

SUPPORTING INFORMATION

Mitigating microbiological risks of potential pathogens carrying antibiotic resistance genes and virulence factors in receiving rivers: Benefits of wastewater treatment plant upgrade

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Text S1 Information on three receiving rivers

Three wastewater-dominant rivers (>80% wastewater in composition), i.e., the Tonghui River, Liangshui River, and Qing River, have received domestic effluent discharge from Beijing city for over 60 years. The Tonghui River flows 20 km from the Dongbianmen Datong Bridge to the Tongzhou District and includes 22 effluent outlets. The Gaobeidian Wastewater Treatment Plant (WWTP) is the largest along the Tonghui River, and its effluent is the dominant source of water in the river. The Liangshui River originates from Houniwa village in the Fengtai District of Beijing. It flows through the Fengtai, Daxing, and Tongzhou districts and then enters the North Canal upstream of the Yulinzhuang Gate. The Liangshui River is 56.8 km long, with a watershed area of 624 km². Effluent from the Xiaohongmen WWTP is the dominant source of water in the river. The Qing River, a tributary of the Wenyu River, flows 23.6 km from the Jingmi diversion canal to the convergence of the Wenyu River. Effluent from the Qing River WWTP is the main source of water in the river. The study sites in the three rivers are all located in urban areas of Beijing, and thus show similar urban land-use type. The depths of the Qing, Tonghui, and Liangshui rivers during the wet season are 8.2, 10.5, and 13.8 m, respectively. The influence of sediment is negligible as most sampling sites have stony river bottoms.

Text S2 Water parameter analyses

Water temperature, conductivity, total dissolved solids (TDS), and pH were recorded

in situ with a multi-parameter water quality sonde (MYRON Co., USA). The water parameters in the receiving rivers were stable before and after WWTP upgrade (WWTP-UP). All water samples were transported to the laboratory to measure dissolved organic carbon (DOC), total nitrogen (TN), and total phosphorus (TP) concentrations, which were determined using alkaline potassium persulfate digestion UV spectrophotometry, ammonium molybdate spectrophotometry, and total organic carbon (TOC) analyzer (Shimadzu TOC-VCPH, Japan), respectively.

Text S3 Shotgun sequencing

For shotgun metagenomic sequencing, total DNA extracted from samples collected in the Tonghui, Liangshui, and Qing rivers was used for metagenomic sequencing. Paired-end libraries (350- or 300-insert size) were constructed using an Illumina TruSeq DNA Sample Preparation Kit (Illumina, Inc., San Diego, CA, USA). Cluster generation was performed on the Illumina cBot automated cluster generation system according to the manufacturer's instructions. The prepared templates were loaded on the Illumina HiSeq X-Ten system for sequencing (150 paired-end reads). A total of 116 DNA samples were sequenced. Following sequencing, raw reads from each metagenome were filtered to remove reads containing adapters and duplications, >10% ambiguous bases, or low-quality bases (Q-score \leq 32 and occupying >40% of total bases).

Text S4 Genomic binning

Contigs assembled using MEGAHIT v1.1.3 were clustered into metagenome-assembled genomes (MAGs) using MetaBAT v2 (Kang et al., 2019), MaxBin v2.0 (Wu et al., 2016), and CONCOCT (Alneberg et al., 2014). MAGs recovered from the same sample using the above three programs were together refined using the *bin_refinement* module in the MetaWRAP pipeline to retain only the best representative MAGs (completeness > 50%; contaminant < 10%); dRep was then used to dereplicate (cutoff for filtering: minimum genome completeness 50%; maximum genome contamination 10%) the MAGs from all samples by identifying groups of highly similar MAGs, choosing the best representative for each MAG set to generate a nonredundant MAG set. The coverage of nonredundant MAGs in each sample was estimated using a mapping-based method (BBMap v38.43, under slow mode/high sensitivity) and customized scripts. The abundance of each MAG in each sample was calculated based on the read mapping percentage and MAG coverage.

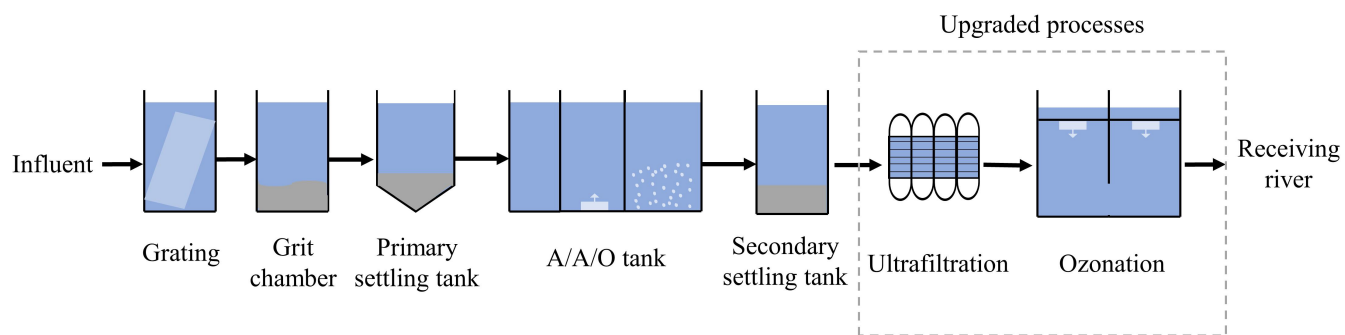


Fig. S1 Schematic of investigated WWTPs with upgraded processes.

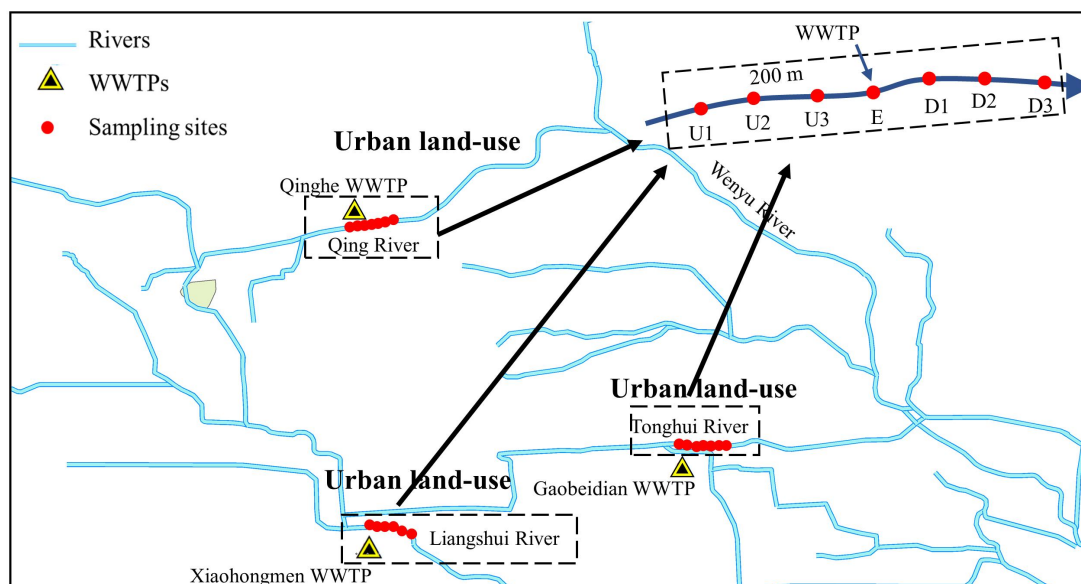


Fig. S2 Sampling sites in the Tonghui, Liangshui, and Qing rivers. Map was modified with permission from Wang et al. (2020).

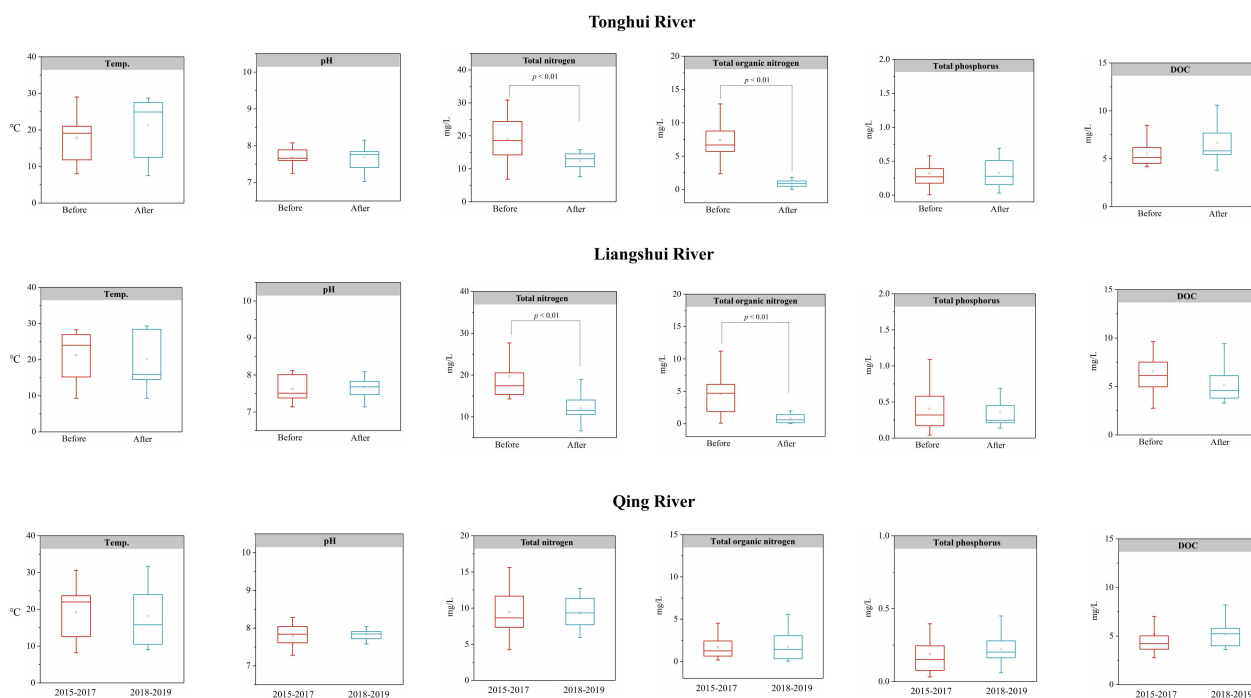


Fig. S3 Variation in water quality indices in Tonghui and Liangshui rivers before and after WWTP-UP and in Qing River correspond to the same sampling time (2015-2017 and 2018-2019) as the other two receiving rivers.

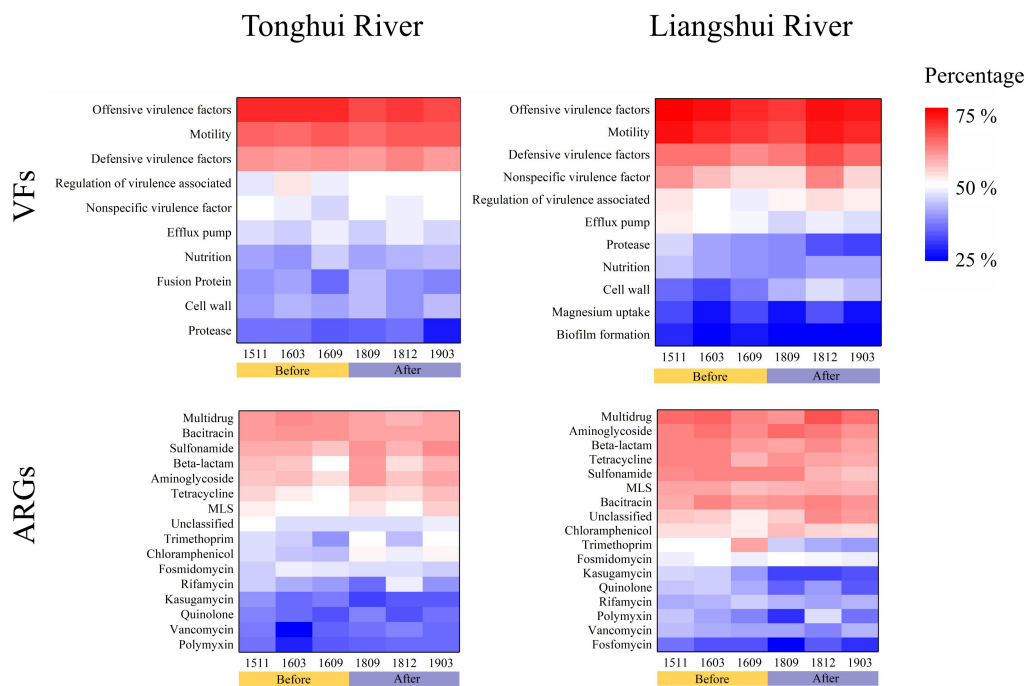


Fig. S4 Heatmaps of percentages of VF and ARG types in the Tonghui and Liangshui rivers before and after WWTP-UP. Color gradient from red to blue represents higher to lower percentages. MLS: macrolide-lincosamide-streptogramin.

Qing River

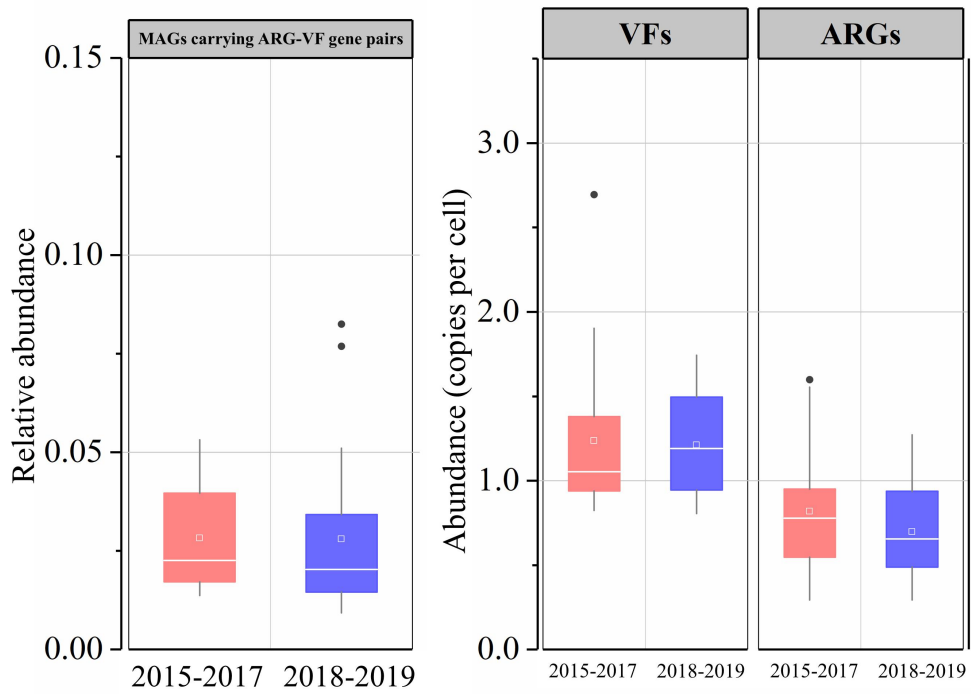


Fig. S5 Relative abundance of total MAGs carrying ARG-VF gene pairs, VFes, and ARGs in the Qing River correspond to the same sampling time (2015-2017 and 2018-2019) as the other two receiving rivers.

Table S1 Information on three WWTPs.⁵

WWTP	Parameter		Receiving river	Treatment process		Upgrade time
	Capacity (m ³ /d)	Discharge into river (m ³ /d)		Before upgrade	After upgrade	
Gaobeidian	1 000 000	490 000	Tonghui River	A/A/O	A/A/O + membrane filtration + ozonation	2017.04
Xiaohongmen	600 000	300 000	Liangshui River	A/A/O	A/A/O + membrane filtration + ozonation	2017.11
Qing	400 000	248 000	Qing River	A/A/O	A/A/O + membrane filtration + ozonation	2013.12

Table S2 Basic information on MAG mapping reads.

Sample ID	Month	River	Upgrade	Mapping read count	Total read number	Mapping ratio
1511WGD1	November	Tonghui	Before	6080910	19335451	31.45%
1511WGD2	November	Tonghui	Before	5515988	19562974	28.20%
1511WGE	November	Tonghui	Before	8182789	19552540	41.85%
1511WGU1	November	Tonghui	Before	9693604	19194841	50.50%
1511WGU2	November	Tonghui	Before	8517639	19274144	44.19%
1511WXD1	November	Liangshui	Before	12118507	19070893	63.54%
1511WXD2	November	Liangshui	Before	8076241	19545039	41.32%
1511WXE	November	Liangshui	Before	11891403	19516498	60.93%
1511WXU1	November	Liangshui	Before	5158863	19216930	26.85%
1511WXU2	November	Liangshui	Before	5979702	19201071	31.14%
1511WQD1	November	Qing	After	8595166	19184720	44.80%
1511WQD2	November	Qing	After	7731119	19259609	40.14%
1511WQU1	November	Qing	After	7389813	19310054	38.27%
1511WQU2	November	Qing	After	9563656	19219441	49.76%
1603WGD1	March	Tonghui	Before	6737841	19115086	35.25%
1603WGD2	March	Tonghui	Before	6170880	19009488	32.46%
1603WGD3	March	Tonghui	Before	6473408	19134666	33.83%
1603WGE	March	Tonghui	Before	6270401	19134257	32.77%
1603WGU0	March	Tonghui	Before	11893602	19134335	62.16%

1603WGU1	March	Tonghui	Before	6286329	19018269	33.05%
1603WGU2	March	Tonghui	Before	9930169	18905970	52.52%
1603WXD1	March	Liangshui	Before	7907029	17410226	45.42%
1603WXD2	March	Liangshui	Before	8883409	19342600	45.93%
1603WXD3	March	Liangshui	Before	9154574	19261611	47.53%
1603WXE	March	Liangshui	Before	6504269	19287940	33.72%
1603WXU0	March	Liangshui	Before	9987503	19298669	51.75%
1603WXU1	March	Liangshui	Before	8151138	19026779	42.84%
1603WXU2	March	Liangshui	Before	11499870	19248525	59.74%
1603QD1	March	Qing	After	9197229	19447206	47.29%
1603QD2	March	Qing	After	10234818	18758085	54.56%
1603QE	March	Qing	After	6228682	19355414	32.18%
1603QU1	March	Qing	After	8187583	19371015	42.27%
1603QU2	March	Qing	After	9251931	19141441	48.33%
1609WGD1	September	Tonghui	Before	8055714	19311354	41.71%
1609WGD2	September	Tonghui	Before	7987424	18933521	42.19%
1609WGD3	September	Tonghui	Before	6591457	18637046	35.37%
1609WGE	September	Tonghui	Before	5427848	18895087	28.73%
1609WGU0	September	Tonghui	Before	6291331	19057657	33.01%
1609WGU1	September	Tonghui	Before	6203886	19212543	32.29%
1609WGU2	September	Tonghui	Before	8596645	16772860	51.25%

1609WXD2	September	Liangshui	Before	4629642	18471666	25.06%
1609WXD3	September	Liangshui	Before	4560274	19097424	23.88%
1609WXE	September	Liangshui	Before	7466353	19265987	38.75%
1609WXU0	September	Liangshui	Before	9113518	19091237	47.74%
1609WXU1	September	Liangshui	Before	8500777	19089055	44.53%
1609WXU2	September	Liangshui	Before	9149056	19249365	47.53%
1609WQD1	September	Qing	After	11097936	19170104	57.89%
1609WQD2	September	Qing	After	9996309	19080554	52.39%
1609WQD3	September	Qing	After	12768378	19228917	66.40%
1609WQE	September	Qing	After	11329658	17494404	64.76%
1609WQU0	September	Qing	After	9169658	19215848	47.72%
1609WQU1	September	Qing	After	11433022	19132414	59.76%
1609WQU2	September	Qing	After	11739638	19312839	60.79%
1809WGD1	September	Tonghui	After	5780552	19375842	29.83%
1809WGD2	September	Tonghui	After	7888874	19731310	39.98%
1809WGD3	September	Tonghui	After	6908511	19187926	36.00%
1809WGE	September	Tonghui	After	6691396	19181343	34.88%
1809WGU0	September	Tonghui	After	6266379	19786590	31.67%
1809WGU1	September	Tonghui	After	4531628	18818562	24.08%
1809WGU2	September	Tonghui	After	8749358	19724797	44.36%
1809WXD1	September	Liangshui	After	6512564	19794906	32.90%

1809WXD2	September	Liangshui	After	6476835	19658358	32.95%
1809WXD3	September	Liangshui	After	8669243	19695845	44.02%
1809WXE	September	Liangshui	After	9773395	19216442	50.86%
1809WXU0	September	Liangshui	After	8801353	19148905	45.96%
1809WXU1	September	Liangshui	After	9009800	19774976	45.56%
1809WXU2	September	Liangshui	After	7323805	19689976	37.20%
1809WQD1	September	Qing	After	8209013	19627740	41.82%
1809WQD2	September	Qing	After	10587206	19793194	53.49%
1809WQD3	September	Qing	After	6179126	19595114	31.53%
1809WQE	September	Qing	After	8588973	19748565	43.49%
1809WQU0	September	Qing	After	9472008	19258841	49.18%
1809WQU1	September	Qing	After	8309710	19608037	42.38%
1809WQU2	September	Qing	After	10841167	19335373	56.07%
1812WGD1	December	Tonghui	After	10431759	19095892	54.63%
1812WGD2	December	Tonghui	After	10376266	15249669	68.04%
1812WGD3	December	Tonghui	After	8233844	19644101	41.92%
1812WGE	December	Tonghui	After	6880371	19162033	35.91%
1812WGU0	December	Tonghui	After	8720403	19094262	45.67%
1812WGU1	December	Tonghui	After	8678968	19164253	45.29%
1812WGU2	December	Tonghui	After	10073316	19441895	51.81%
1812WXD1	December	Liangshui	After	15317024	19156541	79.96%

1812WXD2	December	Liangshui	After	5502604	19788080	27.81%
1812WXD3	December	Liangshui	After	6225730	19409533	32.08%
1812WXE	December	Liangshui	After	7522155	19603801	38.37%
1812WXU0	December	Liangshui	After	10164371	19310533	52.64%
1812WXU1	December	Liangshui	After	6203847	19122145	32.44%
1812WXU2	December	Liangshui	After	9600510	19780875	48.53%
1812WQD1	December	Qing	After	8783231	19588666	44.84%
1812WQD2	December	Qing	After	8418306	19273304	43.68%
1812WQD3	December	Qing	After	7577330	18854913	40.19%
1812WQE	December	Qing	After	9395774	19614892	47.90%
1812WQU0	December	Qing	After	10029837	19188306	52.27%
1812WQU1	December	Qing	After	8635967	19230619	44.91%
1812WQU2	December	Qing	After	8067306	19815447	40.71%
1903WGD1	March	Tonghui	After	8172213	19450471	42.02%
1903WGD2	March	Tonghui	After	9009418	19563563	46.05%
1903WGD3	March	Tonghui	After	6545827	18177286	36.01%
1903WGE	March	Tonghui	After	5246827	19196701	27.33%
1903WGU0	March	Tonghui	After	10797788	19594864	55.11%
1903WGU1	March	Tonghui	After	8641217	17467906	49.47%
1903WGU2	March	Tonghui	After	9848492	19632188	50.17%
1903WXD1	March	Liangshui	After	8978227	19239676	46.67%

1903WXD2	March	Liangshui	After	6356170	19244944	33.03%
1903WXD3	March	Liangshui	After	5835551	19768172	29.52%
1903WXE	March	Liangshui	After	6347770	19093462	33.25%
1903WXU0	March	Liangshui	After	9451470	19316938	48.93%
1903WXU1	March	Liangshui	After	9926013	19683436	50.43%
1903WXU2	March	Liangshui	After	10602454	19230844	55.13%
1903WQD1	March	Qing	After	8445045	19127139	44.15%
1903WQD2	March	Qing	After	6655299	19165660	34.73%
1903WQD3	March	Qing	After	9352612	19742511	47.37%
1903WQE	March	Qing	After	7993103	19738872	40.49%
1903WQU0	March	Qing	After	8324897	19083808	43.62%
1903WQU1	March	Qing	After	8217082	19105059	43.01%
1903WQU2	March	Qing	After	11213493	19298903	58.10%

Table S3 List of 32 potential pathogenic genera downloaded from Virulence Factor Database (<http://www.mgc.ac.cn/VFs/download.htm>).

No.	Genus	Family	Phylum
1	<i>Acinetobacter</i>	Moraxellaceae	Gammaproteobacteria
2	<i>Aeromonas</i>	Aeromonadaceae	Gammaproteobacteria
3	<i>Anaplasma</i>	Anaplasmataceae	Alphaproteobacteria
4	<i>Bacillus</i>	Bacillaceae	Firmicutes
5	<i>Bartonella</i>	Bartonellaceae	Alphaproteobacteria
6	<i>Bordetella</i>	Alcaligenaceae	Betaproteobacteria
7	<i>Brucella</i>	Brucellaceae	Alphaproteobacteria
8	<i>Burkholderia</i>	Burkholderiaceae	Betaproteobacteria
9	<i>Campylobacter</i>	Campylobacteraceae	Epsilonproteobacteria
10	<i>Chlamydia</i>	Chlamydiaceae	Chlamydiae
11	<i>Clostridium</i>	Clostridiaceae	Firmicutes
12	<i>Corynebacterium</i>	Corynebacteriaceae	Actinobacteria
13	<i>Coxiella</i>	Coxiellaceae	Gammaproteobacteria
14	<i>Enterococcus</i>	Enterococcaceae	Firmicutes
15	<i>Escherichia</i>	Enterobacteriaceae	Gammaproteobacteria
16	<i>Francisella</i>	Francisellaceae	Gammaproteobacteria
17	<i>Haemophilus</i>	Pasteurellaceae	Gammaproteobacteria
18	<i>Helicobacter</i>	Helicobacteraceae	Epsilonproteobacteria
19	<i>Klebsiella</i>	Enterobacteriaceae	Gammaproteobacteria
20	<i>Legionella</i>	Legionellaceae	Gammaproteobacteria
21	<i>Listeria</i>	Listeriaceae	Firmicutes
22	<i>Mycobacterium</i>	Mycobacteriaceae	Actinobacteria
23	<i>Mycoplasma</i>	Mycoplasmataceae	Tenericutes
24	<i>Neisseria</i>	Neisseriaceae	Betaproteobacteria
25	<i>Pseudomonas</i>	Pseudomonadaceae	Gammaproteobacteria
26	<i>Rickettsia</i>	Rickettsiaceae	Alphaproteobacteria
27	<i>Salmonella</i>	Enterobacteriaceae	Gammaproteobacteria
28	<i>Shigella</i>	Enterobacteriaceae	Gammaproteobacteria
29	<i>Staphylococcus</i>	Staphylococcaceae	Firmicutes
30	<i>Streptococcus</i>	Streptococcaceae	Firmicutes
31	<i>Vibrio</i>	Vibrionaceae	Deltaproteobacteria
32	<i>Yersinia</i>	Enterobacteriaceae	Gammaproteobacteria

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