EU-CH-MNNFC-1604	Activated sludge	ENA	ERR2808667, ERR2808653	Europe	Switzerland	Menznau	2016/4/4	CND	37,066,886	35,842,590	29,794
SL-EU-CH-TEDNF-1604	Activated sludge	ENA	ERR2815005, ERR2814999	Europe	Switzerland	Triengen	2016/4/13	CND	40,164,758	38,751,788	27,773
SL-EU-CH-TENFC-1604	Activated sludge	ENA	ERR2808648, ERR2808650	Europe	Switzerland	Triengen	2016/4/13	CND	40,598,196	39,365,866	32,328

SL-EU-CH-TGDNF-1604	Activated sludge	ENA	ERR2815001, ERR2814990	Europe	Switzerland	Turgi	2016/4/5	CND	41,211,756	39,585,482	26,290
SL-EU-CH-TGNFC-1604	Activated sludge	ENA	ERR2808657, ERR2808655	Europe	Switzerland	Turgi	2016/4/5	CND	38,380,184	37,091,884	27,354
SL-EU-CH-TUDNF-1604	Activated sludge	ENA	ERR2815007, ERR2814995	Europe	Switzerland	Thun	2016/4/19	CND	42,361,878	40,587,262	31,855
SL-EU-CH-TUNFC-1604	Activated sludge	ENA	ERR2808664, ERR2808649	Europe	Switzerland	Thun	2016/4/19	CND	35,922,568	34,524,590	26,869
SL-EU-CH-WTDNF-1604	Activated sludge	ENA	ERR2815004, ERR2814989	Europe	Switzerland	Winterthur	2016/4/7	CND	38,596,332	37,092,092	22,724
SL-EU-CH-WTNFC-1604	Activated sludge	ENA	ERR2808646, ERR2808661	Europe	Switzerland	Winterthur	2016/4/7	CND	35,485,292	33,894,554	22,775
SL-EU-CH-ZRDNF-1604	Activated sludge	ENA	ERR2814988, ERR2814997	Europe	Switzerland	Zurich	2016/4/12	CND	42,302,646	40,579,470	31,880
SL-EU-CH-ZRNFC-1604	Activated sludge	ENA	ERR2808652, ERR2808658	Europe	Switzerland	Zurich	2016/4/12	CND	41,740,594	40,118,870	33,681
SL-EU-DK-AL-1808	Activated sludge	NCBI	SRR11674009	Europe	Denmark	Aalborg	2018/8/29	NA	224,209,072	220,449,658	150,143
SL-EU-DK-CH-1808	Activated sludge	NCBI	SRR11674051	Europe	Denmark	Copenhagen	2018/8/29	NA	221,393,394	218,055,096	153,028
SL-EU-DK-EJ-1808	Activated sludge	NCBI	SRR11674041	Europe	Denmark	Esbjerg	2018/9/7	NA	158,493,062	155,700,782	134,326
SL-EU-DK-EJ-1809	Activated sludge	NCBI	SRR11674038	Europe	Denmark	Esbjerg	2018/8/29	NA	160,911,714	158,696,774	109,829
SL-EU-DK-FD-1808	Activated sludge	NCBI	SRR11674035	Europe	Denmark	Fredericia	2018/8/29	NA	195,419,532	192,043,762	115,134
SL-EU-DK-HD-1808	Activated sludge	NCBI	SRR11674031	Europe	Denmark	Haderslev	2018/8/29	NA	231,054,940	227,649,096	150,962
SL-EU-DK-HS-1808	Activated sludge	NCBI	SRR11674025	Europe	Denmark	Hirtshals	2018/8/29	NA	167,074,062	163,225,590	111,651
SL-EU-DK-OD-1808	Activated sludge	NCBI	SRR11674044	Europe	Denmark	Odense	2018/8/29	NA	195,095,634	191,903,226	122,796
SL-EU-DK-RK-1808	Activated sludge	NCBI	SRR11674054	Europe	Denmark	Roskilde	2018/8/29	NA	177,276,974	174,784,032	140,855
SL-EU-DK-RS-1808	Activated sludge	NCBI	SRR11674048	Europe	Denmark	Risskov	2018/8/28	NA	198,372,884	195,118,374	136,320
SL-JP-IS-C-19S	Activated sludge	DDBJ	DRR274958	Asia	Japan	NA	2019/8/9	EBPR	43,107,402	42,598,926	23,239
SL-JP-IS-C-19W	Activated sludge	DDBJ	DRR213767	Asia	Japan	NA	2019/2/4	EBPR	49,774,226	46,155,544	94,504
SL-JP-KZ-A-19S	Activated sludge	DDBJ	DRR274955	Asia	Japan	NA	2019/8/9	CAS	42,824,652	42,318,540	28,393
SL-JP-KZ-A-19W	Activated sludge	DDBJ	DRR213780	Asia	Japan	NA	2019/2/4	CAS	54,041,134	50,059,226	103,184
SL-JP-SK-BCAS-19S	Activated sludge (CAS)	DDBJ	DRR274968	Asia	Japan	NA	2019/8/9	MBR	41,559,498	41,134,430	23,235
SL-JP-SK-BCAS-19W	Activated sludge (CAS)	DDBJ	DRR213777	Asia	Japan	NA	2019/2/4	MBR	50,054,806	44,700,706	134,733

SL-JP-SK-BMBR-19S	Activated sludge (MBR)	DDBJ	DRR274967	Asia	Japan	NA	2019/8/9	MBR	33,856,182	33,588,894	17,442
SL-JP-SK-BMBR-19W	Activated sludge (MBR)	DDBJ	DRR213776	Asia	Japan	NA	2019/2/4	MBR	42,735,306	36,672,360	148,115
SL-JP-TB-E-19S	Activated sludge	DDBJ	DRR274964	Asia	Japan	NA	2019/8/9	OD	41,679,270	41,258,062	19,092
SL-JP-TB-E-19W	Activated sludge	DDBJ	DRR213773	Asia	Japan	NA	2019/2/4	OD	42,231,346	40,067,456	42,719
SL-JP-TY-D-19S	Activated sludge	DDBJ	DRR274961	Asia	Japan	NA	2019/8/9	A2O	35,703,174	35,355,430	21,755
SL-JP-TY-D-19W	Activated sludge	DDBJ	DRR213770	Asia	Japan	NA	2019/2/4	A2O	39,868,326	34,556,832	155,498
SL-US-E-DC-1711	Activated sludge	NCBI	SRR9827759	Eastern USA	USA	Washington DC	Nov-17	CAS	110,151,404	104,021,036	52,222
SL-US-E-NC-1707	Activated sludge	NCBI	SRR9827769	Eastern USA	USA	North Carolina	Jul-17	CAS	1,467,906	1,389,776	678
SL-US-E-PA-1504	Activated sludge	NCBI	SRR5007116	Eastern USA	USA	Philadelphia, Pennsylvania	2015/4/22	AD	112,075,678	109,033,538	101,381
SL-US-E-VA1-1709	Activated sludge	NCBI	SRR9827765	Eastern USA	USA	Virginia	Sep-17	CAS	82,782,534	78,201,308	32,236
SL-US-E-VA2-1707	Activated sludge	NCBI	SRR9827774	Eastern USA	USA	Virginia	Jul-17	CAS	64,691,476	59,484,814	32,442
SL-US-W-CA3-1510	Activated sludge	NCBI	SRR8351382	Western USA	USA	Oakland, California	2015/7/21	NA	196,648,240	189,615,828	182,740
SL-US-W-CA4-1507	Activated sludge	NCBI	SRR8351385	Western USA	USA	Oakland, California	2015/10/27	NA	323,297,686	311,605,266	236,377
WW-CN-GD-GZ1-1601	Wastewater	ENA	ERR2607423	Asia	China	Guangzhou, Guang Dong	2016/1/28	NA	104,694,020	101,738,622	136,309
WW-CN-GD-GZ10-1710	Wastewater	ENA	ERR8977354	Asia	China	Guangzhou, Guang Dong	2017/10/13	NA	60,798,646	59,802,928	114,753
WW-CN-GD-GZ11-1712	Wastewater	ENA	ERR8977383	Asia	China	Guangzhou, Guang Dong	2017/12/21	NA	53,966,134	53,329,202	120,096
WW-CN-GD-GZ12-1801	Wastewater	ENA	ERR8977384	Asia	China	Guangzhou, Guang Dong	2018/1/10	NA	63,254,790	61,967,566	173,525
WW-CN-GD-GZ13-1802	Wastewater	ENA	ERR8977385	Asia	China	Guangzhou, Guang Dong	2018/2/8	NA	66,861,286	66,226,816	167,943
WW-CN-GD-GZ14-1803	Wastewater	ENA	ERR8977386	Asia	China	Guangzhou, Guang Dong	2018/3/18	NA	58,620,288	58,011,256	114,998
WW-CN-GD-GZ15-1804	Wastewater	ENA	ERR8977416	Asia	China	Guangzhou, Guang Dong	2018/4/10	NA	86,829,740	85,691,114	253,506
WW-CN-GD-GZ16-1805	Wastewater	ENA	ERR8977417	Asia	China	Guangzhou, Guang Dong	2018/5/11	NA	100,752,192	99,830,206	271,225
WW-CN-GD-GZ17-1808	Wastewater	ENA	ERR8977418	Asia	China	Guangzhou, Guang Dong	2018/8/9	NA	67,874,418	67,335,736	137,640
WW-CN-GD-GZ18-1809	Wastewater	ENA	ERR8977419	Asia	China	Guangzhou, Guang Dong	2018/9/1	NA	91,847,518	90,777,046	175,085
WW-CN-GD-GZ19-1810	Wastewater	ENA	ERR8977420	Asia	China	Guangzhou, Guang Dong	2018/10/9	NA	94,098,252	93,376,448	157,997

WW-CN-GD-GZ2-1602	Wastewater	ENA	ERR9855099	Asia	China	Guangzhou, Guang Dong	2016/2/2	NA	75,145,492	74,883,352	85,405
WW-CN-GD-GZ3-1602	Wastewater	ENA	ERR9855084	Asia	China	Guangzhou, Guang Dong	2016/2/2	NA	54,221,990	54,068,136	63,381
WW-CN-GD-GZ4-1706	Wastewater	ENA	ERR9834200	Asia	China	Guangzhou, Guang Dong	2017/6/16	NA	106,818,460	106,426,174	124,879
WW-CN-GD-GZ5-1712	Wastewater	ENA	ERR4682465	Asia	China	Guangzhou, Guang Dong	2017/12/21	NA	76,220,212	75,431,950	144,392
WW-CN-GD-GZ6-1806	Wastewater	ENA	ERR4682898	Asia	China	Guangzhou, Guang Dong	2018/6/5	NA	81,554,262	80,021,572	141,261
WW-CN-GD-GZ7-1707	Wastewater	ENA	ERR8977351	Asia	China	Guangzhou, Guang Dong	2017/7/11	NA	68,992,840	68,127,498	136,349
WW-CN-GD-GZ8-1708	Wastewater	ENA	ERR8977352	Asia	China	Guangzhou, Guang Dong	2017/8/23	NA	71,153,414	69,782,772	136,226
WW-CN-GD-GZ9-1709	Wastewater	ENA	ERR8977353	Asia	China	Guangzhou, Guang Dong	2017/9/30	NA	71,370,132	70,372,672	135,458
WW-CN-JS-NJ1-1806	Wastewater	ENA	ERR4682827	Asia	China	Nanjing, Jiangsu	2018/6/21	NA	74,100,332	73,245,400	214,582
WW-CN-JS-NJ2-1812	Wastewater	ENA	ERR4678619	Asia	China	Nanjing, Jiangsu	2018/12/27	NA	108,003,504	106,886,946	229,653
WW-CN-JS-SZ-1812	Wastewater	ENA	ERR4678623	Asia	China	Suzhou, Jiangsu	2018/12/27	NA	161,413,106	159,674,146	351,040
WW-CN-JS-YZ-1806	Wastewater	ENA	ERR4682828	Asia	China	Yangzhou, Jiangsu	2018/6/22	NA	84,510,590	83,148,666	145,163
WW-EU-BL-AW-1711	Wastewater	ENA	ERR4682345	Europe	Belgium	Antwerp South	2017/11/21	NA	120,235,570	118,480,512	259,514
WW-EU-BL-BG1-1711	Wastewater	ENA	ERR4682344	Europe	Belgium	Bruges	2017/11/22	NA	76,500,282	75,052,282	208,507
WW-EU-BL-BG2-1812	Wastewater	ENA	ERR4678603	Europe	Belgium	Bruges	2018/12/5	NA	82,361,364	81,871,774	199,593
WW-EU-BL-BS-1711	Wastewater	ENA	ERR4682398	Europe	Belgium	Brussels	2017/11/23	NA	55,249,598	54,688,912	131,789
WW-EU-BL-DR-1812	Wastewater	ENA	ERR4678604	Europe	Belgium	Deurne	2018/12/5	NA	76,077,776	75,448,792	149,235
WW-EU-BL-GH1-1711	Wastewater	ENA	ERR4682343	Europe	Belgium	Ghent	2017/11/22	NA	99,371,696	98,076,642	217,523
WW-EU-BL-GH2-1812	Wastewater	ENA	ERR4678605	Europe	Belgium	Ghent	2018/12/5	NA	82,129,646	81,363,668	185,938
WW-EU-BL-HB-1806	Wastewater	ENA	ERR4682783	Europe	Belgium	Harelbeke	2018/6/12	NA	87,955,392	86,984,160	152,933
WW-EU-BL-LV1-1806	Wastewater	ENA	ERR4682782	Europe	Belgium	Leuven	2018/6/20	NA	72,805,532	72,209,124	69,358
WW-EU-BL-LV2-1812	Wastewater	ENA	ERR4678602	Europe	Belgium	Leuven	2018/12/12	NA	86,029,902	85,480,048	156,917
WW-EU-BL-OT1-1806	Wastewater	ENA	ERR4682781	Europe	Belgium	Ostend	2018/6/12	NA	75,747,482	74,913,382	164,069
WW-EU-BL-OT2-1812	Wastewater	ENA	ERR4678601	Europe	Belgium	Ostend	2018/12/9	NA	76,036,220	75,559,026	190,075

WW-EU-CH-BR1-1601	Wastewater	ENA	ERR1713343	Europe	Switzerland	Bern	2016/1/27	NA	22,838,510	21,315,788	168,450
WW-EU-CH-BR2-1706	Wastewater	ENA	ERR2683218	Europe	Switzerland	Bern	2017/6/11	NA	69,885,826	69,369,526	169,815
WW-EU-CH-BR3-1711	Wastewater	ENA	ERR4682340	Europe	Switzerland	Bern	2017/11/21	NA	143,946,026	142,177,344	387,673
WW-EU-CH-BR4-1806	Wastewater	ENA	ERR4682829	Europe	Switzerland	Bern	2018/6/28	NA	92,909,180	91,743,036	194,637
WW-EU-CH-HW-1811	Wastewater	ENA	ERR4678626	Europe	Switzerland	Herrenschwanden	2018/11/22	NA	137,676,584	136,731,144	288,859
WW-EU-CH-LF1-1601	Wastewater	ENA	ERR2607422	Europe	Switzerland	Liebefeld	2016/1/27	NA	22,855,670	21,332,700	168,486
WW-EU-CH-LF2-1601	Wastewater	ENA	ERR2607418	Europe	Switzerland	Liebefeld	2016/1/27	NA	9,118,594	8,530,508	21,844
WW-EU-CH-LF3-1601	Wastewater	ENA	ERR2607419	Europe	Switzerland	Liebefeld	2016/1/27	NA	5,940,172	5,612,576	21,722
WW-EU-CH-LF4-1601	Wastewater	ENA	ERR2607420	Europe	Switzerland	Liebefeld	2016/1/27	NA	23,382,322	19,408,598	2,818,802
WW-EU-CH-LF5-1601	Wastewater	ENA	ERR2607421	Europe	Switzerland	Liebefeld	2016/1/27	NA	25,058,032	22,633,432	52,749
WW-EU-DE-BR1-1608	Wastewater	ENA	ERR2607435	Europe	Germany	Berlin	2016/8/2	NA	21,782,508	17,573,626	372,234
WW-EU-DE-BR10-1812	Wastewater	ENA	ERR4678635	Europe	Germany	Berlin	2018/12/13	NA	82,960,028	82,440,092	168,217
WW-EU-DE-BR2-1608	Wastewater	ENA	ERR2607431	Europe	Germany	Berlin	2016/8/2	NA	14,508,476	13,340,430	37,138
WW-EU-DE-BR3-1608	Wastewater	ENA	ERR2607433	Europe	Germany	Berlin	2016/8/2	NA	48,726,360	39,811,852	2,110,880
WW-EU-DE-BR4-1608	Wastewater	ENA	ERR2607434	Europe	Germany	Berlin	2016/8/2	NA	14,699,158	13,670,846	31,551
WW-EU-DE-BR5-1602	Wastewater	ENA	ERR9855089	Europe	Germany	Berlin	2016/2/9	NA	60,804,422	60,621,408	119,201
WW-EU-DE-BR6-1602	Wastewater	ENA	ERR9855092	Europe	Germany	Berlin	2016/2/9	NA	61,057,318	60,851,550	117,478
WW-EU-DE-BR7-1706	Wastewater	ENA	ERR2683253	Europe	Germany	Berlin	2017/6/14	NA	111,515,298	110,928,884	171,510
WW-EU-DE-BR8-1711	Wastewater	ENA	ERR4682375	Europe	Germany	Berlin	2017/11/28	NA	82,971,882	82,268,258	126,624
WW-EU-DE-BR9-1807	Wastewater	ENA	ERR4682882	Europe	Germany	Berlin	2018/7/4	NA	104,395,630	103,513,192	116,481
WW-EU-DE-DR1-1707	Wastewater	ENA	ERR2683209	Europe	Germany	Dresden	2017/7/9	NA	74,136,604	73,558,022	166,911
WW-EU-DE-DR2-1711	Wastewater	ENA	ERR4682377	Europe	Germany	Dresden	2017/11/29	NA	84,075,358	83,267,476	191,848
WW-EU-DE-DR3-1806	Wastewater	ENA	ERR4682786	Europe	Germany	Dresden	2018/6/21	NA	77,542,252	76,144,250	163,642
WW-EU-DK-AD1-1602	Wastewater	ENA	ERR2607436	Europe	Denmark	Avedore	2016/2/3	NA	105,630,416	102,418,646	206,862

WW-EU-DK-AD2-1706	Wastewater	ENA	ERR2683197	Europe	Denmark	Avedere	2017/6/15	NA	63,284,466	62,645,246	168,502
WW-EU-DK-AD3-1711	Wastewater	ENA	ERR4682464	Europe	Denmark	Avedere	2017/11/18	NA	63,845,530	63,365,560	147,292
WW-EU-DK-AD4-1806	Wastewater	ENA	ERR4682773	Europe	Denmark	Avedere	2018/6/11	NA	94,280,696	93,137,474	263,193
WW-EU-DK-AD5-1811	Wastewater	ENA	ERR4678671	Europe	Denmark	Avedere	2018/11/18	NA	112,905,018	111,357,372	334,108
WW-EU-DK-AG1-1711	Wastewater	ENA	ERR4682463	Europe	Denmark	Amager	2017/11/18	NA	79,634,036	78,828,416	196,886
WW-EU-DK-AG2-1806	Wastewater	ENA	ERR4682775	Europe	Denmark	Amager	2018/6/11	NA	107,450,120	106,781,730	277,933
WW-EU-DK-AG3-1811	Wastewater	ENA	ERR4678673	Europe	Denmark	Amager	2018/11/18	NA	47,353,118	47,088,284	117,900
WW-EU-DK-AG4-1811	Wastewater	ENA	ERR4678674	Europe	Denmark	Amager	2018/11/18	NA	67,696,244	67,295,088	169,127
WW-EU-DK-CH1-1602	Wastewater	ENA	ERR1713349	Europe	Denmark	Copenhagen	2016/2/3	NA	105,568,222	102,357,148	206,806
WW-EU-DK-CH2-1602	Wastewater	ENA	ERR1713350	Europe	Denmark	Copenhagen	2016/2/3	NA	119,189,178	116,091,652	272,796
WW-EU-DK-CH3-1602	Wastewater	ENA	ERR1713351	Europe	Denmark	Copenhagen	2016/2/3	NA	55,299,398	53,798,810	119,865
WW-EU-DK-DH-1602	Wastewater	ENA	ERR2607437	Europe	Denmark	Damhusaaen	2016/2/3	NA	119,305,388	116,206,806	272,856
WW-EU-DK-LT-1602	Wastewater	ENA	ERR2607438	Europe	Denmark	Lynetten	2016/2/3	NA	55,366,594	53,865,398	119,955
WW-EU-DK-VB1-1711	Wastewater	ENA	ERR4682462	Europe	Denmark	Valby	2017/11/18	NA	62,059,984	61,089,618	171,081
WW-EU-DK-VB2-1806	Wastewater	ENA	ERR4682774	Europe	Denmark	Valby	2018/6/11	NA	104,471,920	102,939,044	245,539
WW-EU-DK-VB3-1811	Wastewater	ENA	ERR4678672	Europe	Denmark	Valby	2018/11/18	NA	62,168,750	61,302,964	167,017
WW-EU-ES-BL1-1602	Wastewater	ENA	ERR1713354	Europe	Spain	Barcelona	2016/2/10	NA	34,447,192	32,418,728	240,874
WW-EU-ES-BL10-1806	Wastewater	ENA	ERR4682780	Europe	Spain	Barcelona	2018/6/21	NA	104,254,798	103,469,174	157,183
WW-EU-ES-BL11-1811	Wastewater	ENA	ERR4678573	Europe	Spain	Barcelona	2018/11/29	NA	87,558,762	86,749,550	84,858
WW-EU-ES-BL2-1610	Wastewater	ENA	ERR2607447	Europe	Spain	Barcelona	2016/10/2	NA	34,501,462	32,472,490	240,938
WW-EU-ES-BL3-1610	Wastewater	ENA	ERR2607442	Europe	Spain	Barcelona	2016/10/2	NA	66,196,302	64,744,308	94,554
WW-EU-ES-BL4-1610	Wastewater	ENA	ERR2607443	Europe	Spain	Barcelona	2016/10/2	NA	61,486,266	60,021,458	88,615
WW-EU-ES-BL5-1610	Wastewater	ENA	ERR2607444	Europe	Spain	Barcelona	2016/10/2	NA	27,732,326	26,216,620	38,340
WW-EU-ES-BL6-1610	Wastewater	ENA	ERR2607445	Europe	Spain	Barcelona	2016/10/2	NA	26,038,608	24,512,800	35,585

WW-EU-ES-BL7-1610	Wastewater	ENA	ERR2607446	Europe	Spain	Barcelona	2016/10/2	NA	61,444,694	59,082,272	1,155,570
WW-EU-ES-BL8-1706	Wastewater	ENA	ERR2683214	Europe	Spain	Barcelona	2017/6/8	NA	127,486,032	126,430,722	255,579
WW-EU-ES-BL9-1711	Wastewater	ENA	ERR4682364	Europe	Spain	Barcelona	2017/11/30	NA	83,602,852	82,586,440	154,245
WW-EU-ES-LL-1812	Wastewater	ENA	ERR4678574	Europe	Spain	Lleida	2018/12/3	NA	86,595,004	86,002,264	69,084
WW-EU-FR-NT1-1706	Wastewater	ENA	ERR2683216	Europe	France	Nantes	2017/6/19	NA	85,105,120	84,651,670	189,902
WW-EU-FR-NT2-1711	Wastewater	ENA	ERR4682347	Europe	France	Nantes	2017/11/20	NA	138,369,044	137,182,320	328,936
WW-EU-FR-NT3-1806	Wastewater	ENA	ERR4682788	Europe	France	Nantes	2018/6/11	NA	103,502,248	101,400,272	214,594
WW-EU-FR-NT4-1811	Wastewater	ENA	ERR4678565	Europe	France	Nantes	2018/11/8	NA	80,225,530	79,443,010	214,754
WW-EU-FR-SP1-1706	Wastewater	ENA	ERR2683217	Europe	France	Saint Philibert	2017/6/20	NA	64,707,698	64,252,606	139,386
WW-EU-FR-SP2-1711	Wastewater	ENA	ERR4682348	Europe	France	Saint Philibert	2017/11/22	NA	148,593,892	147,389,066	147,847
WW-EU-FR-SP3-1806	Wastewater	ENA	ERR4682789	Europe	France	Saint Philibert	2018/6/20	NA	76,648,044	75,396,470	145,041
WW-EU-FR-SP4-1811	Wastewater	ENA	ERR4678566	Europe	France	Saint Philibert	2018/11/26	NA	106,401,992	105,724,242	219,512
WW-EU-SE-GB1-1602	Wastewater	ENA	ERR1713392	Europe	Sweden	Gothenburg	2016/2/11	NA	72,677,334	68,321,136	635,033
WW-EU-SE-GB2-1611	Wastewater	ENA	ERR2607539	Europe	Sweden	Gothenburg	2016/11/2	NA	72,727,920	68,371,244	635,123
WW-EU-SE-GB3-1611	Wastewater	ENA	ERR2607537	Europe	Sweden	Gothenburg	2016/11/2	NA	44,066,812	42,898,808	156,186
WW-EU-SE-GB4-1611	Wastewater	ENA	ERR2607538	Europe	Sweden	Gothenburg	2016/11/2	NA	19,087,754	17,872,800	62,855
WW-EU-SE-GB5-1706	Wastewater	ENA	ERR2683250	Europe	Sweden	Gothenburg	2017/6/8	NA	67,391,606	67,002,404	187,154
WW-EU-SE-GB5-1711	Wastewater	ENA	ERR4682333	Europe	Sweden	Gothenburg	2017/11/19	NA	108,469,296	107,295,064	278,691
WW-EU-SE-GB6-1806	Wastewater	ENA	ERR4682776	Europe	Sweden	Gothenburg	2018/6/15	NA	120,551,584	116,404,820	261,185
WW-EU-SE-GB7-1811	Wastewater	ENA	ERR4678560	Europe	Sweden	Gothenburg	2018/6/15	NA	120,791,142	119,760,542	344,561
WW-EU-SE-US1-1602	Wastewater	ENA	ERR2607540	Europe	Sweden	Uppsala	2016/2/2	NA	99,217,324	96,663,566	237,920
WW-EU-SE-US2-1706	Wastewater	ENA	ERR2683220	Europe	Sweden	Uppsala	2017/6/12	NA	96,275,900	95,686,826	231,732
WW-EU-SE-US3-1711	Wastewater	ENA	ERR4682403	Europe	Sweden	Uppsala	2017/11/27	NA	95,525,130	94,785,920	244,011
WW-EU-SE-US4-1806	Wastewater	ENA	ERR4682777	Europe	Sweden	Uppsala	2018/6/18	NA	103,341,558	101,033,788	209,290

WW-JP-IS-C-19S	Wastewater	DDBJ	DRR274957	Asia	Japan	NA	43686	EBPR	37,302,142	36,947,894	83,685
WW-JP-IS-C-19W	Wastewater	DDBJ	DRR213766	Asia	Japan	NA	43500	EBPR	44,018,626	41,036,944	171,117
WW-JP-KZ-A-19S	Wastewater	DDBJ	DRR274954	Asia	Japan	NA	43686	CAS	42,406,540	42,014,942	94,210
WW-JP-KZ-A-19W	Wastewater	DDBJ	DRR213779	Asia	Japan	NA	43500	CAS	44,030,850	41,616,828	138,073
WW-JP-SK-B-19S	Wastewater	DDBJ	DRR274966	Asia	Japan	NA	43686	MBR	41,132,798	40,838,246	90,896
WW-JP-SK-B-19W	Wastewater	DDBJ	DRR213775	Asia	Japan	NA	43500	MBR	41,339,928	33,501,182	258,702
WW-JP-TB-E-19S	Wastewater	DDBJ	DRR274963	Asia	Japan	NA	43686	OD	39,426,972	39,018,002	66,061
WW-JP-TB-E-19W	Wastewater	DDBJ	DRR213772	Asia	Japan	NA	43500	OD	46,682,324	44,205,368	139,810
WW-JP-TY-D-19S	Wastewater	DDBJ	DRR274960	Asia	Japan	NA	43686	A2O	38,109,512	37,759,590	84,657
WW-JP-TY-D-19W	Wastewater	DDBJ	DRR213769	Asia	Japan	NA	43500	A2O	42,643,554	38,125,238	193,645
WW-US-E-GA-1602	Wastewater	ENA	ERR1713397	Eastern USA	USA	Atlanta, Georgia	42402	NA	89,242,094	86,799,648	163,460
WW-US-E-IL-1602	Wastewater	ENA	ERR1713399	Eastern USA	USA	Chicago, Illinois	42424	NA	38,021,060	33,521,346	590,316
WW-US-W-CO1-1603	Wastewater	ENA	ERR1713406	Western USA	USA	Boulder, Colorado	42457	NA	91,474,464	88,651,882	129,007
WW-US-W-CO2-1603	Wastewater	ENA	ERR1713405	Western USA	USA	Strasburg, Colorado	42457	NA	128,396,526	124,691,142	268,556
WW-US-W-TX1-1602	Wastewater	ENA	ERR1713400	Western USA	USA	El Paso, Texas	2016/2/23	NA	55,635,314	53,838,740	135,259
WW-US-W-TX2-1602	Wastewater	ENA	ERR1713402	Western USA	USA	El Paso, Texas	2016/2/22	NA	44,243,860	42,573,134	148,453
WW-US-W-WA-1602	Wastewater	ENA	ERR1713398	Western USA	USA	Seattle, Washington	2016/2/1	NA	46,722,572	44,990,264	164,669

CAS = conventional activated sludge; A2O = anaerobic/anoxic/oxic; EBPR = enhanced biological phosphorus removal; OD = oxidation ditch, MBR = membrane bioreactor; AD = anaerobic digestion; CND = combined nitrification-denitrification;

Supplementary Table 2. Average proportions of the core ARGs in activated sludge and untreated wastewater across countries. ARGs with an average proportion exceeding 1% in either wastewater or activated sludge were selected and indicated. *P*-values were calculated by Welch's t-test. ARGs with significantly higher proportion in activated sludge than in wastewater were indicated in bold.

ARG	Wastewater (n=124)		Activated Sludge (n=57)		<i>P</i> -value by t-test
	Average	Standard deviation	Average	Standard deviation	
<i>sul1</i>	3.2%	2.4%	9.9%	5.0%	<i>0.000**</i>
<i>OXA</i>	6.4%	2.3%	7.5%	5.8%	<i>0.156</i>
<i>qacEdelta1</i>	2.7%	1.9%	7.3%	3.6%	<i>0.000**</i>
<i>aadA6</i>	7.6%	4.9%	7.1%	12.5%	<i>0.796</i>
<i>AAC(6')-Ib7</i>	1.2%	0.6%	4.5%	2.5%	<i>0.000**</i>
<i>oleC</i>	0.3%	0.4%	4.4%	3.3%	<i>0.000**</i>
<i>oleB</i>	0.2%	0.3%	2.8%	2.4%	<i>0.000**</i>
<i>tlrC</i>	0.2%	0.2%	2.6%	1.9%	<i>0.000**</i>
<i>srnB</i>	0.6%	0.5%	2.5%	1.1%	<i>0.000**</i>
<i>qacL</i>	4.0%	2.9%	2.4%	2.4%	<i>0.000**</i>
<i>ErmF</i>	1.3%	1.0%	2.2%	6.0%	<i>0.216</i>
<i>sul2</i>	1.2%	1.0%	2.0%	1.8%	<i>0.001*</i>
<i>TEM</i>	0.2%	0.1%	2.0%	8.4%	<i>0.098</i>
<i>carA</i>	0.4%	0.2%	1.8%	1.4%	<i>0.000**</i>
<i>APH(6)-Id</i>	1.5%	0.7%	1.4%	1.1%	<i>0.792</i>
<i>msrE</i>	5.1%	3.3%	1.4%	1.3%	<i>0.000**</i>
<i>APH(3'')-Ib</i>	1.4%	0.7%	1.3%	1.0%	<i>0.437</i>
<i>ANT(3'')-IIa</i>	0.98%	0.5%	1.2%	0.8%	<i>0.026</i>
<i>mphE</i>	4.7%	3.2%	1.2%	1.2%	<i>0.000**</i>
<i>aadS</i>	0.8%	0.7%	1.0%	1.7%	<i>0.365</i>
<i>IMP</i>	0.0%	0.0%	1.0%	1.0%	<i>0.000**</i>
<i>aadA11</i>	2.1%	1.9%	0.8%	0.8%	<i>0.000**</i>
<i>ErmB</i>	5.1%	4.4%	0.6%	0.6%	<i>0.000**</i>
<i>mel</i>	1.5%	1.1%	0.4%	0.7%	<i>0.000**</i>
<i>tet(Q)</i>	1.1%	1.0%	0.3%	0.2%	<i>0.000**</i>
<i>tet(39)</i>	1.4%	1.2%	0.2%	0.2%	<i>0.000**</i>
<i>mefJ</i>	1.2%	1.9%	0.1%	0.3%	<i>0.000**</i>

**** $p < 0.01$, *** $p < 0.05$

Supplementary Table 3. Principal component loadings of ARGs in Figure 4a (PCA on ARG profiles of both activated sludge and influent wastewater).

(a). Top 30 positive loading of PC1 (ARGs relatively abundant in activated sludge)

No.	ARG	PC1 loading	MAR	Drug class*	Resistance mechanisms
1	srmB	0.113	1	macrolide	target protection
2	AxyY	0.1116	4	aminoglycoside, cephalosporin , fluoroquinolone , macrolide	efflux
3	MexW	0.1074	5	disinfecting agents and antiseptics, fluoroquinolone , macrolide , phenicol, tetracycline	efflux
4	smeB	0.1064	4	minoglycoside, cephalosporin , cephamycin, penam	efflux
5	mexQ	0.1062	6	carbapenem, diaminopyrimidine, disinfecting agents and antiseptics, macrolide , phenicol, tetracycline	efflux
6	MexD	0.103	9	aminocoumarin, aminoglycoside, cephalosporin , diaminopyrimidine, fluoroquinolone , macrolide , penam, phenicol, tetracycline	efflux
7	tlrC	0.1014	2	lincosamide, macrolide	target protection
8	oleC	0.1009	1	macrolide	efflux
9	AAC(6')-Ib7	0.0959	1	aminoglycoside	inactivation
10	smeR	0.0952	4	aminoglycoside, cephalosporin , cephamycin, penam	efflux
11	smeE	0.0949	4	fluoroquinolone , macrolide , phenicol, tetracycline	efflux
12	oleB	0.0939	1	macrolide	target protection
13	MexF	0.0936	3	diaminopyrimidine, fluoroquinolone , phenicol	efflux
14	mexY	0.0914	10	aminoglycoside, carbapenem, cephalosporin , cephamycin, disinfecting agents and antiseptics, fluoroquinolone , macrolide , penam, phenicol, tetracycline	efflux
15	sulI	0.091	2	sulfonamide, sulfone	target replacement
16	amrB	0.0903	1	aminoglycoside	efflux
17	MexB	0.0902	14	aminocoumarin, carbapenem, cephalosporin , cephamycin, diaminopyrimidine, fluoroquinolone , macrolide , monobactam, penam, penem, peptide, phenicol, sulfonamide, tetracycline	efflux
18	MexK	0.09	3	disinfecting agents and antiseptics, macrolide , tetracycline	efflux
19	Paer_CpxR	0.0869	15	aminocoumarin, aminoglycoside, carbapenem, cephalosporin , cephamycin, diaminopyrimidine, fluoroquinolone , macrolide , monobactam, penam, penem, peptide, phenicol, sulfonamide, tetracycline	efflux
20	carA	0.0866	1	macrolide	target protection
21	MuxC	0.0859	4	aminocoumarin, macrolide , monobactam, tetracycline	efflux
22	ceoB	0.0857	2	aminoglycoside, fluoroquinolone	efflux
23	IMP	0.0824	5	Carbapenem, cephalosporin , cephamycin, penam, penem	inactivation
24	MexI	0.0815	3	disinfecting agents and antiseptics, fluoroquinolone , tetracycline	efflux
25	smeS	0.0771	4	aminoglycoside, cephalosporin , cephamycin, penam	efflux
26	MuxB	0.0746	4	aminocoumarin, macrolide , monobactam, tetracycline	efflux
27	aadA9	0.0725	1	aminoglycoside	inactivation
28	Cstr_tetA	0.0666	2	penam, tetracycline	efflux
29	smeC	0.0633	4	aminoglycoside, cephalosporin , cephamycin, penam	efflux
30	aadA25	0.0631	1	aminoglycoside	inactivation

*Drug classes listed as highest-priority critically important antimicrobials by WHO are shown in bold.

Table 3 (b). Top 30 negative loading of PC1 (ARGs relatively abundant in wastewater)

No.	ARG	PC1 loading	MAR	Drug class*	Resistance mechanisms
1	emrR	-0.1258	1	fluoroquinolone	efflux
2	TolC	-0.1241	15	Aminocoumarin, aminoglycoside, carbapenem, cephalosporin , cephamycin, disinfecting agents and antiseptics, fluoroquinolone , glycylcycline, macrolide , penam, penem, peptide, phenicol, rifamycin, tetracycline	efflux
3	cpxA	-0.124	2	aminocoumarin, aminoglycoside	efflux
4	Kpne_KpnH	-0.124	8	Aminoglycoside, carbapenem, cephalosporin , fluoroquinolone , macrolide , penam, penem, peptide	efflux
5	H-NS	-0.1238	6	cephalosporin , cephamycin, fluoroquinolone , macrolide , penam, tetracycline	efflux
6	Ecol_acrA	-0.1235	8	diaminopyrimidine, fluoroquinolone , phenicol	efflux
7	baeS	-0.123	2	aminocoumarin, aminoglycoside	efflux
8	AcrE	-0.1227	4	cephalosporin , cephamycin, fluoroquinolone , penam	efflux
9	AcrS	-0.1223	9	cephalosporin , cephamycin, disinfecting agents and antiseptics, fluoroquinolone , glycylcycline, penam, phenicol, rifamycin, tetracycline	efflux
10	AcrF	-0.1217	4	cephalosporin , cephamycin, fluoroquinolone , penam	efflux
11	mdtH	-0.1214	1	fluoroquinolone	efflux
12	baeR	-0.1213	2	aminocoumarin, aminoglycoside	efflux
13	emrA	-0.1212	1	fluoroquinolone	efflux
14	gadX	-0.1205	3	fluoroquinolone , macrolide , penam	efflux
15	evgS	-0.12	4	fluoroquinolone , macrolide , penam, tetracycline	efflux
16	emrY	-0.12	1	tetracycline	efflux
17	evgA	-0.1187	4	fluoroquinolone , macrolide , penam, tetracycline	efflux
18	acrB	-0.1179	8	cephalosporin , disinfecting agents and antiseptics, fluoroquinolone , glycylcycline, penam, phenicol, rifamycin, tetracycline	efflux
19	kdpE	-0.1167	1	aminoglycoside	efflux
20	gadW	-0.1143	3	fluoroquinolone , macrolide , penam	efflux
21	marA	-0.1142	12	carbapenem, cephalosporin , cephamycin, disinfecting agents and antiseptics, fluoroquinolone , glycylcycline, monobactam, penam, penem, phenicol, rifamycin, tetracycline	efflux
22	emrK	-0.1133	1	tetracycline	efflux
23	acrD	-0.1117	1	aminoglycoside	efflux
24	mdtM	-0.1115	5	disinfecting agents and antiseptics, fluoroquinolone, lincosamide, nucleoside, phenicol	efflux
25	SHV	-0.1114	3	carbapenem, cephalosporin , penam	inactivation
26	Tet32	-0.1077	1	tetracycline	target protection
27	Ecol_emrE	-0.1052	1	macrolide	efflux
28	Kpne_acrA	-0.1015	8	cephalosporin , disinfecting agents and antiseptics, fluoroquinolone , glycylcycline, penam, phenicol, rifamycin, tetracycline	efflux
29	oqxA	-0.0988	5	diaminopyrimidine, fluoroquinolone , glycylcycline, nitrofurantoin, tetracycline	efflux
30	emrB	-0.0979	1	fluoroquinolone	efflux

*Drug classes listed as highest-priority critically important antimicrobials by WHO are shown in bold.

Table 3 (c). Top 30 positive loading of PC2

No.	ARG	PC2 loading	MAR	Drug class*	Resistance mechanisms
1	sul1	0.1147	2	sulfonamide, sulfone	target replacement
2	aadA8b	0.1136	1	aminoglycoside	inactivation
3	QnrS15	0.1126	1	fluoroquinolone	target protection
4	tet(A)	0.1126	1	tetracycline	efflux
5	aadA2	0.1082	1	aminoglycoside	inactivation
6	AAC(6')-IIa	0.1071	1	aminoglycoside	inactivation
7	aadA3	0.107	1	aminoglycoside	inactivation
8	oqxB	0.1002	5	diaminopyrimidine, fluoroquinolone , glycylcycline, nitrofurantoin, tetracycline	efflux
9	mdtF	0.1001	3	fluoroquinolone , macrolide , penam	efflux
10	acrD	0.0995	1	aminoglycoside	efflux
11	aadA24	0.0991	1	aminoglycoside	inactivation
12	EreA	0.0989	1	macrolide	inactivation
13	sul2	0.0972	2	sulfonamide, sulfone	target replacement
14	emrB	0.0945	1	fluoroquinolone	efflux
15	mdtM	0.0925	5	disinfecting agents and antiseptics, fluoroquinolone , lincosamide, nucleoside, phenicol	efflux
16	oqxA	0.0908	5	diaminopyrimidine, fluoroquinolone , glycylcycline, nitrofurantoin, tetracycline	efflux
17	IDC	0.0903	2	carbapenem, cephalosporin	inactivation
18	acrB	0.0891	8	cephalosporin , disinfecting agents and antiseptics, fluoroquinolone , glycylcycline, penam, phenicol, rifamycin, tetracycline	efflux
19	AAC(3)-IId	0.0884	1	aminoglycoside	inactivation
20	MexD	0.0878	9	aminocoumarin, aminoglycoside, cephalosporin , diaminopyrimidine, fluoroquinolone , macrolide , penam, phenicol, tetracycline	efflux
21	kdpE	0.0874	1	aminoglycoside	inactivation
22	aadA8	0.0857	1	aminoglycoside	inactivation
23	baeR	0.0838	2	aminocoumarin, aminoglycoside	efflux
24	baeS	0.0836	2	aminocoumarin, aminoglycoside	efflux
25	tet(C)	0.0836	1	tetracycline	efflux
26	mdtH	0.083	1	fluoroquinolone	efflux
27	Kpne_KpnG	0.083	8	aminoglycoside, carbapenem, cephalosporin , fluoroquinolone , macrolide , penam, penem, peptide	efflux
28	amrB	0.0828	1	aminoglycoside	efflux
29	Ecol_mdjA	0.0827	2	disinfecting agents and antiseptics, tetracycline	efflux
30	smeB	0.0823	4	aminoglycoside, cephalosporin , cephamycin, penam	efflux

*Drug classes listed as highest-priority critically important antimicrobials by WHO are shown in bold.

Table 3 (d). Top 30 negative loading of PC2

No.	ARG	PC2 loading	MAR	Drug class*	Resistance mechanisms
1	mel	-0.1368	2	macrolide, streptogramin	target protection
2	tet(O)	-0.1293	1	tetracycline	target protection
3	tet(O/W/O)	-0.1259	1	tetracycline	target protection
4	mphG	-0.1221	1	macrolide	inactivation
5	tet(O/M/O)	-0.1216	1	tetracycline	target protection
6	ErmB	-0.1196	5	lincosamide, macrolide , streptogramin A, streptogramin B, streptogramin	target alteration
7	mphE	-0.1167	1	macrolide	inactivation
8	tet(O/W/32/O)	-0.1136	1	tetracycline	target protection
9	tet(W/32/O)	-0.1122	1	tetracycline	target protection
10	tet(W/N/W)	-0.1112	1	tetracycline	target protection
11	mefC	-0.1112	1	macrolide	efflux
12	mefJ	-0.1111	1	macrolide	efflux
13	msr(I)	-0.1092	3	macrolide , phosphonic acid, streptogramin	target protection
14	msrE	-0.1071	2	macrolide, streptogramin	target protection
15	tet(W)	-0.1059	1	tetracycline	target protection
16	tet(O/W)	-0.0999	1	tetracycline	target protection
17	tet(Q)	-0.0937	1	tetracycline	target protection
18	EreD	-0.0934	1	macrolide	inactivation
19	tet(S)	-0.0866	1	tetracycline	target protection
20	APH(3')-VIa	-0.0865	1	aminoglycoside	inactivation
21	AAC(3)-Ia	-0.0864	1	aminoglycoside	inactivation
22	adeK	-0.0845	10	carbapenem, cephalosporin , diaminopyrimidine, fluoroquinolone , lincosamide, macrolide , penem, phenicol, rifamycin, tetracycline	efflux
23	mtrD	-0.084	2	macrolide , penam	efflux
24	aadA27	-0.0838	1	aminoglycoside	inactivation
25	CblA	-0.0801	1	cephalosporin	inactivation
26	ErmG	-0.08	5	Lincosamide, macrolide, streptogramin A, streptogramin B, streptogramin	Target alteration
27	tet(M)	-0.0776	1	tetracycline	target protection
28	EBR	-0.0766	3	carbapenem, cephalosporin, penam	inactivation
29	adeJ	-0.0739	10	carbapenem, cephalosporin , diaminopyrimidine, fluoroquinolone , lincosamide, macrolide , penem, phenicol, rifamycin, tetracycline	efflux
30	tet(40)	-0.0733	1	tetracycline	efflux

*Drug classes listed as highest-priority critically important antimicrobials by WHO are shown in bold.

Supplementary Table 4. Principal component loadings of ARGs in Figure 4b (PCA on ARG profiles of activated sludge only).

(a). Top 30 positive loading of PC1

No.	ARG	PC1 loading	MAR	Drug class*	Resistance mechanisms
1	AxyY	0.0911	4	aminoglycoside, cephalosporin , fluoroquinolone , macrolide	efflux
2	tet(W/N/W)	0.081	1	tetracycline	target protection
3	tet(O/W/32/O)	0.0809	1	tetracycline	target protection
4	tet(W)	0.0731	1	tetracycline	target protection
5	MexB	0.0713	14	aminocoumarin, carbapenem, cephalosporin , cephamycin, diaminopyrimidine, fluoroquinolone , macrolide , monobactam, penam, penem, peptide, phenicol, sulfonamide, tetracycline	efflux
6	srmB	0.0673	1	macrolide	target protection
7	smeE	0.0663	4	fluoroquinolone , macrolide , phenicol, tetracycline	efflux
8	smeS	0.0663	4	aminoglycoside, cephalosporin , cephamycin, penam	efflux
9	tlrC	0.0649	2	lincosamide, macrolide	target protection
10	Bpse_Omp38	0.0628	6	carbapenem, cephalosporin , cephamycin, monobactam, penam, penem	Reduced permeability
11	aadA25	0.0586	1	aminoglycoside	inactivation
12	aadA11	0.0583	1	aminoglycoside	inactivation
13	smeC	0.0573	4	aminoglycoside, cephalosporin , cephamycin, penam	efflux
14	aadA15	0.0554	1	aminoglycoside	inactivation
15	ErmB	0.0549	5	lincosamide, macrolide , streptogramin A, streptogramin B, streptogramin	target alteration
16	aadA6/aadA10	0.0539	1	aminoglycoside	inactivation
17	tet(O/M/O)	0.0538	1	tetracycline	target protection
18	MexL	0.0536	3	disinfecting agents and antiseptics, macrolide , tetracycline	efflux
19	oleC	0.0532	1	macrolide	efflux
20	tet(O/W/O)	0.0527	1	tetracycline	target protection
21	APH(6)-Id	0.0503	1	aminoglycoside	inactivation
22	Cstr_tetA	0.0492	2	penam, tetracycline	efflux
23	OprM	0.0475	16	aminocoumarin, aminoglycoside, carbapenem, cephalosporin , cephamycin, diaminopyrimidine, disinfecting agents and antiseptics, fluoroquinolone , macrolide , monobactam, penam, penem, peptide, phenicol, sulfonamide, tetracycline	efflux
24	MexK	0.0474	3	disinfecting agents and antiseptics, macrolide , tetracycline	efflux
25	oleB	0.047	1	macrolide	target protection
26	tet(W/32/O)	0.0469	1	tetracycline	target protection
27	APH(3'')-Ib	0.0445	1	aminoglycoside	inactivation
28	Kpne_acrA	0.0411	8	cephalosporin , disinfecting agents and antiseptics, fluoroquinolone , glycylicycline, penam, phenicol, rifamycin, tetracycline	efflux
29	ceoB	0.0403	2	aminoglycoside, fluoroquinolone	efflux
30	mexQ	0.0396	6	carbapenem, diaminopyrimidine, disinfecting agents and antiseptics, macrolide , phenicol, tetracycline	efflux

*Drug classes listed as highest-priority critically important antimicrobials by WHO are shown in bold.

Table 4 (b). Top 30 negative loading of PC1 (ARGs relatively abundant in activated sludge of Japan)

No.	ARG	PC1 loading	MAR	Drug class*	Resistance mechanisms
1	evgS	-0.1872	4	fluoroquinolone , macrolide , penam, tetracycline	efflux
2	AcrS	-0.1802	9	cephalosporin , cephamycin, disinfecting agents and antiseptics, fluoroquinolone , glycylicycline, penam, phenicol, rifamycin, tetracycline	efflux
3	evgA	-0.1768	4	fluoroquinolone , macrolide , penam, tetracycline	efflux
4	mdtM	-0.1733	5	disinfecting agents and antiseptics, fluoroquinolone , lincosamide, nucleoside, phenicol	efflux
5	gadW	-0.1714	3	fluoroquinolone , macrolide , penam	efflux
6	Ecol_mdfA	-0.1693	2	disinfecting agents and antiseptics, tetracycline	efflux
7	TolC	-0.165	15	aminocoumarin, aminoglycoside, carbapenem, cephalosporin , cephamycin, disinfecting agents and antiseptics, fluoroquinolone , glycylicycline, macrolide , penam, penem, peptide, phenicol, rifamycin, tetracycline	efflux
8	Kpne_KpnH	-0.1644	8	aminoglycoside, carbapenem, cephalosporin , fluoroquinolone , macrolide , penam, penem, peptide	efflux
9	gadX	-0.164	3	fluoroquinolone , macrolide , penam	efflux
10	emrY	-0.1623	1	tetracycline	efflux
11	EC	-0.1591	1	cephalosporin	inactivation
12	mdtE	-0.157	3	fluoroquinolone , macrolide , penam	efflux
13	kdpE	-0.156	1	aminoglycoside	efflux
14	emrB	-0.1536	1	fluoroquinolone	efflux
15	cpxA	-0.1488	2	aminocoumarin, aminoglycoside	efflux
16	mdtH	-0.1455	1	fluoroquinolone	efflux
17	emrK	-0.1442	1	tetracycline	efflux
18	emrA	-0.1429	1	fluoroquinolone	efflux
19	emrR	-0.1428	1	fluoroquinolone	efflux
20	AcrE	-0.14	4	cephalosporin , cephamycin, fluoroquinolone , penam	efflux
21	baeS	-0.1396	2	aminocoumarin, aminoglycoside	efflux
22	VEB	-0.1342	2	cephalosporin , monobactam	inactivation
23	baeR	-0.1337	2	aminocoumarin, aminoglycoside	efflux
24	aadS	-0.1331	1	aminoglycoside	inactivation
25	mphE	-0.1158	1	macrolide	inactivation
26	Ecol_acrA	-0.1112	8	cephalosporin , disinfecting agents and antiseptics, fluoroquinolone , glycylicycline, penam, phenicol, rifamycin, tetracycline	efflux
27	marA	-0.1096	12	carbapenem, cephalosporin , cephamycin, disinfecting agents and antiseptics, fluoroquinolone , glycylicycline, monobactam, penam, penem, phenicol, rifamycin, tetracycline	efflux
28	acrB	-0.1087	8	cephalosporin , disinfecting agents and antiseptics, fluoroquinolone , glycylicycline, penam, phenicol, rifamycin, tetracycline	efflux
29	H-NS	-0.1067	6	cephalosporin , cephamycin, fluoroquinolone , macrolide , penam, tetracycline	efflux
30	AcrF	-0.1018	4	cephalosporin , cephamycin, fluoroquinolone , penam	efflux

*Drug classes listed as highest-priority critically important antimicrobials by WHO are shown in bold.

Table 4 (c). Top 30 positive loading of PC2 (ARGs relatively abundant in activated sludge of China and the west of the USA)

No.	ARG	PC2 loading	MAR	Drug class*	Resistance mechanisms
1	tet(C)	0.1569	1	tetracycline	efflux
2	EreA	0.1455	1	macrolide	inactivation
3	ErmF	0.1166	5	incosamide, macrolide , streptogramin A, streptogramin B, streptogramin	target alteration
4	Tet(36)	0.1122	1	tetracycline	target protection
5	mel	0.1044	2	macrolide , streptogramin	target protection
6	aadA16	0.104	1	aminoglycoside	inactivation
7	TLA	0.1033	3	cephalosporin , fluoroquinolone , monobactam	inactivation
8	EreD	0.1028	1	macrolide	inactivation
9	sul3	0.1011	2	sulfonamide, sulfone	target replacement
10	RSD2	0.0988	2	carbapenem, cephalosporin	inactivation
11	Erm(35)	0.0978	5	lincosamide, macrolide , streptogramin A, streptogramin B, streptogramin	target alteration
12	ErmG	0.0966	5	lincosamide, macrolide , streptogramin A, streptogramin B, streptogramin	target alteration
13	AAC(6')-Ia	0.0957	1	aminoglycoside	inactivation
14	Erm(47)	0.0949	4	lincosamide, macrolide , streptogramin B, streptogramin	target alteration
15	ANT(6)-Ib	0.0934	1	aminoglycoside	inactivation
16	aadA6	0.0918	1	aminoglycoside	inactivation
17	QnrD1	0.0914	1	fluoroquinolone	target protection
18	Tet(44)	0.0809	1	tetracycline	target protection
19	aphA15	0.0805	1	aminoglycoside	inactivation
20	TEM	0.0789	4	cephalosporin , monobactam, penam, penem	inactivation
21	Erm(51)	0.0781	4	lincosamide, macrolide , streptogramin B, streptogramin	target alteration
22	tet(M)	0.0729	1	tetracycline	target protection
23	tet(X)	0.0719	1	tetracycline	target protection
24	EreB	0.0719	1	macrolide	inactivation
25	AAC(6')-IIa	0.0716	1	aminoglycoside	inactivation
26	aadA13	0.0714	1	aminoglycoside	inactivation
27	mef(B)	0.0697	1	macrolide	efflux
28	mphF	0.0671	1	macrolide	inactivation
29	tet(40)	0.0656	1	tetracycline	efflux
30	QnrS15	0.064	1	fluoroquinolone	target protection

*Drug classes listed as highest-priority critically important antimicrobials by WHO are shown in bold.

Table 4 (d). Top 30 negative loading of PC2 (ARGs relatively abundant in activated sludge of Europe and east USA)

No.	ARG	PC2 loading	MAR	Drug class*	Resistance mechanisms
1	srmB	-0.1631	1	macrolide	target protection
2	smeB	-0.1568	4	aminoglycoside, cephalosporin , cephamycin, penam	efflux
3	AAC(6')-Ib7	-0.1505	1	aminoglycoside	inactivation
4	smeR	-0.1498	4	aminoglycoside, cephalosporin , cephamycin, penam	efflux
5	MexB	-0.1394	14	aminocoumarin, carbapenem, cephalosporin , cephamycin, diaminopyrimidine, fluoroquinolone , macrolide , monobactam, penam, penem, peptide, phenicol, sulfonamide, tetracycline	efflux
6	MexD	-0.1332	9	aminocoumarin, aminoglycoside, cephalosporin , diaminopyrimidine, fluoroquinolone , macrolide , penam, phenicol, tetracycline	efflux
7	MexW	-0.1275	5	disinfecting agents and antiseptics, fluoroquinolone , macrolide , phenicol, tetracycline	efflux
8	smeC	-0.127	4	aminoglycoside, cephalosporin , cephamycin, penam	efflux
9	smeE	-0.1243	4	fluoroquinolone , macrolide , phenicol, tetracycline	efflux
10	smeS	-0.1219	4	aminoglycoside, cephalosporin , cephamycin, penam	efflux
11	IMP	-0.1216	5	carbapenem, cephalosporin , cephamycin, penam, penem	inactivation
12	AxyY	-0.1183	4	aminoglycoside, cephalosporin , fluoroquinolone , macrolide	efflux
13	aadA25	-0.1117	1	aminoglycoside	inactivation
14	tlrC	-0.1103	2	lincosamide, macrolide	target protection
15	acrD	-0.1099	1	aminoglycoside	efflux
16	aadA8	-0.1094	1	aminoglycoside	inactivation
17	Bpse_Omp38	-0.1077	6	carbapenem, cephalosporin , cephamycin, monobactam, penam, penem	Reduced permeability
18	sul1	-0.1073	2	sulfonamide, sulfone	target replacement
19	aadA15	-0.1047	1	aminoglycoside	inactivation
20	TxR	-0.1035	1	tetracycline	efflux
21	MOX	-0.1016	3	cephalosporin , cephamycin, penam	inactivation
22	MexY	-0.0986	10	aminoglycoside, carbapenem, cephalosporin , cephamycin, disinfecting agents and antiseptics, fluoroquinolone , macrolide , penam, phenicol, tetracycline	efflux
23	aadA9	-0.0971	1	aminoglycoside	inactivation
24	aadA22	-0.0961	1	aminoglycoside	inactivation
25	ErmB	-0.0951	5	lincosamide, macrolide , streptogramin A, streptogramin B, streptogramin	target alteration
26	aadA6/aadA10	-0.0932	1	aminoglycoside	inactivation
27	smeF	-0.0929	4	fluoroquinolone , macrolide , phenicol, tetracycline	efflux
28	ANT(3'')-IIa	-0.0928	1	aminoglycoside	inactivation
29	mexQ	-0.0928	6	carbapenem, diaminopyrimidine, disinfecting agents and antiseptics, macrolide , phenicol, tetracycline	efflux
30	APH(3'')-Ib	-0.092	1	aminoglycoside	inactivation

*Drug classes listed as highest-priority critically important antimicrobials by WHO are shown in bold.

Supplementary Table 5. Principal component loadings of ARGs in Figure 4c (PCA on ARG profiles of influent wastewater only).

(a). Top 30 positive loading of PC1 (ARGs relatively abundant in wastewater of Japan)

No.	ARG	PC1 loading	MAR	Drug class*	Resistance mechanisms
1	OXA	0.0896	3	carbapenem, cephalosporin , penam	inactivation
2	mphE	0.0803	1	macrolide	inactivation
3	msrE	0.0724	2	macrolide , streptogramin	target protection
4	TxR	0.067	1	tetracycline	efflux
5	mphG	0.067	1	macrolide	inactivation
6	APH(3')-VIa	0.0656	1	aminoglycoside	inactivation
7	APH(3')-VIb	0.0616	1	aminoglycoside	inactivation
8	mefC	0.061	1	macrolide	efflux
9	MexB	0.0593	14	aminocoumarin, carbapenem, cephalosporin , cephamycin, diaminopyrimidine, fluoroquinolone , macrolide , monobactam, penam, penem, peptide, phenicol, sulfonamide, tetracycline	efflux
10	ErmG	0.0551	5	lincosamide, macrolide , streptogramin A, streptogramin B, streptogramin	target alteration
11	ErmF	0.0551	5	lincosamide, macrolide , streptogramin A, streptogramin B, streptogramin	target alteration
12	NPS	0.0543	2	cephalosporin , penam	inactivation
13	CblA	0.0537	1	cephalosporin	inactivation
14	MuxC	0.052	4	aminocoumarin, macrolide , monobactam, tetracycline	efflux
15	tet(Q)	0.0501	1	tetracycline	target protection
16	QnrS2	0.0495	1	fluroquinolone	target protection
17	AAC(3)-Ia	0.0485	1	aminoglycoside	inactivation
18	FOX	0.0483	2	cephalosporin , cephamycin	inactivation
19	aadA7	0.0469	1	aminoglycoside	inactivation
20	aadA9	0.0469	1	aminoglycoside	inactivation
21	carA	0.0457	1	macrolide	target protection
22	QnrVC5	0.0444	1	fluroquinolone	target protection
23	msr(I)	0.0441	3	macrolide , phosphonic acid, streptogramin	target protection
24	QnrVC4	0.0441	1	fluroquinolone	target protection
25	srmB	0.044	1	macrolide	target protection
26	ErmB	0.0433	5	lincosamide, macrolide, streptogramin A, streptogramin B, streptogramin	target alteration
27	AAC(6')	0.0433	1	aminoglycoside	inactivation
28	mefJ	0.0424	1	macrolide	efflux
29	mexQ	0.0405	6	carbapenem, diaminopyrimidine, disinfecting agents and antiseptics, macrolide , phenicol, tetracycline	efflux
30	MOX	0.0402	3	cephalosporin , cephamycin, penam	inactivation

*Drug classes listed as highest-priority critically important antimicrobials by WHO are shown in bold.

Table 5 (b). Top 30 negative loading of PC1 (ARGs relatively abundant in wastewater of China)

No.	ARG	PC1 loading	MAR	Drug class*	Resistance mechanisms
1	mdtM	-0.146	5	disinfecting agents and antiseptics, fluoroquinolone , lincosamide, nucleoside, phenicol	efflux
2	baeS	-0.1445	2	aminocoumarin, aminoglycoside	efflux
3	Kpne_KpnH	-0.1442	8	aminoglycoside, carbapenem, cephalosporin , fluoroquinolone , macrolide , penam, penem, peptide	efflux
4	Ecol_mdjA	-0.1438	2	disinfecting agents and antiseptics, tetracycline	efflux
5	TolC	-0.1436	15	aminocoumarin, aminoglycoside, carbapenem, cephalosporin , cephamycin, disinfecting agents and antiseptics, fluoroquinolone , glycylcycline, macrolide , penam;penem, peptide;phenicol, rifamycin, tetracycline	efflux
6	acrD	-0.1427	1	aminoglucoside	efflux
7	mdtE	-0.1427	3	fluoroquinolone , macrolide , penam	efflux
8	baeR	-0.1426	2	aminocoumarin, aminoglycoside	efflux
9	acrB	-0.1425	8	cephalosporin , disinfecting agents and antiseptics, fluoroquinolone , glycylcycline, penam, phenicol, rifamycin, tetracycline	efflux
10	acrE	-0.1421	4	cephalosporin , cephamycin, fluoroquinolone , penam	efflux
11	cpxA	-0.1419	2	aminocoumarin, aminoglycoside	efflux
12	mdtH	-0.1416	1	fluoroquinolone	efflux
13	mdtF	-0.1414	3	fluoroquinolone , macrolide , penam	efflux
14	emrA	-0.1413	1	fluoroquinolone	efflux
15	kdpE	-0.1406	1	aminoglycoside	efflux
16	EC	-0.1402	1	cephalosporin	inactivation
17	Ecol_acrA	-0.14	8	cephalosporin , disinfecting agents and antiseptics, fluoroquinolone , glycylcycline, penam, phenicol, rifamycin, tetracycline	efflux
18	emrB	-0.137	1	fluoroquinolone	efflux
19	AcrF	-0.1359	4	cephalosporin , cephamycin, fluoroquinolone , penam	efflux
20	emrR	-0.1341	1	fluoroquinolone	efflux
21	marA	-0.1339	12	carbapenem, cephalosporin , cephamycin, disinfecting agents and antiseptics, fluoroquinolone , glycylcycline, monobactam, penam, penem, phenicol, rifamycin, tetracycline	efflux
22	H-NS	-0.1336	6	cephalosporin , cephamycin, fluoroquinolone , macrolide , penam, tetracycline	efflux
23	gadX	-0.1315	3	fluoroquinolone , macrolide , penam	efflux
24	emrY	-0.1307	1	tetracycline	efflux
25	gadW	-0.1301	3	fluoroquinolone , macrolide , penam	efflux
26	AcrS	-0.1293	9	cephalosporin , cephamycin, disinfecting agents and antiseptics, fluoroquinolone , glycylcycline, penam, phenicol, rifamycin, tetracycline	efflux
27	evgS	-0.1272	4	fluoroquinolone , macrolide , penam, tetracycline	efflux
28	emrK	-0.1267	1	tetracycline	efflux
29	QnrS15	-0.1207	1	fluoroquinolone	target protection
30	evgA	-0.1202	4	fluoroquinolone , macrolide , penam, tetracycline	efflux

*Drug classes listed as highest-priority critically important antimicrobials by WHO are shown in bold.

Table 5 (c). Top 30 positive loading of PC2 (ARGs relatively abundant in wastewater of EU and USA)

No.	ARG	PC2 loading	MAR	Drug class*	Resistance mechanisms
1	tet(W)	0.1192	1	tetracycline	target protection
2	tet(W/N/W)	0.1188	1	tetracycline	target protection
3	ErmB	0.1101	5	lincosamide, macrolide , streptogramin A, streptogramin B, streptogramin	target alteration
4	mtrD	0.1046	2	macrolide , penam	efflux
5	meI	0.1044	2	macrolide , streptogramin	target protection
6	tet(W/32/O)	0.1039	1	tetracycline	target protection
7	tet(O/W)	0.1022	1	tetracycline	target protection
8	tet(O/W/32/O)	0.1018	1	tetracycline	target protection
9	tet(O/W/O)	0.0986	1	tetracycline	target protection
10	aadA4	0.083	1	aminoglycoside	inactivation
11	APH(6)-Ic	0.0798	1	aminoglycoside	inactivation
12	aadA11	0.0783	1	aminoglycoside	inactivation
13	tet(O/M/O)	0.0757	1	tetracycline	target protection
14	AAC(3)-Ia	0.0748	1	aminoglycoside	inactivation
15	aadA6/aadA10	0.0732	1	aminoglycoside	inactivation
16	tet(O)	0.0731	1	tetracycline	target protection
17	mefJ	0.0724	1	macrolide	efflux
18	LCR	0.0713	2	cephalosporin , penam	inactivation
19	TxR	0.0697	1	tetracycline	efflux
20	AIM	0.0688	3	cephalosporin , cephamycin, penam	inactivation
21	OprZ	0.0678	4	aminoglycoside, cephalosporin , fluoroquinolone , macrolide	efflux
22	evgS	0.0665	4	fluoroquinolone , macrolide , penam, tetracycline	efflux
23	AcrF	0.0647	4	cephalosporin , cephamycin, fluoroquinolone , penam	efflux
24	evgA	0.0647	4	fluoroquinolone , macrolide , penam, tetracycline	efflux
25	MexB	0.062	14	aminocoumarin, carbapenem, cephalosporin , cephamycin, diaminyrimidine, fluoroquinolone , macrolide , monobactam, penam, penem, peptide, phenicol, sulfonamide, tetracycline	efflux
26	msr(I)	0.061	3	macrolide , phosphonic acid, streptogramin	target protection
27	emrK	0.0609	1	tetracycline	efflux
28	gadX	0.0601	3	fluoroquinolone , macrolide , penam	efflux
29	ACI	0.0591	3	cephalosporin , penam, penem	inactivation
30	OXY-1	0.0578	3	cephalosporin , monobactam, penam	inactivation

*Drug classes listed as highest-priority critically important antimicrobials by WHO are shown in bold.

Table 5 (d). Top 30 negative loading of PC2 (ARGs relatively abundant in wastewater of China and Japan)

No.	ARG	PC2 loading	MAR	Drug class*	Resistance mechanisms
1	VEB	-0.1559	2	cephalosporin , monobactam	inactivation
2	sul1	-0.1548	2	sulfonamide, sulfone	target replacement
3	AAC(6')-Ib7	-0.147	1	aminoglycoside	inactivation
4	aadA8b	-0.146	1	aminoglycoside	inactivation
5	aadS	-0.1455	1	aminoglycoside	inactivation
6	aadA8	-0.1437	1	aminoglycoside	inactivation
7	aadA3	-0.1409	1	aminoglycoside	inactivation
8	aadA2	-0.1398	1	aminoglycoside	inactivation
9	AAC(6')-IIa	-0.1382	1	aminoglycoside	inactivation
10	aadA24	-0.1344	1	aminoglycoside	inactivation
11	ANT(3'')-IIa	-0.131	1	aminoglycoside	inactivation
12	EreA	-0.1205	1	macrolide	inactivation
13	AAC(6')-IIc	-0.1183	1	aminoglycoside	inactivation
14	GES	-0.1167	3	carbapenem, cephalosporin , penam	inactivation
15	Sul4	-0.1164	1	sulfonamide	target replacement
16	Tet(C)	-0.1133	1	tetracycline	efflux
17	AAC6_IeAPH2_Ia	-0.1102	1	aminoglycoside	inactivation
18	ErmF	-0.1093	5	lincosamide, macrolide , streptogramin A, streptogramin B, streptogramin	target alteration
19	APH(3')-IIa	-0.1051	1	aminoglycoside	inactivation
20	EreB	-0.1046	1	macrolide	inactivation
21	AAC(6')-Ib8	-0.1038	1	aminoglycoside	inactivation
22	AAC(6')	-0.1025	1	aminoglycoside	inactivation
23	RSD2	-0.1013	2	carbapenem, cephalosporin	inactivation
24	Tet(36)	-0.1005	1	tetracycline	target protection
25	QnrS2	-0.0994	1	fluoroquinolone	target protection
26	Tet(G)	-0.0985	1	tetracycline	efflux
27	AAC(6')-II	-0.0968	1	aminoglycoside	inactivation
28	ErmX	-0.0967	5	lincosamide, macrolide , streptogramin A, streptogramin B, streptogramin	target alteration
29	Tet(X3)	-0.0967	2	glycylcycline, tetracycline	inactivation
30	ANT3II_ANT6II	-0.0962	1	aminoglycoside	inactivation

*Drug classes listed as highest-priority critically important antimicrobials by WHO are shown in bold.

Supplementary Table 6. Principal component loadings of microbial community in Figure 4d (PCA on 16S-based microbial community of both activated sludge and influent wastewater).

(a). Top 30 positive loading of PC1

No.	Phylum	Class	Order	Family	Genus	PC1	PC2
1	Chloroflexi	Dehalococcoidia				0.040	-0.009
2	Crenarchaeota					0.040	-0.008
3	Bacteroidota	Rhodothermia	Rhodothermales	Rhodothermaceae	Salinibacter	0.039	-0.007
4	Crenarchaeota	Nitrososphaeria				0.039	-0.011
5	Chloroflexi	Ktedonobacteria				0.039	-0.004
6	Desulfobacterota	Desulfobacteria				0.039	-0.011
7	Bacteroidota	Rhodothermia				0.039	-0.011
8	Crenarchaeota	Bathyarchaeia				0.039	-0.003
9	Bacteroidota	Rhodothermia	Rhodothermales			0.039	-0.007
10	Bacteroidota	Rhodothermia	Rhodothermales	Rhodothermaceae		0.039	-0.007
11	Planctomycetota	vadinHA49				0.039	-0.010
12	Thermoplasmatota	Thermoplasmata	Marine Group II			0.039	-0.006
13	Desulfobacterota	Desulfobacteria	Desulfobacterales			0.039	-0.011
14	Actinobacteriota	Acidimicrobiia	Actinomarinales			0.038	-0.007
15	Aenigmarchaeota					0.038	-0.013
16	Firmicutes	Clostridia	Clostridia vadinBB60 group			0.038	-0.002
17	Thermoplasmatota					0.038	-0.012
18	Actinobacteriota	Actinobacteria	Propionibacteriales	Propionibacteriaceae	Cutibacterium	0.038	0.001
19	Asgardarchaeota					0.038	-0.007
20	Chloroflexi	Ktedonobacteria	Ktedonobacterales	Ktedonobacteraceae		0.038	-0.010
21	Nitrospirota	Thermodesulfovibrionia				0.038	-0.008
22	Chloroflexi	Ktedonobacteria	Ktedonobacterales			0.038	-0.002
23	Nitrospirota	Thermodesulfovibrionia	uncultured			0.038	-0.005
24	Thermoplasmatota	Thermoplasmata				0.038	-0.013
25	Desulfobacterota	Desulfobacteria	Desulfatiglandales			0.038	-0.009
26	Desulfobacterota	Desulfobacteria	Desulfatiglandales	Desulfatiglandaceae		0.038	-0.009
27	Desulfobacterota	Desulfobacteria	Desulfatiglandales	Desulfatiglandaceae	Desulfatiglans	0.038	-0.009
28	Spirochaetota	Spirochaetia	Spirochaetales	Spirochaetaceae	Spirochaeta	0.038	0.001
29	Acidobacteriota	Acidobacteriae	Acidobacteriales			0.037	-0.017
30	Crenarchaeota	Thermoprotei				0.037	-0.013

Table 6 (b). Top 30 negative loading of PC1

No.	Phylum	Class	Order	Family	Genus	PC1	PC2
1	Proteobacteria	Gammaproteobacteria	Burkholderiales	Aquaspirillaceae	Rivicola	-0.005	0.002
2	Proteobacteria	Gammaproteobacteria	Burkholderiales	Aquaspirillaceae		-0.004	0.004
3	Actinobacteriota	Acidimicrobiia	Microtrichales	Microtrichaceae	Candidatus Microthrix	-0.004	-0.019
4	Halobacterota	Methanosarcinia	Methanosarciniales	Methanosarcinaceae	ANME-3	-0.004	0.002
5	Proteobacteria	Gammaproteobacteria	Burkholderiales	Comamonadaceae	Leptothrix	-0.004	-0.019
6	Bacteroidota	Bacteroidia	Cytophagales	Microscillaceae		-0.004	0.000
7	Bacteroidota	Bacteroidia	Cytophagales	Microscillaceae	uncultured	-0.004	0.000
8	Bacteroidota	Bacteroidia	Cytophagales			-0.004	0.000
9	Proteobacteria	Gammaproteobacteria	Burkholderiales	Sutterellaceae	AAP99	-0.004	-0.018
10	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Rhodanobacteraceae	Aquimonas	-0.003	-0.002
11	Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Ursidibacter	-0.003	0.001
12	Proteobacteria	Gammaproteobacteria	Aeromonadales	Aeromonadaceae		-0.003	0.003
13	Proteobacteria	Gammaproteobacteria	Aeromonadales	Aeromonadaceae	Aeromonas	-0.003	0.003
14	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Roseobacter clade NAC11-7 lineage	-0.003	0.002
15	Bacteroidota	Bacteroidia				-0.003	0.001
16	Proteobacteria	Gammaproteobacteria	Aeromonadales			-0.003	0.003
17	Bacteroidota					-0.003	0.001
18	Proteobacteria	Alphaproteobacteria	Rhodobacterales			-0.003	0.002
19	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae		-0.003	0.002
20	Proteobacteria	Gammaproteobacteria	Beggiatoales	Beggiatoaceae	Candidatus Parabeggiatoa	-0.003	-0.001
21	Proteobacteria	Gammaproteobacteria	Thiomicrospirales	Thiomicrospiraceae	Thiomicrothrix	-0.003	0.003
22	Firmicutes	Desulfotomaculia	Desulfotomaculales	g_Desulfotomaculum		-0.003	0.001
23	Halobacterota	Methanosarcinia	Methanosarciniales	Methanosarcinaceae		-0.003	0.001
24	Proteobacteria	Gammaproteobacteria	Beggiatoales	Beggiatoaceae	Beggiatoa	-0.003	-0.002
25	Proteobacteria	Gammaproteobacteria	Beggiatoales			-0.003	-0.002
26	Proteobacteria	Gammaproteobacteria	Beggiatoales	Beggiatoaceae		-0.003	-0.002
27	Proteobacteria	Gammaproteobacteria	Thiomicrospirales			-0.002	0.005
28	Proteobacteria	Gammaproteobacteria	Thiomicrospirales	Thiomicrospiraceae		-0.002	0.005
29	Proteobacteria	Gammaproteobacteria	Enterobacterales	Morganellaceae	Proteus	-0.002	0.002
30	Proteobacteria	Gammaproteobacteria	Thiomicrospirales	Thiomicrospiraceae	uncultured	-0.002	0.006

Table 6 (c). Top 30 positive loading of PC2 (Microbial species relatively abundant in untreated wastewater)

No.	Phylum	Class	Order	Family	Genus	PC1	PC2
1	Firmicutes					0.017	0.054
2	Firmicutes	Bacilli				0.015	0.052
3	Firmicutes	Clostridia				0.019	0.052
4	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Blautia	0.016	0.052
5	Firmicutes	Bacilli	Lactobacillales			0.014	0.052
6	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	0.014	0.051
7	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae		0.014	0.051
8	Firmicutes	Bacilli	Acholeplasmatales	Acholeplasmataceae	Candidatus Phytoplasma	0.020	0.051
9	Verrucomicrobiota	Verrucomicrobiae	Verrucomicrobiales	Akkermansiaceae		0.013	0.051
10	Verrucomicrobiota	Verrucomicrobiae	Verrucomicrobiales	Akkermansiaceae	Akkermansia	0.013	0.051
11	Actinobacteriota	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	0.012	0.050
12	Actinobacteriota	Actinobacteria	Bifidobacteriales			0.012	0.050
13	Actinobacteriota	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae		0.012	0.050
14	Firmicutes	Clostridia	Clostridiales	Clostridiaceae		0.017	0.050
15	Firmicutes	Bacilli	Erysipelotrichales			0.018	0.050
16	Firmicutes	Bacilli	Erysipelotrichales	Erysipelotrichaceae	Holdemanella	0.014	0.049
17	Actinobacteriota	Coriobacteriia	Coriobacteriales			0.016	0.049
18	Firmicutes	Bacilli	Erysipelotrichales	Erysipelatoclostridiaceae	Catenibacterium	0.012	0.049
19	Proteobacteria	Gammaproteobacteria	Burkholderiales	Comamonadaceae	Brachymonas	0.012	0.049
20	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium sensu stricto 12	0.013	0.049
21	Actinobacteriota	Coriobacteriia	Coriobacteriales	Coriobacteriaceae	Collinsella	0.013	0.049
22	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	0.013	0.049
23	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae		0.013	0.049
24	Actinobacteriota	Coriobacteriia				0.016	0.049
25	Firmicutes	Bacilli	Acholeplasmatales			0.019	0.049
26	Firmicutes	Bacilli	Acholeplasmatales	Acholeplasmataceae		0.019	0.049
27	Firmicutes	Bacilli	Erysipelotrichales	Erysipelatoclostridiaceae		0.017	0.049
28	Cyanobacteria	Cyanobacteriia	Cyanobacteriales	Phormidiaceae	Arthrospira PCC-7345	0.012	0.049
29	Actinobacteriota	Coriobacteriia	Coriobacteriales	Coriobacteriaceae		0.013	0.049
30	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium sensu stricto 1	0.017	0.049

Table 6 (d). Top 30 negative loading of PC2 (Microbial species relatively abundant in activated sludge)

No.	Phylum	Class	Order	Family	Genus	PC1	PC2
1	Myxococcota	Polyangia	Haliangiales			0.010	-0.038
2	Myxococcota	Polyangia	Haliangiales	Haliangiaceae		0.010	-0.038
3	Myxococcota	Polyangia	Haliangiales	Haliangiaceae	Haliangium	0.010	-0.038
4	Myxococcota	Polyangia	Polyangiales	Polyangiaceae	Sorangium	0.018	-0.038
5	Myxococcota	Polyangia				0.024	-0.037
6	Myxococcota					0.026	-0.037
7	Myxococcota	Polyangia	Polyangiales	Sandaracinaceae		0.025	-0.037
8	Verrucomicrobiota	Verrucomicrobiae	Pedosphaerales	Pedosphaeraceae	Oikopleura	0.026	-0.037
9	Cyanobacteria	Vampirivibrionia	Obscuribacterales			0.022	-0.037
10	Cyanobacteria	Vampirivibrionia	Obscuribacterales	Obscuribacteraceae		0.022	-0.037
11	Bacteroidota	Bacteroidia	Chitinophagales	uncultured		0.024	-0.037
12	Bacteroidota	Bacteroidia	Chitinophagales			0.027	-0.037
13	Bacteroidota	Bacteroidia	Chitinophagales	37-13		0.024	-0.037
14	Cyanobacteria	Vampirivibrionia	Obscuribacterales	Obscuribacteraceae	Candidatus Obscuribacter	0.019	-0.036
15	Proteobacteria	Gammaproteobacteria	Burkholderiales	Rhodocyclaceae	Sulfuritalea	0.026	-0.036
16	Proteobacteria	Gammaproteobacteria	Burkholderiales	Nitrosomonadaceae	oc32	0.020	-0.036
17	Myxococcota	Polyangia	Polyangiales	Phaselicystidaceae		0.015	-0.036
18	Myxococcota	Polyangia	Polyangiales	Phaselicystidaceae	Phaselicystis	0.015	-0.036
19	Myxococcota	Polyangia	mle1-27			0.020	-0.036
20	Myxococcota	Polyangia	Polyangiales	Sandaracinaceae	uncultured	0.023	-0.035
21	Bacteroidota	Bacteroidia	Chitinophagales	Saprospiraceae	uncultured	0.028	-0.035
22	Bacteroidota	Bacteroidia	Chitinophagales	Saprospiraceae		0.028	-0.035
23	Verrucomicrobiota	Verrucomicrobiae	Pedosphaerales	Pedosphaeraceae	uncultured	0.029	-0.035
24	Myxococcota	Polyangia	Polyangiales	Sandaracinaceae	Sandaracinus	0.028	-0.034
25	Bacteroidota	Bacteroidia	Chitinophagales	Chitinophagaceae	Terrimonas	0.015	-0.034
26	Bacteroidota	Bacteroidia	Sphingobacteriales	AKYH767		0.012	-0.034
27	Proteobacteria	Gammaproteobacteria	Burkholderiales	Nitrosomonadaceae	IS-44	0.030	-0.034
28	Spirochaetota	Leptospirae				0.018	-0.034
29	Spirochaetota	Leptospirae	Leptospirales			0.018	-0.034
30	Spirochaetota	Leptospirae	Leptospirales	Leptospiraceae		0.018	-0.034

Supplementary Table 7. Principal component loadings of ARGs in Figure 4e (PCA on 16S-based microbial community of activated sludge only).

(a). Top 30 positive loading of PC1 (Microbial species relatively abundant in activated sludge of Denmark in Europe and Western USA)

No.	Phylum	Class	Order	Family	Genus	PC1	PC4
1	Firmicutes	Negativicutes				0.031	0.000
2	Firmicutes	Negativicutes	Veillonellales-Selenomonadales			0.031	-0.001
3	Verrucomicrobiota	Verrucomicrobiae	Pedosphaerales			0.031	-0.001
4	Verrucomicrobiota	Verrucomicrobiae	Pedosphaerales	Pedosphaeraceae		0.031	-0.001
5	Firmicutes	Bacilli	Paenibacillales	Paenibacillaceae	Paenibacillus	0.031	-0.002
6	Firmicutes	Clostridia	Oscillospirales			0.031	-0.002
7	Firmicutes	Bacilli	Paenibacillales			0.031	0.002
8	Firmicutes	Bacilli	Paenibacillales	Paenibacillaceae		0.031	0.002
9	Firmicutes	Negativicutes	Veillonellales-Selenomonadales	Selenomonadaceae		0.031	-0.002
10	Crenarchaeota	Nitrososphaeria				0.031	0.004
11	Firmicutes	Clostridia	f__Hungateiclostridiaceae	g__Fastidiosipila		0.031	0.004
12	Chloroflexi	Dehalococcoidia				0.031	0.001
13	Chloroflexi	Dehalococcoidia	SAR202 clade			0.031	0.000
14	Firmicutes	Clostridia	Clostridiales			0.031	0.006
15	Crenarchaeota					0.031	0.001
16	Firmicutes	Negativicutes	Veillonellales-Selenomonadales	Veillonellaceae		0.031	-0.001
17	Firmicutes	Clostridia				0.030	0.002
18	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae		0.030	0.002
19	Bacteroidota	Rhodothermia				0.030	0.001
20	Proteobacteria	Gammaproteobacteria	Gammaproteobacteria Incertae Sedis	Unknown Family	uncultured	0.030	0.000
21	Bacteroidota	Bacteroidia	Bacteroidales	Muribaculaceae		0.030	-0.001
22	Crenarchaeota	Thermoprotei				0.030	0.003
23	Chloroflexi	Ktedonobacteria				0.030	0.000
24	Gemmatimonadota	BD2-11 terrestrial group				0.030	0.000
25	Cyanobacteria	Cyanobacteriia	Synechococcales			0.030	0.000
26	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	uncultured	0.030	-0.002
27	Firmicutes	Clostridia	Clostridia UCG-014			0.030	-0.002
28	Acidobacteriota	Acidobacteriae	Acidobacteriales			0.030	0.010
29	Proteobacteria	Gammaproteobacteria	Enterobacterales	Erwiniaceae		0.030	0.003
30	Bacteroidota	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	0.030	0.003

Table 7 (b). Top 30 negative loading of PC1 (Microbial species relatively abundant in activated sludge of Japan during summer and Switzerland in Europe)

No.	Phylum	Class	Order	Family	Genus	PC1	PC4
1	Proteobacteria	Gammaproteobacteria	Burkholderiales	Comamonadaceae	Leptothrix	-0.006	0.015
2	Actinobacteriota	Acidimicrobiia	Microtrichales	Microtrichaceae	Candidatus Microthrix	-0.005	0.009
3	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Rhodanobacteraceae	Aquimonas	-0.004	-0.002
4	Proteobacteria	Gammaproteobacteria	Burkholderiales	Sutterellaceae	AAP99	-0.003	-0.001
5	Proteobacteria	Alphaproteobacteria	Caulobacterales	Hyphomonadaceae	Hirschia	-0.003	0.020
6	Actinobacteriota	Actinobacteria	Micrococcales	Beutenbergiaceae		-0.002	0.003
7	Bacteroidota	Bacteroidia	Cytophagales	Spirosomaceae	uncultured	-0.002	-0.007
8	Proteobacteria	Gammaproteobacteria	Beggiatoales	Beggiatoaceae	Beggiatoa	-0.002	0.000
9	Halobacterota	Methanomicrobia	Methanomicrobiales	Methanomicrobiaceae	Methanoculleus	-0.002	-0.001
10	Desulfobacterota	Syntrophobacteria	Syntrophobacterales	Syntrophobacteraceae	Syntrophobacter	-0.002	-0.001
11	Proteobacteria	Gammaproteobacteria	Beggiatoales			-0.002	-0.002
12	Proteobacteria	Gammaproteobacteria	Beggiatoales	Beggiatoaceae		-0.002	-0.002
13	Proteobacteria	Gammaproteobacteria	Beggiatoales	Beggiatoaceae	Candidatus Parabeggiatoa	-0.002	-0.032
14	Halobacterota	Methanomicrobia	Methanomicrobiales	Methanomicrobiaceae		-0.002	-0.001
15	Desulfobacterota	Syntrophobacteria	Syntrophobacterales	Syntrophobacteraceae		-0.002	-0.001
16	Desulfobacterota	Syntrophobacteria				-0.001	-0.001
17	Desulfobacterota	Syntrophobacteria	Syntrophobacterales			-0.001	-0.001
18	Proteobacteria	Gammaproteobacteria	Burkholderiales	Rhodocyclaceae	uncultured	-0.001	0.016
19	Proteobacteria	Gammaproteobacteria	Burkholderiales	Comamonadaceae	Hydrogenophaga	-0.001	0.000
20	Chloroflexi	Chloroflexia	Chloroflexales	Roseiflexaceae	Kouleothrix	-0.001	-0.020
21	Actinobacteriota	Actinobacteria	Frankiales	Sporichthyaceae	Longivirga	-0.001	0.046
22	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Methylocella	-0.001	0.036
23	Planctomycetota	Brocadiae	Brocadiales	Brocadaceae	Candidatus Brocadia	-0.001	0.035
24	Planctomycetota	Brocadiae	Brocadiales	Brocadaceae		-0.001	0.034
25	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Beijerinckia	-0.001	0.036
26	Halobacterota	Methanomicrobia				-0.001	-0.002
27	Halobacterota	Methanomicrobia	Methanomicrobiales			-0.001	-0.002
28	Desulfobacterota	Syntrophia	Syntrophales	Smithellaceae		-0.001	-0.005
29	Desulfobacterota	Syntrophia	Syntrophales	Smithellaceae	Smithella	-0.001	-0.005
30	Planctomycetota	Phycisphaerae	S-70			-0.001	0.026

Table 7 (c). Top 30 positive loading of PC4 (Microbial species relatively abundant in activated sludge of most of China, and eastern US)

No.	Phylum	Class	Order	Family	Genus	PC1	PC4
1	Actinobacteriota	Thermoleophila	Solirubrobacterales	67-14		0.010	0.065
2	Actinobacteriota	Thermoleophila	Solirubrobacterales			0.013	0.063
3	Acidobacteriota	Vicinamibacteria	Subgroup 17			0.008	0.063
4	Actinobacteriota	Thermoleophila				0.016	0.061
5	Armatimonadota	Fimbriimonadia	Fimbriimonadales	Fimbriimonadaceae		0.020	0.061
6	Proteobacteria	Gammaproteobacteria	Gammaproteobacteria Incertae Sedis	Unknown Family	Acidibacter	0.018	0.060
7	Proteobacteria	Gammaproteobacteria	PLTA13			0.005	0.059
8	Armatimonadota	Fimbriimonadia	Fimbriimonadales	Fimbriimonadaceae	Fimbriimonas	0.007	0.058
9	Nitrospirota	Nitrospira				0.001	0.057
10	Nitrospirota	Nitrospira	Nitrospirales			0.001	0.057
11	Nitrospirota	Nitrospira	Nitrospirales	Nitrospiraceae		0.001	0.057
12	Nitrospirota	Nitrospira	Nitrospirales	Nitrospiraceae	Nitrospira	0.001	0.057
13	Proteobacteria	Alphaproteobacteria	Tistrellales	Geminicoccaceae	Candidatus Alysiosphaera	0.012	0.057
14	Nitrospirota					0.004	0.057
15	Actinobacteriota	Acidimicrobiia	IMCC26256			0.010	0.056
16	Chloroflexi	JG30-KF-CM66				0.015	0.056
17	Actinobacteriota	Acidimicrobiia				0.011	0.055
18	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae		0.014	0.055
19	Armatimonadota	Fimbriimonadia				0.014	0.054
20	Armatimonadota	Fimbriimonadia	Fimbriimonadales			0.014	0.054
21	Patescibacteria	Microgenomatia	Candidatus Levybacteria			0.015	0.054
22	Methyloirabilota	Methyloirabilia	Methyloirabiales			0.007	0.054
23	Methyloirabilota	Methyloirabilia	Methyloirabiales	Methyloirabilaceae		0.007	0.054
24	Methyloirabilota	Methyloirabilia	Methyloirabiales	Methyloirabilaceae	Candidatus Methyloirabilis	0.002	0.054
25	Chloroflexi	Chloroflexia	Thermomicrobiales			0.009	0.053
26	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	uncultured	0.016	0.053
27	Actinobacteriota	Acidimicrobiia	Microtrichales			0.007	0.052
28	Chloroflexi	KD4-96				0.007	0.052
29	Planctomycetota	Planctomycetes	Gemmatales	Gemmataceae	uncultured	0.018	0.052
30	Chloroflexi	Chloroflexia	Thermomicrobiales	JG30-KF-CM45		0.006	0.051

Table 7 (d). Top 30 negative loading of PC4 (Microbial species relatively abundant in activated sludge of Japan during winter)

No.	Phylum	Class	Order	Family	Genus	PC1	PC4
1	Bacteroidota	Bacteroidia				0.008	-0.086
2	Bacteroidota	Bacteroidia	Cytophagales			0.002	-0.086
3	Bacteroidota					0.008	-0.086
4	Bacteroidota	Bacteroidia	Cytophagales	Microscillaceae		0.002	-0.086
5	Bacteroidota	Bacteroidia	Cytophagales	Microscillaceae	uncultured	0.002	-0.086
6	Proteobacteria	Gammaproteobacteria	Alteromonadales	Pseudoalteromonadaceae	Pseudoalteromonas	0.004	-0.084
7	Proteobacteria	Gammaproteobacteria	Alteromonadales	Pseudoalteromonadaceae		0.004	-0.084
8	Proteobacteria	Gammaproteobacteria	Alteromonadales			0.020	-0.065
9	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	Candidatus Photodesmus	0.008	-0.059
10	Proteobacteria	Gammaproteobacteria	Burkholderiales	Aquaspirillaceae	Rivicola	0.000	-0.058
11	Verrucomicrobiota	Verrucomicrobiae	Opitutales	Opitutaceae	Opitutus	0.013	-0.054
12	Proteobacteria	Gammaproteobacteria	Burkholderiales	Aquaspirillaceae		0.004	-0.051
13	Proteobacteria	Gammaproteobacteria	Aeromonadales	Aeromonadaceae	Tolumonas	0.008	-0.050
14	Proteobacteria	Alphaproteobacteria	Rickettsiales	Rickettsiaceae	Candidatus Megaira	0.001	-0.048
15	Bacteroidota	Bacteroidia	Sphingobacteriales	NS11-12 marine group		0.011	-0.045
16	Bacteroidota	Bacteroidia	Chitinophagales	Chitinophagaceae	Edaphobaculum	0.014	-0.044
17	Campilobacterota	Campylobacteria	Campylobacterales	Sulfurospirillaceae		0.022	-0.044
18	Campilobacterota	Campylobacteria	Campylobacterales	Sulfurospirillaceae	Sulfurospirillum	0.022	-0.044
19	Actinobacteriota	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	Pseudonocardia	0.019	-0.043
20	Proteobacteria	Gammaproteobacteria	Enterobacterales	Morganellaceae	Proteus	0.019	-0.043
21	Spirochaetota	Leptospirae	Leptospirales	Leptospiraceae	Turneriella	0.012	-0.042
22	Proteobacteria	Alphaproteobacteria	Paracaedibacterales	Paracaedibacteraceae	Candidatus Paracaedibacter	0.014	-0.042
23	Bacteroidota	Bacteroidia	Sphingobacteriales	env.OPS 17		0.016	-0.042
24	Proteobacteria	Gammaproteobacteria	Thiomicrospirales	Thiomicrospiraceae	Thiomicrothrix	0.012	-0.041
25	Proteobacteria	Gammaproteobacteria	Thiotrichales	Thiotrichaceae	Thiothrix	0.001	-0.039
26	Proteobacteria	Gammaproteobacteria	Thiotrichales			0.004	-0.038
27	Proteobacteria	Gammaproteobacteria	Thiotrichales	Thiotrichaceae		0.004	-0.038
28	Bacteroidota	Bacteroidia	Sphingobacteriales			0.023	-0.038
29	Proteobacteria	Alphaproteobacteria	Rickettsiales	Rickettsiaceae		0.015	-0.037
30	Bacteroidota	Bacteroidia	Cytophagales	Cytophagaceae	Cytophaga	0.009	-0.035

Supplementary Table 8. Principal component loadings of ARGs in Figure 4f (PCA on 16S-based microbial community of influent wastewater only).

(a). Top 30 positive loading of PC1 (Microbial species relatively abundant in most of untreated wastewater of Europe)

No.	Phylum	Class	Order	Family	Genus	PC1	PC4
1	Chloroflexi	Dehalococcoidia				0.041	0.009
2	Spirochaetota	Spirochaetia				0.040	0.012
3	Spirochaetota	Spirochaetia	Spirochaetales			0.040	0.012
4	Spirochaetota	Spirochaetia	Spirochaetales	Spirochaetaceae		0.040	0.012
5	Verrucomicrobiota	Omnitrophia	Omnitrophales			0.040	-0.001
6	Verrucomicrobiota	Omnitrophia				0.040	-0.001
7	Thermoplasmatota	Thermoplasmata				0.039	0.023
8	Cyanobacteria					0.039	-0.022
9	Crenarchaeota					0.039	0.018
10	Thermoplasmatota					0.039	0.023
11	Bacteroidota	Rhodothermia	Rhodothermales	Rhodothermaceae	Salinibacter	0.039	0.016
12	Verrucomicrobiota					0.039	-0.013
13	Cyanobacteria	Cyanobacteriia				0.039	-0.023
14	Halobacterota	Halobacteria				0.039	0.012
15	Halobacterota	Halobacteria	Halobacterales			0.039	0.012
16	Firmicutes	Bacilli	Mycoplasmatales	Mycoplasmataceae	Ureaplasma	0.039	-0.006
17	Planctomycetota					0.038	-0.018
18	Verrucomicrobiota	Omnitrophia	Omnitrophales	Omnitrophaceae		0.038	-0.007
19	Verrucomicrobiota	Omnitrophia	Omnitrophales	Omnitrophaceae	Candidatus Omnitrophus	0.038	-0.007
20	Crenarchaeota	Bathyarchaeia				0.038	0.015
21	Desulfobacterota	Desulfobacteria				0.038	0.017
22	Nanoarchaeota					0.038	0.016
23	Nanoarchaeota	Nanoarchaeia				0.038	0.016
24	Planctomycetota	vadinHA49				0.038	0.009
25	Nanoarchaeota	Nanoarchaeia	Woesearchaeales			0.038	0.015
26	Actinobacteriota	Thermoleophilia	Gaiellales			0.038	0.008
27	Cyanobacteria	Cyanobacteriia	Chloroplast			0.037	-0.023
28	Proteobacteria	Gammaproteobacteria	Francisellales			0.037	-0.012
29	Proteobacteria	Gammaproteobacteria	Francisellales	Francisellaceae		0.037	-0.012
30	Sumerlaeota					0.037	0.002

Table 8 (b). Top 30 negative loading of PC1 (Microbial species relatively abundant in most of untreated wastewater of Western USA and Japan during summer)

No.	Phylum	Class	Order	Family	Genus	PC1	PC4
1	Proteobacteria	Gammaproteobacteria	Burkholderiales	Aquaspirillaceae	Rivicola	-0.011	0.032
2	Halobacterota	Methanosarcinia	Methanosarciniales	Methanosarcinaceae	ANME-3	-0.010	0.061
3	Proteobacteria	Gammaproteobacteria	Burkholderiales	Aquaspirillaceae		-0.010	0.032
4	Bacteroidota	Bacteroidia	Cytophagales			-0.009	0.066
5	Bacteroidota	Bacteroidia	Cytophagales	Microscillaceae		-0.009	0.066
6	Bacteroidota	Bacteroidia	Cytophagales	Microscillaceae	uncultured	-0.009	0.066
7	Halobacterota	Methanosarcinia	Methanosarciniales	Methanosarcinaceae		-0.009	0.062
8	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Ruegeria	-0.009	0.052
9	Bacteroidota	Bacteroidia				-0.009	0.066
10	Bacteroidota					-0.009	0.066
11	Firmicutes	Bacilli	Staphylococcales	Staphylococcaceae		-0.008	0.063
12	Firmicutes	Bacilli	Staphylococcales	Staphylococcaceae	Staphylococcus	-0.008	0.063
13	Firmicutes	Bacilli	Staphylococcales			-0.008	0.063
14	Proteobacteria	Gammaproteobacteria	Ectothiorhodospirales	Ectothiorhodospiraceae	Thioalkalivibrio	-0.007	-0.005
15	Firmicutes	Desulfotomaculia	Desulfotomaculales	g_Desulfohalotomaculum		-0.007	0.064
16	Proteobacteria	Gammaproteobacteria	Aeromonadales	Aeromonadaceae		-0.007	0.066
17	Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Ursidibacter	-0.007	0.062
18	Proteobacteria	Gammaproteobacteria	Enterobacterales	Morganellaceae	Proteus	-0.007	0.064
19	Proteobacteria	Gammaproteobacteria	Aeromonadales	Aeromonadaceae	Aeromonas	-0.007	0.064
20	Campilobacterota	Campylobacteria	Campylobacteriales	uncultured		-0.007	0.032
21	Proteobacteria	Gammaproteobacteria	Aeromonadales			-0.007	0.066
22	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Roseobacter clade NAC11-7 lineage	-0.007	0.063
23	Proteobacteria	Gammaproteobacteria	Burkholderiales	Oxalobacteraceae	Herminiimonas	-0.007	0.002
24	Proteobacteria	Alphaproteobacteria	Rhodobacterales			-0.007	0.063
25	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae		-0.007	0.063
26	Proteobacteria	Gammaproteobacteria	Thiomicrospirales	Thiomicrospiraceae	Thiomicrothabdis	-0.007	0.064
27	Firmicutes	Desulfotomaculia	Desulfotomaculales			-0.006	0.065
28	Proteobacteria	Gammaproteobacteria	Pasteurellales			-0.006	0.062
29	Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae		-0.006	0.062
30	Firmicutes	Desulfotomaculia				-0.006	0.065

Table 8 (c). Top 30 positive loading of PC4 (Microbial species relatively abundant in untreated wastewater of Japan during winter)

No.	Phylum	Class	Order	Family	Genus	PC1	PC4
1	Proteobacteria	Gammaproteobacteria	Alteromonadales	Shewanellaceae	Shewanella	0.000	0.067
2	Proteobacteria	Gammaproteobacteria	Alteromonadales	Shewanellaceae		0.001	0.067
3	Proteobacteria	Gammaproteobacteria	Aeromonadales			-0.007	0.066
4	Bacteroidota					-0.009	0.066
5	Bacteroidota	Bacteroidia				-0.009	0.066
6	Proteobacteria	Gammaproteobacteria	Aeromonadales	Aeromonadaceae		-0.007	0.066
7	Actinobacteriota	Actinobacteria	Micrococcales	Micrococcaceae	Pseudarthrobacter	0.009	0.066
8	Bacteroidota	Bacteroidia	Cytophagales			-0.009	0.066
9	Bacteroidota	Bacteroidia	Cytophagales	Microscillaceae		-0.009	0.066
10	Bacteroidota	Bacteroidia	Cytophagales	Microscillaceae	uncultured	-0.009	0.066
11	Firmicutes	Desulfotomaculia				-0.006	0.065
12	Firmicutes	Desulfotomaculia	Desulfotomaculales			-0.006	0.065
13	Proteobacteria	Gammaproteobacteria	Aeromonadales	Aeromonadaceae	Aeromonas	-0.007	0.064
14	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Stenotrophomonas	-0.006	0.064
15	Proteobacteria	Gammaproteobacteria	Enterobacterales	Morganellaceae	Proteus	-0.007	0.064
16	Proteobacteria	Gammaproteobacteria	Thiomicrospirales	Thiomicrospiraceae	Thiomicrothabdis	-0.007	0.064
17	Proteobacteria	Gammaproteobacteria	Thiomicrospirales			-0.006	0.064
18	Proteobacteria	Gammaproteobacteria	Thiomicrospirales	Thiomicrospiraceae		-0.006	0.064
19	Proteobacteria	Gammaproteobacteria	Thiomicrospirales	Thiomicrospiraceae	uncultured	-0.005	0.064
20	Firmicutes	Desulfotomaculia	Desulfotomaculales	g__Desulfohalotomaculum		-0.007	0.064
21	Halobacterota	Methanosarcinia				-0.006	0.063
22	Halobacterota	Methanosarcinia	Methanosarciniales			-0.006	0.063
23	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Roseobacter clade NAC11-7 lineage	-0.007	0.063
24	Proteobacteria	Alphaproteobacteria	Rhodobacterales			-0.007	0.063
25	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae		-0.007	0.063
26	Halobacterota					0.002	0.063
27	Firmicutes	Bacilli	Staphylococcales			-0.008	0.063
28	Firmicutes	Bacilli	Staphylococcales	Staphylococcaceae		-0.008	0.063
29	Firmicutes	Bacilli	Staphylococcales	Staphylococcaceae	Staphylococcus	-0.008	0.063
30	Proteobacteria	Gammaproteobacteria	Burkholderiales	Burkholderiaceae	Burkholderia-Caballeronia-Paraburkholderia	-0.006	0.062

Table 8 (d). Top 30 negative loading of PC4 (Microbial species relatively abundant in most of untreated wastewater of China and Eastern USA)

No.	Phylum	Class	Order	Family	Genus	PC1	PC4
1	Proteobacteria	Gammaproteobacteria	Burkholderiales	Comamonadaceae	Alicyclophilus	0.014	-0.045
2	Actinobacteriota	Actinobacteria	Propionibacteriales	Propionibacteriaceae	Propioniciclava	0.022	-0.045
3	Firmicutes	Negativicutes	Veillonellales-Selenomonadales	Selenomonadaceae	Anaerovibrio	0.007	-0.044
4	Firmicutes	Bacilli	Bacillales	Bacillaceae	Halobacillus	0.008	-0.044
5	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiales Incertae Sedis		0.014	-0.043
6	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Ketogulonicigenium	0.019	-0.043
7	Proteobacteria	Gammaproteobacteria	Methylococcales	Methylococcaceae		0.002	-0.042
8	Proteobacteria	Gammaproteobacteria	Burkholderiales	SC-I-84		0.018	-0.041
9	Firmicutes	Negativicutes	Veillonellales-Selenomonadales	Selenomonadaceae	Schwartzia	0.010	-0.041
10	Actinobacteriota	Actinobacteria	Propionibacteriales	Propionibacteriaceae	Micropruina	0.010	-0.041
11	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiales Incertae Sedis	uncultured	0.008	-0.040
12	Proteobacteria	Gammaproteobacteria	Gammaproteobacteria Incertae Sedis	Unknown Family	Acidibacter	0.009	-0.040
13	Proteobacteria	Alphaproteobacteria	Rhizobiales	A0839		0.004	-0.040
14	Proteobacteria	Gammaproteobacteria	Burkholderiales	Comamonadaceae	Ideonella	0.004	-0.039
15	Bacteroidota	Bacteroidia	Chitinophagales	Chitinophagaceae	Niabella	0.006	-0.039
16	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Gemmobacter	0.020	-0.039
17	Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	Roseomonas	0.018	-0.039
18	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Rhodobacter	0.019	-0.039
19	Proteobacteria	Gammaproteobacteria	Burkholderiales	Methylophilaceae	uncultured	0.004	-0.038
20	Proteobacteria	Gammaproteobacteria	Methylococcales			0.007	-0.037
21	Actinobacteriota	Actinobacteria	Propionibacteriales	Propionibacteriaceae		0.031	-0.037
22	Actinobacteriota	Actinobacteria	Frankiales	Sporichthyaceae	Longivirga	0.001	-0.037
23	Cyanobacteria	Cyanobacteriia	Cyanobacteriales	Nostocaceae	Rivularia PCC-7116	0.012	-0.036
24	Actinobacteriota	Coriobacteriia	Coriobacteriales	Atopobiaceae		0.027	-0.036
25	Euryarchaeota	Methanobacteria	Methanobacteriales	Methanobacteriaceae	Methanobacterium	0.012	-0.036
26	Actinobacteriota	Coriobacteriia	Coriobacteriales	Atopobiaceae	Olsenella	0.023	-0.036
27	Actinobacteriota	Acidimicrobiia	Microtrichales	Microtrichaceae	uncultured	0.007	-0.036
28	Chloroflexi	Anaerolineae	SJA-15			0.009	-0.036
29	Proteobacteria	Gammaproteobacteria	Methylococcales	Methylococcaceae	Methyloparacoccus	0.004	-0.035
30	Actinobacteriota	Actinobacteria	Propionibacteriales			0.033	-0.035

Supplementary Table 9. Frequency of horizontal gene transfer (HGT) reported in past studies. The maximum HGT rate μ was estimated from the highest frequency f_{HGT} and contact time t by $\mu = \frac{\ln(1+f_{HGT})}{t}$

Reference	Donor	Recipient	Temperature [°C]	Contact time [hr]	Frequency of HGT [log]	Quantification methods	Max HGT rate, estimated [1/d]
Li et al. 2019 ¹	<i>E. coli</i>	<i>E. coli</i>	26	4	-1.5	Molecular	0.18
Kenzaka et al. 2007 ²	Phage	<i>E. coli</i>	37	0.33	-3 to -4	Molecular	0.07
	Phage	<i>E. coli</i>	37	0.33	-6 to -7	Culture	7.2×10⁻⁰⁵
Johnsen & Kroer 2007 ³	<i>E. coli</i>	<i>Pseudomonas putida</i>	29	18	-3 to -4	Culture	1.3×10⁻⁰³
Hutinel et al. 2021 ⁴	WWTP Effluent	<i>E. coli</i>	30	3	-4 to -6	Culture	8.0×10⁻⁰⁴
Jutkina et al. 2016 ⁵	WWTP Effluent	<i>E. coli</i>	30	3	-5 to -6	Culture	8.0×10⁻⁰⁵
	WWTP Effluent	<i>E. coli</i>	30	16	-5 to -6	Culture	1.5×10⁻⁰⁵
Mishra et al. 2021 ⁶	<i>E. coli</i>	<i>E. coli</i>	37	2	-12 to -13	Culture	1.2×10⁻¹¹
	<i>E. coli</i>	<i>E. coli</i>	30	2	-12 to -13	Culture	1.2×10⁻¹¹
	<i>E. coli</i>	<i>E. coli</i>	20	6	-13 to -14	Culture	4.0×10⁻¹³

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