Multiple Discharges of Treated Municipal Wastewater Have a Small Effect on the Quantities of Numerous Antibiotic Resistance Determinants in the Upper Mississippi River

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Table S1. A brief description of the wastewater treatment facilities described in this study.

Fig. 1 Number	Facility	Average Flow Rate (MGD)	Discharge Location (miles)	Disinfection Technology	Other Tertiary Technologies
1	Bemidji	1	54.5	UV	Chemical P removal; Effluent filtration
2	Grand Rapids	6	161.2	Chlorination/ Dechlorination	Tertiary ponds
3	Aitkin	0.4	285.9	Chlorination/ Dechlorination	
4	Brainerd	7	343.2	UV	Biological P removal
5	Little Falls	1	376.3	Chlorination/ Dechlorination	
6	St. Cloud	10	422.2	UV	Biological P removal
7	Elk River	1.1	457.5	UV	Sand filtration; Chemical P removal
8	Metropolitan	180	505	Chlorination/ Dechlorination	Biological P removal
9	Empire	24	517.7	UV	Biological P removal
10	Eagles Point	10	522.2	UV	Chemical P removal
11	Hastings	1.5	527.2	Chlorination/ Dechlorination	
12	Wabasha	0.2	581.2	UV	Chemical and Biological P removal
13	Winona	4	615.2	Chlorination/ Dechlorination	
14	LaCrosse	10	641.2	UV	Biological P removal

Table S2. A brief description of the sample locations from which Mississippi River samples were collected.

Fig. 1 Letter	Description	Downstream location (miles)
A	Itasca State Park	0
B	Three Rivers Scenic	87
C	Aitkin	267.5
D	Fort Ripley	359
E	Royalton	387
F	Elk River	458
G	University of Minnesota	489
H	Prairie Island	534
I	Lake Pepin	565
J	Wabasha	578
K	Winona	614
L	Goose Island	646

Table S3. The primer sequences, expected amplicon size, and annealing temperature for each real-time PCR method used in this work.

Target	Primer Sequence (5'→3')	Size (bp)	Annealing Temp (°C)	SI Reference
16S rRNA gene (338F, 518R)	**F:** CCT ACG GGA GGC AGC AG **R:** ATT ACC GCG GCT GCT GG	202	60	*1*
bla Q1	**F:** TAC GGG GTC TGA CGC TCA **R:** TAA GCA TTG GTA ACT TGT	150	55	*2*
erm(B)	**F:** GAT ACC GTT TAC GAA ATT GG **R:** GAA TCG AGA CTT GAG TGT GC	364	58	*3*
IncA/C	**F:** TTC ATC AGC TCC AGC TTC TT **R:** CAG ATT CAT GAT CGA TTC GTT T	126	60	This study
intI1	**F:** CCT CCC GCA CGA TGA TC **R:** TCC ACG CAT CGT CAG GC	280	60	*4*
qnrA	**F:** AGG ATT TCT CAC GCC AGG ATT **R:** CGC TTT CAA TGA AAC TGC A	124	60	*5*
sul1	**F:** CCG TTG GCC TTC CTG TAA AG **R:** TTG CCG ATC GCG TGA AGT	67	60	*6*
tet(A)	**F:** GCT ACA TCC TGC TTG CCT TC **R:** CAT AGA TCG CCG TGA AGA GG	210	60	*7*
tet(W)	**F:** GAG AGC CTG CTA TAT GCC AGC **R:** GGG CGT ATC CAC AAT GTT AAC	168	60	*8*
tet(X)	**F:** AGC CTT ACC AAT GGG TGT AAA **R:** TTC TTA CCT TGG ACA TCC CG	278	60	*7, 9*

Table S4. Flow rates in the Mississippi River for 11 (July 2012 and August 2012) or 13 (May 2012) stations maintained by the United States Geological Survey (waterdata.usgs.gov) or the United States Army Corps of Engineers (www.mvp-wc.usace.army.mil). Reported flow rates are the arithmetic means of four consecutive days that match the dates that river and wastewater effluent samples were collected.

Station Description	Downstream Distance (miles)	Flow Rate, May 2012 (ft^3 s^{-1})	Flow Rate, July 2012 (ft^3 s^{-1})	Flow Rate, Aug. 2012 (ft^3 s^{-1})
Near Bemidji	64.2	493	129	105
Willow Beach at Ball Club	128.2	1565	920	953
Grand Rapids	162.4	1575	2088	1069
Aitkin	285.9	4297	6017	2042
Brainerd	337.2	5216	7165	2664
Royalton	388.2	9665	9182	3626
St. Cloud	416.2	11037	9385	3627
Anoka	476.8	19667	12526	4846
St. Paul, Smith Ave	501.2	38561	N/A	N/A
Prescott, WI	527.2	47227	N/A	N/A
Winona, MN	612.8	59725	28700	13900
Lock and Dam 7	635.7	66425	30650	14850
Lock and Dam 8	659	69150	34550	15175

Table S5. Quantities of 16S rRNA genes per milliliter detected in wastewater effluents on three different sample dates.

Facility	May 2012	July 2012	August 2012
Bemidji	1.4×10^{6}	7.1×10^{8}	4.1×10^{8}
Grand Rapids	3.4×10^{7}	1.0×10^{8}	5.2×10^{7}
Aitkin	1.2×10^{7}	1.9×10^{8}	1.4×10^{8}
Brainerd	2.2×10^{7}	1.5×10^{10}	2.1×10^{2}
Little Falls	2.4×10^{2}	1.8×10^{9}	1.2×10^{8}
St. Cloud	6.6×10^{5}	9.7×10^{7}	4.2×10^{9}
Elk River	5.6×10^{6}	7.5×10^{8}	1.5×10^{8}
Metropolitan	4.0×10^{6}	3.4×10^{8}	1.4×10^{8}
Empire	3.3×10^{5}	4.0×10^{8}	6.4×10^{7}
Eagles Point	7.4×10^{6}	5.5×10^{9}	1.6×10^{7}
Hastings	5.9×10^{6}	3.4×10^{8}	2.3×10^{7}
Wabasha	8.4×10^{5}	3.2×10^{7}	1.5×10^{7}
Winona	1.2×10^{7}	3.8×10^{9}	2.2×10^{8}
LaCrosse	4.0×10^{7}	6.1×10^{8}	1.8×10^{8}

Table S6. Quantities of 16S rRNA genes per mL detected in the Mississippi River on three different sample dates. BD = below detection, N/A = not available

Facility	May 2012	July 2012	August 2012
Itasca State Park	2.4×10^5	5.9×10^7	2.2×10^8
Three Rivers Scenic	3.0×10^5	4.7×10^7	2.6×10^7
Aitkin	4.8×10^5	5.9×10^7	1.6×10^7
Fort Ripley	7.7×10^5	6.7×10^7	3.4×10^7
Royalton	4.0×10^5	7.5×10^7	3.9×10^8
Elk River	2.0×10^6	7.9×10^7	2.0×10^8
University of Minnesota	1.1×10^6	1.1×10^8	1.8×10^8
Prairie Island	2.2×10^6	1.6×10^8	6.9×10^8
Lake Pepin	NA	6.4×10^7	1.1×10^8
Wabasha	7.5×10^5	4.6×10^7	1.0×10^8
Winona	4.5×10^5	5.7×10^7	5.4×10^7
Goose Island	2.2×10^6	6.9×10^7	9.0×10^7

Table S7. Quantities of *intI1* genes per milliliter detected in wastewater effluents on three different sample dates.

Facility	May 2012	July 2012	August 2012
Bemidji	1.9×10^5	8.6×10^6	4.4×10^7
Grand Rapids	2.6×10^6	3.9×10^4	3.9×10^5
Aitkin	5.9×10^5	4.2×10^5	1.7×10^6
Brainerd	1.4×10^6	3.8×10^7	3.6×10^6
Little Falls	2.6×10^6	1.2×10^7	2.4×10^7
St. Cloud	4.3×10^4	1.7×10^5	1.6×10^6
Elk River	1.4×10^5	4.0×10^5	3.9×10^5
Metropolitan	1.6×10^5	3.1×10^5	6.0×10^5
Empire	2.5×10^4	2.1×10^5	1.2×10^5
Eagles Point	7.2×10^5	7.8×10^6	8.7×10^5
Hastings	1.8×10^5	3.0×10^5	7.2×10^5
Wabasha	3.3×10^4	1.2×10^4	3.5×10^4
Winona	9.4×10^5	5.4×10^6	1.2×10^6
LaCrosse	2.4×10^6	1.7×10^5	5.1×10^5

Table S8. Quantities of *tet*(A) genes per milliliter detected in wastewater effluents on three different sample dates.

Facility	May 2012	July 2012	August 2012
Bemidji	2.6×10^3	2.8×10^5	1.0×10^5
Grand Rapids	3.1×10^5	6.4×10^3	2.0×10^4
Aitkin	6.4×10^3	1.2×10^4	1.7×10^5
Brainerd	1.7×10^4	6.0×10^6	3.8×10^5
Little Falls	5.3×10^5	4.9×10^5	5.6×10^6
St. Cloud	1.1×10^3	9.1×10^3	4.0×10^5
Elk River	5.0×10^3	5.4×10^4	5.3×10^5
Metropolitan	3.3×10^3	2.4×10^4	1.2×10^5
Empire	1.3×10^3	2.1×10^4	9.7×10^4
Eagles Point	8.5×10^3	3.0×10^5	1.2×10^5
Hastings	6.4×10^3	2.9×10^4	1.5×10^5
Wabasha	4.8×10^3	4.6×10^3	2.1×10^4
Winona	2.9×10^4	2.8×10^5	8.8×10^4
LaCrosse	8.4×10^4	1.6×10^4	1.1×10^5

Table S9. Quantities of *tet*(W) genes per milliliter detected in wastewater effluents on three different sample dates.

Facility	May 2012	July 2012	August 2012
Bemidji	2.9×10^4	2.9×10^5	3.3×10^4
Grand Rapids	3.3×10^4	5.0×10^2	5.2×10^3
Aitkin	3.6×10^5	5.4×10^4	2.6×10^5
Brainerd	2.9×10^5	4.2×10^6	1.3×10^5
Little Falls	5.3×10^5	8.0×10^5	2.0×10^6
St. Cloud	1.5×10^4	1.2×10^4	1.3×10^5
Elk River	9.5×10^4	1.1×10^5	3.4×10^4
Metropolitan	4.8×10^4	5.8×10^4	7.5×10^4
Empire	6.1×10^3	4.3×10^4	1.5×10^4
Eagles Point	6.2×10^4	4.5×10^5	1.3×10^4
Hastings	4.8×10^4	7.7×10^4	4.8×10^4
Wabasha	2.2×10^4	5.2×10^3	4.1×10^3
Winona	1.1×10^5	1.5×10^5	1.3×10^4
LaCrosse	1.2×10^6	7.5×10^4	1.9×10^4

Table S10. Quantities of *tet*(X) genes per milliliter detected in wastewater effluents on three different sample dates.

Facility	May 2012	July 2012	August 2012
Bemidji	5.3×10^{3}	1.1×10^{6}	2.4×10^{4}
Grand Rapids	4.2×10^{3}	1.6×10^{4}	4.4×10^{4}
Aitkin	1.3×10^{5}	1.4×10^{5}	4.6×10^{5}
Brainerd	5.8×10^{4}	1.1×10^{7}	2.9×10^{5}
Little Falls	1.5×10^{5}	3.0×10^{7}	3.6×10^{6}
St. Cloud	1.5×10^{3}	8.0×10^{5}	6.5×10^{5}
Elk River	4.8×10^{3}	2.4×10^{5}	2.0×10^{4}
Metropolitan	1.8×10^{4}	1.2×10^{5}	3.4×10^{5}
Empire	5.8×10^{2}	2.7×10^{5}	2.3×10^{4}
Eagles Point	3.0×10^{4}	1.0×10^{6}	3.0×10^{5}
Hastings	4.1×10^{3}	2.2×10^{5}	1.2×10^{5}
Wabasha	2.6×10^{3}	2.6×10^{4}	5.9×10^{3}
Winona	1.5×10^{5}	4.5×10^{6}	1.9×10^{5}
LaCrosse	8.0×10^{5}	2.3×10^{5}	3.7×10^{4}

Table S11. Quantities of *sul1* genes per milliliter detected in wastewater effluents on three different sample dates.

Facility	May 2012	July 2012	August 2012
Bemidji	4.4×10^4	7.3×10^5	2.5×10^5
Grand Rapids	7.6×10^5	4.5×10^4	2.6×10^5
Aitkin	2.2×10^5	8.3×10^4	1.7×10^5
Brainerd	4.8×10^5	1.4×10^7	1.7×10^6
Little Falls	5.8×10^5	7.1×10^5	2.8×10^6
St. Cloud	1.0×10^5	1.0×10^5	3.1×10^5
Elk River	4.0×10^5	6.9×10^5	8.2×10^5
Metropolitan	9.1×10^4	8.2×10^4	8.5×10^4
Empire	2.6×10^4	9.4×10^4	7.6×10^4
Eagles Point	2.2×10^5	1.0×10^6	1.1×10^5
Hastings	1.2×10^5	1.1×10^5	1.2×10^5
Wabasha	3.8×10^4	1.9×10^4	1.6×10^4
Winona	2.1×10^5	9.1×10^5	1.7×10^5
LaCrosse	1.2×10^6	8.6×10^4	8.9×10^4

Table S12. Quantities of *erm*(B) genes per milliliter detected in wastewater effluents on three different sample dates. N/A = not available

Facility	May 2012	July 2012	August 2012
Bemidji	1.6×10^{3}	1.5×10^{4}	5.6×10^{3}
Grand Rapids	7.4×10^{3}	9.9×10^{1}	5.6×10^{3}
Aitkin	6.8×10^{4}	1.5×10^{4}	1.3×10^{5}
Brainerd	6.0×10^{4}	9.7×10^{5}	3.6×10^{4}
Little Falls	1.6×10^{5}	9.7×10^{4}	BD
St. Cloud	3.6×10^{3}	1.6×10^{3}	6.1×10^{4}
Elk River	3.7×10^{3}	7.4×10^{3}	5.8×10^{4}
Metropolitan	6.6×10^{3}	5.6×10^{3}	3.3×10^{4}
Empire	5.4×10^{2}	3.2×10^{3}	1.1×10^{4}
Eagles Point	1.6×10^{4}	8.7×10^{4}	1.4×10^{4}
Hastings	1.1×10^{4}	1.7×10^{4}	5.9×10^{4}
Wabasha	1.1×10^{3}	1.3×10^{2}	6.4×10^{2}
Winona	6.2×10^{4}	5.2×10^{4}	2.0×10^{4}
LaCrosse	6.0×10^{4}	4.7×10^{3}	8.9×10^{3}

Table S13. Quantities of *qnrA* genes per milliliter detected in wastewater effluents on three different sample dates. BD = below detection, N/A = not available

Facility	May 2012	July 2012	August 2012
Bemidji	1.1×10^1	2.9×10^1	2.0×10^3
Grand Rapids	2.0×10^1	BD	BD
Aitkin	2.1×10^1	1.2×10^1	7.4×10^2
Brainerd	1.1×10^2	3.1×10^3	8.2×10^2
Little Falls	8.2×10^1	1.7×10^2	3.5×10^3
St. Cloud	4.4×10^0	2.5×10^1	3.5×10^3
Elk River	6.7×10^1	1.2×10^1	5.5×10^2
Metropolitan	1.0×10^2	3.9×10^2	2.8×10^3
Empire	9.2×10^0	3.2×10^2	1.0×10^3
Eagles Point	5.5×10^3	1.6×10^4	2.8×10^4
Hastings	1.6×10^3	4.5×10^2	BD
Wabasha	1.1×10^0	6.9×10^0	BD
Winona	9.6×10^1	5.0×10^2	3.0×10^2
LaCrosse	1.6×10^3	8.8×10^2	3.7×10^3

Table S14. Quantities of *intI1* genes per 16S rRNA genes detected in wastewater effluents on three different sample dates.

Facility	May 2012	July 2012	August 2012
Bemidji	1.8×10^{-1}	1.2×10^{-2}	1.1×10^{-1}
Grand Rapids	7.5×10^{-2}	3.8×10^{-4}	7.5×10^{-3}
Aitkin	4.7×10^{-2}	2.2×10^{-3}	1.2×10^{-2}
Brainerd	6.4×10^{-2}	2.6×10^{-3}	1.8×10^{-2}
Little Falls	1.1×10^{-1}	6.7×10^{-3}	2.0×10^{-2}
St. Cloud	6.6×10^{-2}	1.7×10^{-3}	3.8×10^{-3}
Elk River	2.6×10^{-2}	5.4×10^{-4}	2.7×10^{-3}
Metropolitan	4.1×10^{-2}	9.1×10^{-4}	4.2×10^{-3}
Empire	7.6×10^{-2}	5.3×10^{-4}	1.9×10^{-3}
Eagles Point	9.7×10^{-2}	1.4×10^{-3}	5.5×10^{-3}
Hastings	3.1×10^{-2}	8.6×10^{-4}	3.1×10^{-3}
Wabasha	3.9×10^{-2}	3.7×10^{-4}	2.4×10^{-3}
Winona	7.6×10^{-2}	1.4×10^{-3}	5.5×10^{-3}
LaCrosse	5.9×10^{-2}	2.8×10^{-4}	2.9×10^{-3}

Table S15. Quantities of *tet*(A) genes per 16S rRNA genes detected in wastewater effluents on three different sample dates.

Facility	May 2012	July 2012	August 2012
Bemidji	2.5×10^{-3}	3.1×10^{-4}	2.4×10^{-4}
Grand Rapids	9.2×10^{-3}	6.3×10^{-5}	3.8×10^{-4}
Aitkin	5.1×10^{-4}	6.1×10^{-5}	1.2×10^{-3}
Brainerd	7.7×10^{-4}	4.1×10^{-4}	1.8×10^{-3}
Little Falls	2.2×10^{-2}	2.7×10^{-4}	4.7×10^{-3}
St. Cloud	1.8×10^{-3}	9.4×10^{-5}	9.5×10^{-4}
Elk River	8.9×10^{-4}	7.2×10^{-5}	3.6×10^{-3}
Metropolitan	8.4×10^{-4}	7.1×10^{-5}	8.2×10^{-4}
Empire	3.8×10^{-3}	5.2×10^{-5}	1.5×10^{-3}
Eagles Point	1.1×10^{-3}	5.4×10^{-5}	7.5×10^{-4}
Hastings	1.1×10^{-3}	8.5×10^{-5}	6.5×10^{-4}
Wabasha	5.7×10^{-3}	1.4×10^{-4}	1.5×10^{-3}
Winona	2.4×10^{-3}	7.4×10^{-5}	3.9×10^{-4}
LaCrosse	2.1×10^{-3}	2.7×10^{-5}	6.4×10^{-4}

Table S16. Quantities of *tet*(W) genes per 16S rRNA genes detected in wastewater effluents on three different sample dates. BD = below detection, N/A = not available

Facility	May 2012	July 2012	August 2012
Bemidji	2.8×10^{-2}	4.0×10^{-4}	7.9×10^{-5}
Grand Rapids	9.5×10^{-4}	4.9×10^{-6}	1.0×10^{-4}
Aitkin	2.9×10^{-2}	2.8×10^{-4}	1.9×10^{-3}
Brainerd	1.3×10^{-2}	2.9×10^{-4}	6.0×10^{-4}
Little Falls	2.2×10^{-2}	4.4×10^{-4}	1.7×10^{-3}
St. Cloud	2.2×10^{-2}	1.3×10^{-4}	3.2×10^{-4}
Elk River	1.7×10^{-2}	1.4×10^{-4}	2.3×10^{-4}
Metropolitan	1.2×10^{-2}	1.7×10^{-4}	5.2×10^{-4}
Empire	1.8×10^{-3}	1.1×10^{-4}	2.3×10^{-4}
Eagles Point	8.4×10^{-3}	8.3×10^{-5}	8.1×10^{-5}
Hastings	8.2×10^{-2}	2.2×10^{-4}	2.1×10^{-4}
Wabasha	2.6×10^{-2}	1.6×10^{-4}	2.8×10^{-4}
Winona	8.6×10^{-3}	3.8×10^{-5}	6.0×10^{-5}
LaCrosse	2.9×10^{-2}	1.2×10^{-4}	1.1×10^{-4}

Table S17. Quantities of *tet*(X) genes per 16S rRNA genes detected in wastewater effluents on three different sample dates.

Facility	May 2012	July 2012	August 2012
Bemidji	5.2×10^{-3}	1.5×10^{-4}	5.8×10^{-5}
Grand Rapids	1.2×10^{-4}	1.6×10^{-4}	8.5×10^{-4}
Aitkin	1.0×10^{-2}	7.4×10^{-5}	3.3×10^{-3}
Brainerd	2.6×10^{-3}	7.6×10^{-4}	1.4×10^{-3}
Little Falls	6.1×10^{-3}	1.6×10^{-4}	3.0×10^{-3}
St. Cloud	2.3×10^{-3}	8.3×10^{-5}	1.5×10^{-3}
Elk River	8.5×10^{-4}	3.2×10^{-5}	1.4×10^{-4}
Metropolitan	4.5×10^{-3}	3.6×10^{-4}	2.4×10^{-3}
Empire	1.8×10^{-3}	6.7×10^{-5}	3.5×10^{-4}
Eagles Point	4.1×10^{-3}	1.9×10^{-4}	1.9×10^{-3}
Hastings	6.9×10^{-4}	6.4×10^{-5}	5.0×10^{-4}
Wabasha	3.1×10^{-3}	8.0×10^{-6}	4.1×10^{-4}
Winona	1.2×10^{-2}	1.2×10^{-4}	8.3×10^{-4}
LaCrosse	2.0×10^{-2}	3.8×10^{-5}	2.1×10^{-4}

Table S18. Quantities of *sul1* genes per 16S rRNA genes detected in wastewater effluents on three different sample dates.

Facility	May 2012	July 2012	August 2012
Bemidji	4.2×10^{-2}	1.0×10^{-3}	6.1×10^{-4}
Grand Rapids	2.2×10^{-2}	4.4×10^{-4}	5.0×10^{-3}
Aitkin	1.8×10^{-2}	4.3×10^{-4}	1.2×10^{-3}
Brainerd	2.2×10^{-2}	9.7×10^{-4}	7.9×10^{-3}
Little Falls	2.4×10^{-2}	3.9×10^{-4}	2.3×10^{-3}
St. Cloud	1.5×10^{-1}	1.0×10^{-3}	7.3×10^{-4}
Elk River	7.0×10^{-2}	9.2×10^{-4}	5.6×10^{-3}
Metropolitan	2.3×10^{-2}	2.4×10^{-4}	5.9×10^{-4}
Empire	7.9×10^{-2}	2.4×10^{-4}	1.2×10^{-3}
Eagles Point	2.9×10^{-2}	1.9×10^{-4}	6.9×10^{-4}
Hastings	2.1×10^{-2}	3.2×10^{-4}	5.3×10^{-4}
Wabasha	4.5×10^{-2}	5.8×10^{-4}	1.1×10^{-3}
Winona	1.7×10^{-2}	2.4×10^{-4}	7.4×10^{-4}
LaCrosse	3.0×10^{-2}	1.4×10^{-4}	5.0×10^{-4}

Table S19. Quantities of *erm*(B) genes per 16S rRNA genes detected in wastewater effluents on three different sample dates. BD = below detection, N/A = not available

Facility	May 2012	July 2012	August 2012
Bemidji	1.6×10^{-3}	2.1×10^{-5}	1.3×10^{-5}
Grand Rapids	2.2×10^{-4}	9.8×10^{-7}	1.1×10^{-4}
Aitkin	5.4×10^{-3}	7.6×10^{-5}	9.6×10^{-4}
Brainerd	2.8×10^{-3}	6.7×10^{-5}	1.7×10^{-4}
Little Falls	6.7×10^{-3}	5.3×10^{-5}	BD
St. Cloud	5.4×10^{-3}	1.6×10^{-5}	1.4×10^{-4}
Elk River	6.5×10^{-4}	9.9×10^{-6}	4.0×10^{-4}
Metropolitan	1.7×10^{-3}	1.6×10^{-5}	2.3×10^{-4}
Empire	1.6×10^{-3}	7.9×10^{-6}	1.7×10^{-4}
Eagles Point	2.1×10^{-3}	1.6×10^{-5}	8.5×10^{-5}
Hastings	1.8×10^{-3}	4.9×10^{-5}	2.6×10^{-4}
Wabasha	1.3×10^{-3}	4.1×10^{-6}	4.4×10^{-5}
Winona	5.0×10^{-3}	1.4×10^{-5}	9.0×10^{-5}
LaCrosse	1.5×10^{-3}	7.6×10^{-6}	5.1×10^{-5}

Table S20. Quantities of *qnrA* genes per 16S rRNA genes detected in wastewater effluents on three different sample dates. BD = below detection, N/A = not available

Facility	May 2012	July 2012	August 2012
Bemidji	1.1×10^{-5}	2.6×10^{-8}	2.6×10^{-8}
Grand Rapids	5.8×10^{-7}	BD	BD
Aitkin	1.7×10^{-6}	1.3×10^{-7}	1.3×10^{-7}
Brainerd	4.8×10^{-6}	8.2×10^{-8}	8.2×10^{-8}
Little Falls	3.4×10^{-6}	1.1×10^{-7}	1.1×10^{-7}
St. Cloud	6.7×10^{-6}	2.5×10^{-7}	2.5×10^{-7}
Elk River	1.2×10^{-5}	1.5×10^{-8}	1.5×10^{-8}
Metropolitan	2.5×10^{-5}	3.0×10^{-7}	3.0×10^{-7}
Empire	2.8×10^{-5}	2.7×10^{-7}	2.7×10^{-7}
Eagles Point	7.4×10^{-4}	3.4×10^{-6}	3.4×10^{-6}
Hastings	2.7×10^{-4}	3.3×10^{-7}	3.3×10^{-7}
Wabasha	1.3×10^{-6}	1.2×10^{-7}	1.2×10^{-7}
Winona	7.7×10^{-6}	4.1×10^{-8}	4.0×10^{-8}
LaCrosse	3.9×10^{-5}	5.7×10^{-7}	5.7×10^{-7}

Table S21. Quantities of *intI1* genes per mL detected in the Mississippi River on three different sample dates. BD = below detection, N/A = not available

Facility	May 2012	July 2012	August 2012
Itasca State Park	1.0×10^3	2.3×10^3	5.1×10^4
Three Rivers Scenic	3.3×10^3	1.1×10^3	3.0×10^3
Aitkin	3.5×10^3	1.6×10^3	2.2×10^3
Fort Ripley	2.6×10^3	1.5×10^4	4.8×10^3
Royalton	7.1×10^2	5.3×10^3	6.0×10^3
Elk River	5.3×10^3	8.7×10^3	6.7×10^4
University of Minnesota	2.5×10^3	7.1×10^3	3.0×10^4
Prairie Island	5.4×10^3	1.5×10^3	6.8×10^4
Lake Pepin	7.1×10^3	1.4×10^3	3.1×10^4
Wabasha	3.9×10^3	4.8×10^2	9.4×10^3
Winona	2.4×10^3	1.7×10^3	7.0×10^3
Goose Island	1.7×10^4	1.5×10^3	1.0×10^4

Table S22. Quantities of *tet*(A) genes per mL detected in the Mississippi River on three different sample dates. BD = below detection, N/A = not available

Facility	May 2012	July 2012	August 2012
Itasca State Park	4.5×10^1	7.6×10^1	2.0×10^3
Three Rivers Scenic	2.3×10^2	4.1×10^1	4.4×10^2
Aitkin	1.6×10^2	2.4×10^2	N/A
Fort Ripley	4.5×10^1	3.6×10^2	4.1×10^2
Royalton	2.2×10^1	6.6×10^1	1.2×10^3
Elk River	3.3×10^1	4.2×10^2	3.3×10^3
University of Minnesota	5.6×10^1	5.5×10^2	1.5×10^3
Prairie Island	8.8×10^1	5.9×10^1	3.4×10^3
Lake Pepin	4.8×10^2	1.1×10^2	1.7×10^4
Wabasha	1.8×10^2	2.6×10^2	1.3×10^3
Winona	5.8×10^1	1.6×10^2	3.1×10^2
Goose Island	4.9×10^2	1.0×10^2	3.1×10^2

Table S23. Quantities of *tet*(W) genes per mL detected in the Mississippi River on three different sample dates. BD = below detection, N/A = not available

Facility	May 2012	July 2012	August 2012
Itasca State Park	8.3×10^2	4.2×10^2	1.3×10^2
Three Rivers Scenic	4.1×10^3	6.2×10^2	3.3×10^1
Aitkin	2.6×10^3	8.6×10^1	N/A
Fort Ripley	1.1×10^2	5.0×10^3	1.9×10^2
Royalton	4.3×10^3	2.9×10^3	1.6×10^2
Elk River	2.4×10^2	1.1×10^3	4.9×10^2
University of Minnesota	6.2×10^2	1.4×10^3	4.2×10^2
Prairie Island	6.1×10^3	1.2×10^2	2.6×10^2
Lake Pepin	3.4×10^3	6.0×10^2	2.0×10^3
Wabasha	3.0×10^3	2.5×10^2	1.5×10^2
Winona	3.0×10^3	1.1×10^2	1.1×10^2
Goose Island	5.5×10^2	3.6×10^2	4.8×10^1

Table S24. Quantities of *tet*(X) genes per mL detected in the Mississippi River on three different sample dates. BD = below detection, N/A = not available

Facility	May 2012	July 2012	August 2012
Itasca State Park	8.3×10^0	1.2×10^1	3.4×10^1
Three Rivers Scenic	4.1×10^2	9.5×10^0	4.4×10^1
Aitkin	2.6×10^1	4.4×10^1	N/A
Fort Ripley	1.1×10^0	6.0×10^1	2.4×10^1
Royalton	4.3×10^0	2.2×10^2	1.4×10^2
Elk River	2.4×10^1	1.0×10^2	1.5×10^2
University of Minnesota	6.2×10^1	2.5×10^2	9.2×10^1
Prairie Island	6.1×10^1	BD	1.9×10^2
Lake Pepin	3.4×10^2	4.8×10^1	1.4×10^3
Wabasha	3.0×10^1	5.2×10^1	4.0×10^2
Winona	3.0×10^1	BD	3.5×10^2
Goose Island	3.8×10^2	1.3×10^1	3.1×10^1

Table S25. Quantities of *sul1* genes per mL detected in the Mississippi River on three different sample dates. BD = below detection, N/A = not available

Facility	May 2012	July 2012	August 2012
Itasca State Park	1.8×10^3	1.3×10^3	1.2×10^4
Three Rivers Scenic	3.9×10^3	1.2×10^3	1.1×10^3
Aitkin	3.6×10^3	1.6×10^3	N/A
Fort Ripley	2.7×10^3	1.3×10^4	1.3×10^3
Royalton	1.2×10^3	6.1×10^3	1.4×10^3
Elk River	2.5×10^3	3.3×10^3	3.0×10^3
University of Minnesota	2.5×10^3	2.7×10^3	2.4×10^3
Prairie Island	2.8×10^3	6.4×10^2	4.6×10^3
Lake Pepin	7.5×10^3	1.1×10^3	1.5×10^4
Wabasha	3.7×10^3	1.9×10^2	1.7×10^3
Winona	3.2×10^3	1.5×10^3	1.6×10^3
Goose Island	7.5×10^3	1.5×10^3	1.8×10^3

Table S26. Quantities of *erm*(B) genes per mL detected in the Mississippi River on three different sample dates. BD = below detection, N/A = not available

Facility	May 2012	July 2012	August 2012
Itasca State Park	2.0×10^{2}	8.0×10^{0}	4.7×10^{2}
Three Rivers Scenic	9.5×10^{2}	4.0×10^{1}	BD
Aitkin	7.4×10^{2}	2.5×10^{1}	N/A
Fort Ripley	7.8×10^{1}	5.8×10^{1}	8.6×10^{1}
Royalton	4.2×10^{0}	1.1×10^{2}	1.8×10^{2}
Elk River	3.1×10^{1}	1.3×10^{2}	4.8×10^{2}
University of Minnesota	3.4×10^{2}	9.2×10^{1}	1.9×10^{2}
Prairie Island	4.3×10^{2}	3.8×10^{1}	2.3×10^{2}
Lake Pepin	7.7×10^{2}	5.0×10^{1}	4.1×10^{2}
Wabasha	1.3×10^{3}	4.2×10^{1}	BD
Winona	2.8×10^{2}	1.3×10^{1}	8.5×10^{1}
Goose Island	3.8×10^{3}	3.0×10^{1}	BD

Table S27. Quantities of *qnrA* genes per mL detected in the Mississippi River on three different sample dates. BD = below detection, N/A = not available

Facility	May 2012	July 2012	August 2012
Itasca State Park	7.1×10^{0}	BD	3.8×10^{2}
Three Rivers Scenic	6.5×10^{0}	5.1×10^{0}	BD
Aitkin	BD	BD	N/A
Fort Ripley	BD	4.1×10^{1}	BD
Royalton	BD	9.6×10^{0}	BD
Elk River	6.4×10^{0}	BD	BD
University of Minnesota	1.5×10^{1}	1.4×10^{1}	BD
Prairie Island	$3.8 \times 10\text{-}^{1}$	1.6×10^{0}	BD
Lake Pepin	BD	9.1×10^{-1}	BD
Wabasha	3.2×10^{0}	BD	BD
Winona	6.1×10^{0}	BD	BD
Goose Island	4.9×10^{0}	4.9×10^{0}	BD

Table S28. Quantities of *intI1* genes per 16S rRNAs detected in the Mississippi River on three different sample dates. BD = below detection, N/A = not available

Facility	May 2012	July 2012	August 2012
Itasca State Park	4.3×10^{-3}	3.9×10^{-5}	2.3×10^{-4}
Three Rivers Scenic	1.1×10^{-2}	2.2×10^{-5}	1.1×10^{-4}
Aitkin	7.2×10^{-3}	2.7×10^{-5}	1.4×10^{-4}
Fort Ripley	3.4×10^{-3}	2.2×10^{-4}	1.4×10^{-4}
Royalton	1.8×10^{-3}	7.1×10^{-5}	1.6×10^{-4}
Elk River	2.8×10^{-3}	1.1×10^{-4}	3.3×10^{-4}
University of Minnesota	2.3×10^{-3}	6.5×10^{-5}	1.7×10^{-4}
Prairie Island	2.5×10^{-3}	9.1×10^{-6}	9.9×10^{-5}
Lake Pepin	N/A	2.1×10^{-5}	2.9×10^{-4}
Wabasha	5.2×10^{-3}	1.0×10^{-5}	9.2×10^{-5}
Winona	5.3×10^{-3}	3.0×10^{-5}	1.3×10^{-4}
Goose Island	7.7×10^{-3}	2.2×10^{-5}	1.1×10^{-4}

Table S29. Quantities of *tet*(A) genes per 16S rRNAs detected in the Mississippi River on three different sample dates. BD = below detection, N/A = not available

Facility	May 2012	July 2012	August 2012
Itasca State Park	1.9×10^{-4}	1.3×10^{-6}	9.2×10^{-6}
Three Rivers Scenic	7.6×10^{-4}	8.8×10^{-7}	1.7×10^{-5}
Aitkin	3.3×10^{-4}	4.1×10^{-6}	N/A
Fort Ripley	5.9×10^{-5}	5.5×10^{-6}	1.2×10^{-5}
Royalton	5.5×10^{-5}	8.7×10^{-7}	3.0×10^{-5}
Elk River	1.7×10^{-5}	5.3×10^{-6}	1.6×10^{-5}
University of Minnesota	5.1×10^{-5}	5.0×10^{-6}	8.6×10^{-6}
Prairie Island	4.0×10^{-5}	3.6×10^{-7}	5.0×10^{-6}
Lake Pepin	N/A	1.7×10^{-6}	1.6×10^{-4}
Wabasha	2.4×10^{-4}	5.5×10^{-6}	1.2×10^{-5}
Winona	1.3×10^{-4}	2.7×10^{-6}	5.8×10^{-6}
Goose Island	2.2×10^{-4}	1.5×10^{-6}	3.5×10^{-6}

Table S30. Quantities of *tet*(W) genes per 16S rRNAs detected in the Mississippi River on three different sample dates. BD = below detection, N/A = not available

Facility	May 2012	July 2012	August 2012
Itasca State Park	3.5×10^{-3}	7.1×10^{-6}	5.8×10^{-7}
Three Rivers Scenic	1.4×10^{-2}	1.3×10^{-5}	1.3×10^{-6}
Aitkin	5.5×10^{-3}	1.5×10^{-6}	N/A
Fort Ripley	1.4×10^{-3}	7.5×10^{-5}	5.6×10^{-6}
Royalton	1.1×10^{-3}	3.9×10^{-5}	4.1×10^{-6}
Elk River	1.2×10^{-3}	1.4×10^{-5}	2.4×10^{-6}
University of Minnesota	5.6×10^{-4}	1.3×10^{-5}	2.3×10^{-6}
Prairie Island	2.8×10^{-4}	7.4×10^{-7}	3.8×10^{-7}
Lake Pepin	N/A	9.3×10^{-6}	1.9×10^{-5}
Wabasha	4.1×10^{-3}	5.4×10^{-6}	1.5×10^{-6}
Winona	6.6×10^{-3}	1.9×10^{-6}	2.1×10^{-6}
Goose Island	2.5×10^{-3}	5.2×10^{-6}	5.3×10^{-7}

Table S31. Quantities of *tet*(X) genes per 16S rRNAs detected in the Mississippi River on three different sample dates. BD = below detection, N/A = not available

Facility	May 2012	July 2012	August 2012
Itasca State Park	3.5×10^{-5}	2.0×10^{-7}	1.6×10^{-7}
Three Rivers Scenic	5.0×10^{-4}	2.0×10^{-7}	1.7×10^{-6}
Aitkin	1.2×10^{-4}	7.5×10^{-7}	N/A
Fort Ripley	7.0×10^{-6}	9.0×10^{-7}	7.0×10^{-7}
Royalton	1.4×10^{-5}	3.0×10^{-6}	3.5×10^{-6}
Elk River	8.9×10^{-6}	1.3×10^{-6}	7.5×10^{-7}
University of Minnesota	3.2×10^{-5}	2.3×10^{-6}	5.2×10^{-7}
Prairie Island	2.3×10^{-5}	BD	2.8×10^{-7}
Lake Pepin	N/A	7.5×10^{-7}	1.3×10^{-5}
Wabasha	5.2×10^{-5}	1.1×10^{-6}	3.9×10^{-6}
Winona	1.8×10^{-4}	BD	6.5×10^{-6}
Goose Island	1.7×10^{-4}	1.9×10^{-7}	3.5×10^{-7}

Table S32. Quantities of *sul1* genes per 16S rRNAs detected in the Mississippi River on three different sample dates. BD = below detection, N/A = not available

Facility	May 2012	July 2012	August 2012
Itasca State Park	7.5×10^{-3}	2.2×10^{-5}	5.2×10^{-5}
Three Rivers Scenic	1.3×10^{-2}	2.5×10^{-5}	4.3×10^{-5}
Aitkin	7.4×10^{-3}	2.8×10^{-5}	N/A
Fort Ripley	3.5×10^{-3}	1.9×10^{-4}	3.8×10^{-5}
Royalton	3.1×10^{-3}	8.0×10^{-5}	3.7×10^{-5}
Elk River	1.3×10^{-3}	4.2×10^{-5}	1.5×10^{-5}
University of Minnesota	2.3×10^{-3}	2.5×10^{-5}	1.3×10^{-5}
Prairie Island	1.3×10^{-3}	3.9×10^{-6}	6.8×10^{-6}
Lake Pepin	N/A	1.6×10^{-5}	1.4×10^{-4}
Wabasha	5.0×10^{-3}	4.0×10^{-6}	1.7×10^{-5}
Winona	7.0×10^{-3}	2.6×10^{-5}	3.0×10^{-5}
Goose Island	3.4×10^{-3}	2.2×10^{-6}	2.1×10^{-5}

Table S33. Quantities of *erm*(B) genes per 16S rRNAs detected in the Mississippi River on three different sample dates. BD = below detection, N/A = not available

Facility	May 2012	July 2012	August 2012
Itasca State Park	8.6×10^{-4}	1.4×10^{-7}	2.1×10^{-6}
Three Rivers Scenic	3.2×10^{-3}	8.4×10^{-7}	BD
Aitkin	1.5×10^{-3}	4.2×10^{-7}	N/A
Fort Ripley	1.0×10^{-4}	8.7×10^{-7}	2.5×10^{-6}
Royalton	1.1×10^{-5}	1.5×10^{-6}	4.7×10^{-6}
Elk River	1.6×10^{-5}	1.6×10^{-6}	2.4×10^{-6}
University of Minnesota	3.1×10^{-4}	8.5×10^{-7}	1.0×10^{-6}
Prairie Island	2.0×10^{-4}	2.3×10^{-7}	3.4×10^{-7}
Lake Pepin	N/A	7.8×10^{-7}	3.9×10^{-6}
Wabasha	1.7×10^{-3}	9.0×10^{-7}	BD
Winona	6.3×10^{-4}	2.4×10^{-7}	1.6×10^{-6}
Goose Island	1.7×10^{-3}	4.3×10^{-7}	BD

Table S34. Quantities of *qnrA* genes per 16S rRNAs detected in the Mississippi River on three different sample dates. BD = below detection, N/A = not available

Facility	May 2012	July 2012	August 2012
Itasca State Park	3.0×10^{-5}	BD	1.7×10^{-3}
Three Rivers Scenic	2.2×10^{-5}	1.1×10^{-7}	BD
Aitkin	BD	BD	N/A
Fort Ripley	BD	6.1×10^{-7}	BD
Royalton	BD	1.3×10^{-7}	BD
Elk River	3.3×10^{-6}	BD	BD
University of Minnesota	1.4×10^{-5}	1.3×10^{-7}	BD
Prairie Island	1.8×10^{-7}	9.5×10^{-9}	BD
Lake Pepin	N/A	1.4×10^{-8}	BD
Wabasha	4.3×10^{-6}	BD	BD
Winona	1.4×10^{-5}	BD	BD
Goose Island	2.2×10^{-6}	7.2×10^{-8}	BD

Table S35. Quantities of IncA/C plasmids per milliliter detected in wastewater effluents on three different sample dates. BD = below detection, N/A = not available

Facility	May 2012	July 2012	August 2012
Bemidji	B.D.	1.3×10^{2}	2.5×10^{2}
Grand Rapids	B.D.	B.D.	8.1×10^{1}
Aitkin	1.9×10^{2}	4.2×10^{1}	4.7×10^{2}
Brainerd	3.3×10^{2}	3.2×10^{3}	5.5×10^{2}
Little Falls	7.2×10^{2}	3.1×10^{1}	1.7×10^{4}
St. Cloud	B.D.	B.D.	2.2×10^{3}
Elk River	2.1×10^{2}	3.9×10^{1}	8.9×10^{1}
Metropolitan	5.0×10^{2}	2.7×10^{2}	5.7×10^{2}
Empire	B.D.	9.6×10^{1}	5.3×10^{1}
Eagles Point	5.7×10^{2}	2.3×10^{3}	1.4×10^{2}
Hastings	1.8×10^{2}	2.9×10^{2}	3.7×10^{2}
Wabasha	B.D.	3.1×10^{1}	8.9×10^{1}
Winona	8.9×10^{2}	5.4×10^{1}	N/A
LaCrosse	3.6×10^{4}	1.1×10^{3}	1.2×10^{2}

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