Supporting Information

Prevalence of class 1 integron and its gene cassettes carrying antibiotic resistance genes in drinking water treatment and distribution systems

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Figure S7. Co-occurrence of resistant gene cassette on the same sequence in PMA+ samples at ARG type level.

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Text S1. Optimal concentration for PMA-qPCR experiment.

The target genes for qPCR were 16S rRNA genes of *E. coli* ATCC25922. Different volumes of PMAxx were added to 1 mL suspension of live and autoclaved dead bacteria and mixed thoroughly to achieve final PMAxx concentrations of 0, 20, 30, 40, 50 and 60 μmol/L respectively. The mixture was incubated in the dark for 10 min, and exposed under a PMA-Light with a 650 W halogen tungsten lamp for 15 min. After PMAxx pretreatment, DNA was extracted using TIANamp Bacteria DNA Kit (Tiangen Biotech, China) then qPCR was performed. The Ct values of dead bacteria increased when the PMAxx concentration was in the range of 0 – 40 μmol/L and decreased while >40 μmol/L, which indicated that 40 μmol/L PMAxx was able to bind to the DNA completely of dead bacteria (Figure S1). Hence 40 μmol/L PMAxx was taken for pre-treatment in this study.

Table S1. Primer and reaction information about integrons and 16S rRNA gene.

Target gene	Primer name	Primer sequence (5′ – 3′)	Amplicon size (bp)	PCR Reaction condition	qPCR Reaction condition	Reference
*intI*1	*intI*1-LC1	GCCTTGATGTTACCCGAGAG	196	94℃ 5min; 35 cycles (94℃ 30s, 55℃ 30s, 72℃ 1min); 72℃ 7min	50℃ 2min; 95℃ 10min; 40 cycles (95℃ 30s, 60℃ 1min)	(An et al., 2018)
	*intI*1-LC5	GATCGGTCGAATGCGTGT				
16S rRNA gene	27F	AGAGTTTGATCCTGGCTCAG	1465	94℃ 5min; 35 cycles (94℃ 30s, 54℃ 30s, 72℃ 1min30s); 72℃ 10min		(Lane, 1991)
	1492R	TACGGCTACCTTGTTACGACTT				
class 1 integron cassettes	5′ CS	GGCATCCAAGCAGCAAG	Uncertain	95℃ 10min; 35cycles (94℃ 30s, 55℃ 30s, 72℃ 2min30s); 72℃ 10min		(An et al., 2018)
	3′ CS	AAGCAGACTTGACCTGA				

Table S2. Concentration of antibiotics and criteria of antibiotic susceptibility analysis.

Antibiotics		Concentration (μg per disk)	Diameter of antibiotic circle (mm)			Quality Control Ranges (mm)
			Susceptible（S）	Intermediate（I）	Resistant（R）	*E. coli* ATCC25922
Aminoglycosides	gentamicin	10	≥15	13-14	≤12	19-26
	tobramycin	10	≥15	13-14	≤12	24-30
Beta-lactam	piperacillin	100	≥21	18-20	≤17	24-30
	ampicillin	10	≥17	14-16	≤13	15-22
	imipenem	10	≥23	20-22	≤19	26-32
Sulfanilamide	sulfamethoxazole	25	≥16	11-15	≤10	23-29
Fluoroquinolone	levofloxacin	5	≥21	17-20	≤16	29-37
Cephalosporin	ceftazidime	30	≥21	18-20	≤17	25-32
Tetracycline	tetracycline	30	≥15	12-14	≤11	18-25
Trimethoprim	trimethoprim	5	≥16	11-15	≤10	21-28

Table S3. The taxonomic annotation and phenotypic antibiotic resistance results of intI1-positive bacterial isolates at species level. R: resistant, I: intermediate, S: susceptible.

Isolates	Species	ampicillin	ceftazidime	gentamicin	imipenem	levofloxacin	piperacillin	tetracycline	tobramycin	sulfamethoxazole	trimethoprim
SW-1	*Escherichia coli*	R	S	S	S	R	R	S	S	R	R
SW-2	*Escherichia fergusonii*	R	S	S	S	S	R	R	S	R	R
SW-3	*Raoultella ornithinolytica*	R	S	S	I	S	S	R	I	R	R
SW-4	*Escherichia coli*	R	S	S	S	S	I	R	S	R	R
SW-5	*Citrobacter freundii*	R	S	R	I	I	R	R	I	R	R
SW-6	unclassified	R	S	I	I	R	I	R	S	R	R
SW-7	*Citrobacter freundii*	R	S	R	I	I	R	R	R	R	R
SW-8	*Citrobacter freundii*	R	S	R	R	I	R	R	I	R	R
SW-9	unclassified	R	S	I	R	R	R	R	I	R	R
SW-10	unclassified	R	S	I	S	R	S	R	I	R	R
SW-11	unclassified	R	S	S	S	S	S	S	S	I	R
SW-12	*Klebsiella aerogenes*	R	S	S	S	S	S	S	S	S	S
SW-13	*Bacillus* sp.	R	S	S	S	S	S	S	S	S	R
SW-14	*Raoultella terrigena*	S	S	S	S	S	S	S	S	S	S
SW-15	*Enterobacter* sp.	I	S	S	S	S	S	S	S	S	S
SW-16	unclassified	S	S	S	S	S	S	S	S	S	S
SW-17	*Raoultella* sp.	S	S	S	S	S	S	S	S	S	S
SW-18	*Kluyvera cryocrescens*	S	S	S	S	S	S	R	S	S	S
ES-1	*Enterobacter* sp. S10	I	S	S	I	S	S	S	S	S	S
ES-2	*Escherichia coli*	R	S	S	S	S	R	R	S	R	R
ES-3	*Raoultella* sp.	R	S	S	I	S	S	S	S	S	S

Isolates	Species	ampicillin	ceftazidime	gentamicin	imipenem	levofloxacin	piperacillin	tetracycline	tobramycin	sulfamethoxazole	trimethoprim
ES-4	*Raoultella ornithinolytica*	S	S	S	S	S	I	S	S	S	S
ES-5	*Pseudomonas protegens*	R	S	S	S	S	S	S	S	S	R
ES-6	*Pseudomonas putida*	R	S	S	S	S	S	S	S	R	R
ES-7	*Klebsiella* sp. MTJW-11	R	S	S	S	S	R	S	S	S	S
ES-8	unclassified	R	S	S	S	S	I	S	S	S	S
ES-9	*Enterobacter ludwigii*	R	S	S	S	S	S	S	S	S	S
ES-10	*Kluyvera cryocrescens*	S	S	S	S	S	S	R	S	S	I
ES-11	*Raoultella ornithinolytica*	R	S	S	S	S	S	S	S	S	S
ES-12	unclassified	R	S	S	S	S	S	S	S	S	S
ES-13	*Raoultella ornithinolytica*	R	S	S	S	S	S	S	S	S	S
ES-14	*Raoultella* sp.	I	S	S	S	S	S	S	S	S	S
ES-15	*Enterobacter ludwigii*	I	S	S	S	S	S	S	S	S	S
ES-16	*Raoultella* sp. XT-12	I	S	S	S	S	S	S	S	S	S
ES-17	*Raoultella terrigena*	S	S	S	S	S	S	S	S	S	S
ES-18	unclassified	I	S	S	S	S	S	R	S	S	S
FW-1	unclassified	R	S	S	S	S	S	S	S	R	R
FW-2	unclassified	R	S	S	I	S	S	S	S	R	R
FW-3	unclassified	R	S	S	I	S	S	S	S	R	R
FW-4	*Pseudomonas* sp.	R	S	S	S	S	S	S	S	R	R
FW-5	unclassified	S	S	S	S	S	S	S	S	S	S
FW-6	unclassified	R	S	S	S	S	I	S	S	S	S

Table S4. Information of MinION sequencing data for drinking water samples.

Sample	Raw reads			Filtered reads			
	No. of Reads	Avg (bp)	Max (bp)/Q score	No. of Reads	Avg (bp)	Mean read quality	Proportion of ARG-carried reads
SW	249,898	619.2	8723 (8.8)	249,474(99.8%)	620.0	9.6	11.79%
ES	615,995	660.6	10714 (10.4)	615,058(99.8%)	661.4	9.5	9.18%
FW	225,229	672.1	6819 (10.7)	224,969(99.8%)	672.7	9.6	11.28%
CW	433,857	785.5	5992 (8.1)	433,551(99.9%)	786.0	9.8	4.81%
TWA	270,671	738.8	8160 (8.8)	270,425(99.9%)	739.4	9.7	2.31%
TWB	346,154	806.0	7075 (10.7)	345,955(99.9%)	806.4	9.9	1.85%

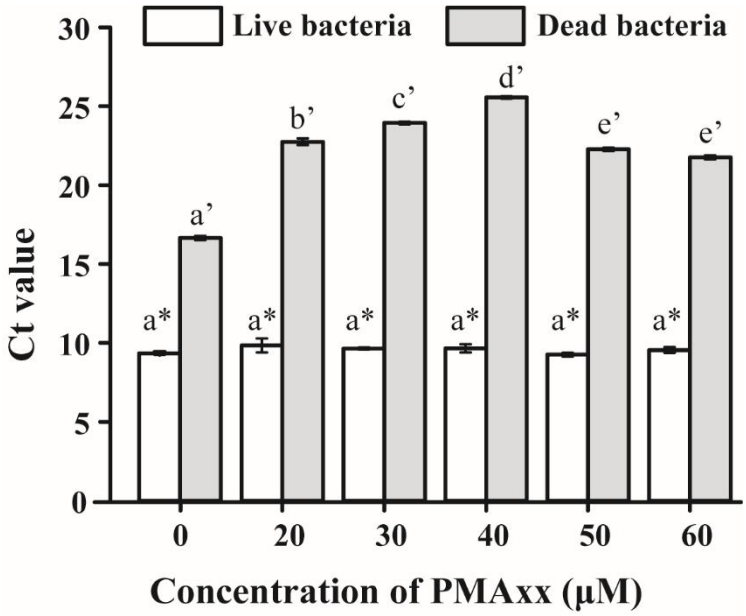

Figure S1. Histogram showing Ct values of live and dead bacteria under PMAxx treatment with different concentrations. (a*: significant difference between live and dead bacteria. a′ b′ c′ d′ e′: significant difference of dead bacteria).

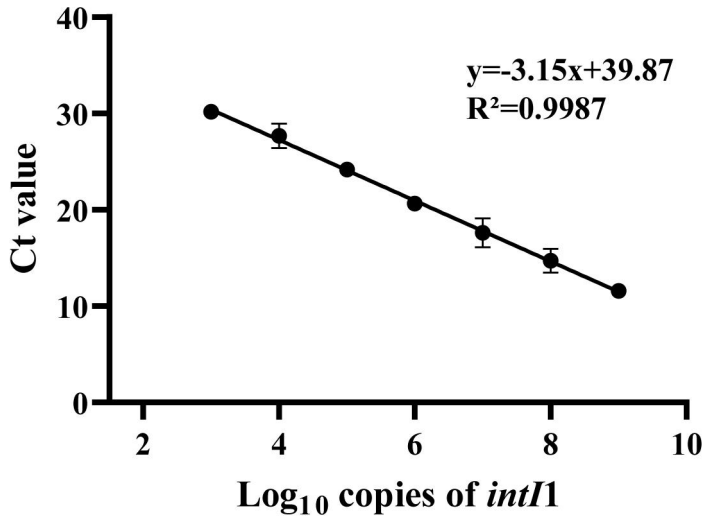

Figure S2. The standard curve (mean ± standard error) of *intI*1. The amplification efficiency was 107.6%.

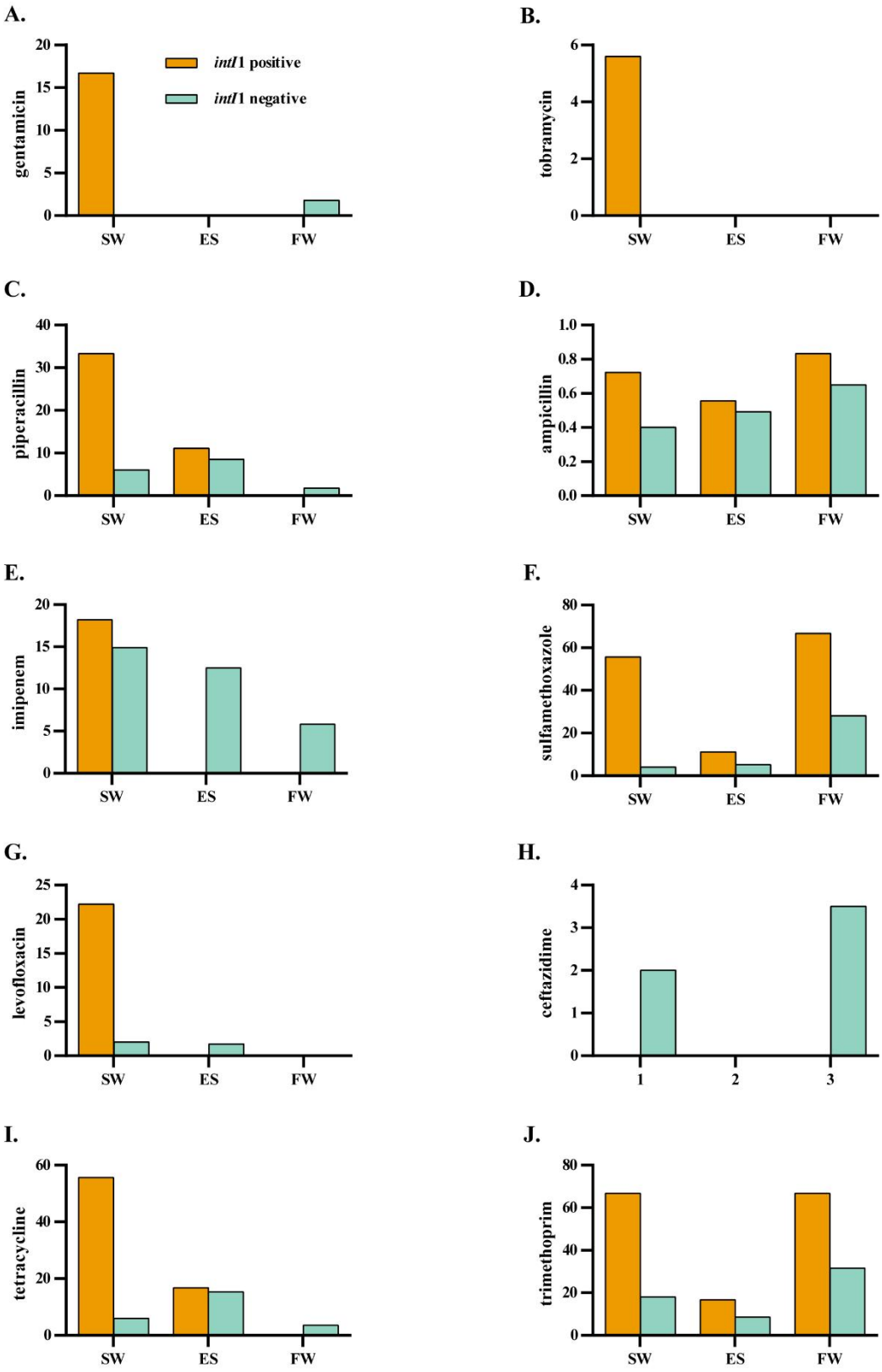

Figure S3. The percentage (%) of *intI*1-positive and negative isolates resistant to antibiotics.

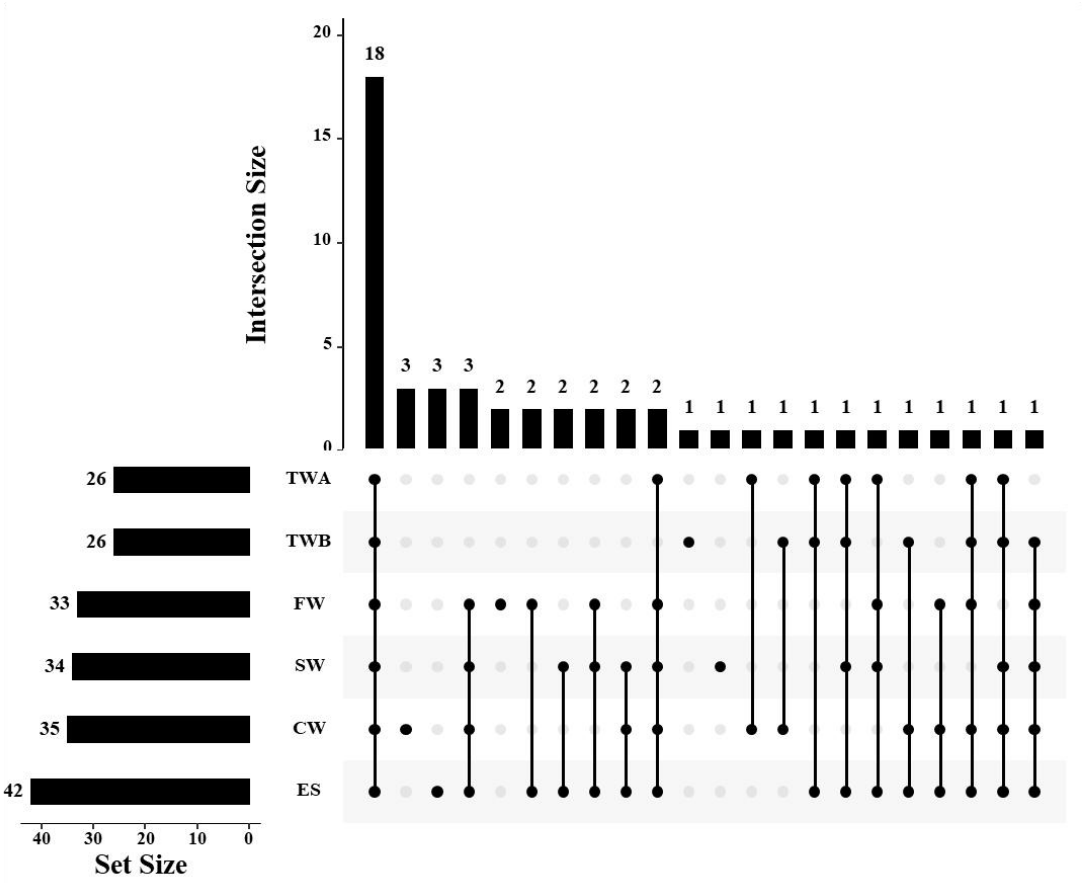

Figure S4. UpSet plot showing the detection of ARG subtypes in drinking water samples.

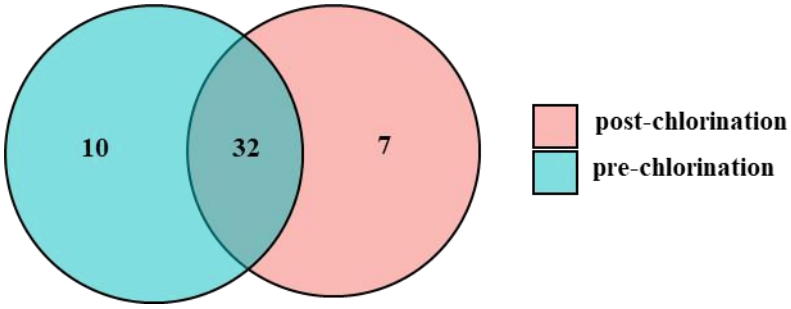

Figure S5. Venn plot showing the detect numbers of ARG subtypes in pre- and post-chlorination samples.

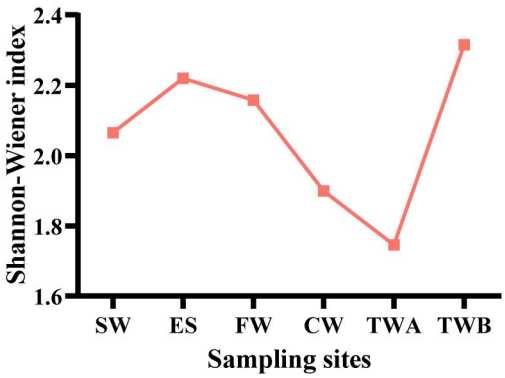

Figure S6. Shannon-Wiener index of class 1 integron-borne ARGs in drinking water samples.

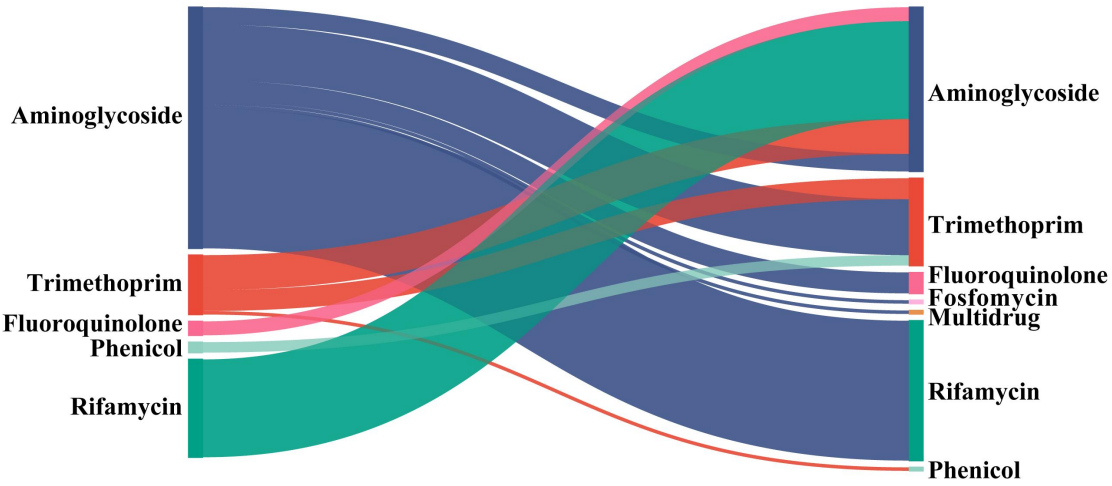

Figure S7. Co-occurrence of resistant gene cassette on the same sequence in PMA+ samples at ARG type level.

References

An X-L, Chen Q-L, Zhu D, Zhu Y-G, Gillings M R, Su J-Q (2018). Impact of wastewater treatment on the prevalence of integrons and the genetic diversity of integron gene cassettes. Applied and Environmental Microbiology, 84(9): e02766-17

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