Supplementary Materials

Employing quantitative microbial risk assessment (QMRA) to derive aquatic thresholds for antibiotic-resistant strains of *Escherichia coli*, *Staphylococcus aureus* and *Salmonella Typhi*: a case study in the Yangtze Estuary

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Supplementary Table 1. Primers of functional genes

Bacteria	Genes	Primers		Length (bp)	Annealing temperatures (℃)
		F	R		
Escherichia coli	rfbE	AGTTTTTTACCCTGTTC ACACGAT	CCTCAGCTATAGGGT GCTTTTGA	67	60
Staphylococcus aureus	rpoB	GGTAATTTCTCATTAGA GTTTGTGGATT	TAGGGTCACGGTTTT TAGATTCTTC	81	60
Salmonella paratyphi B	invA	TGATGCGAGTGGTAAAT TATTCC	CTCCCCCTCTTCATG CGTTA	110	60

Supplementary Table 2. PCR system of 25μL

Items	Volume (μL)
samples	1
Primer F	0.5
Primer R	0.5
Taq pcr master mix (2×, blue dye)	12.5
ddH_2O	10.5

Supplementary Table 3. PCR procedure of functional genes

Stages	Temperature (℃)	Time	No. of circulation
stage 1	95	10 min	1
	95	15 sec	
stage 2	60	30 sec	40
	72	30 sec	
stage 3	72	10 sec	1

Supplementary Table 4. qPCR system of 20μl

Items	Volume (μL)
samples	1
Primer F	0.4
Primer R	0.4
Taq pcr master mix（2x，blue dye）	10
ROX 100× ROX Reference Dye	0.2
ddH2O	8

Supplementary Table 5. qPCR procedure of functional genes

Stages	Temperature (℃)	Time	Circulation
stage 1	95	3 min	1
	95	15 sec	
stage 2	60	30 sec	40
	72	30 sec	
	95	15 sec	
	60	1 min	
stage 3			1
	9`5	15 sec	
	60	15 sec	

Supplementary Table 6. Parameters in the quantitative microbial risk assessment (QMRA) models of *Escherichia coli*, *Staphylococcus aureus*, *Salmonella Typhi* and their four antibiotic-resistant bacteria

Parameters	Description of data	Dimension	Distribution	References
conc (concentration of target pathogen)				
high-flow season				
EC (*Escherichia coli*)	position = 0.00, 50% = 0.06, 75% = 0.33	copies/mL	Gamma	this study
SA (*Staphylococcus aureus*)	position = 0.00, 50% = 19.65, 75% = 22.97	copies/mL	Gamma	this study
ST (Salmonella Typhi)	position = 0.00, 50% = 11.99, 75% = 22.48	copies/mL	Gamma	this study
Low-flow season				
EC (*Escherichia coli*)	position = 0.00, 50% = 3.70, 75% = 4.90	copies/mL	Gamma	this study
SA (*Staphylococcus aureus*)	position = 0.00, 50% = 9.40, 75% = 24.62	copies/mL	Gamma	this study
ST (Salmonella Typhi)	position = 0.00, 50% = 111.23, 75% = 309.72	copies/mL	Gamma	this study
k (the copy number of functional genes in each cell)	minimum=1, maximum=4	/	Uniform	[1]
r (resistant ratio)				
high-flow season				
EC	position = 0.00, 50% = 0.93, 75% = 3.23	%	Gamma	this study
SA	position = 0.00, 50% = 0.78, 75% = 8.79	%	Gamma	this study
ST	position = 0.00, 50% = 2.07, 75% = 7.86	%	Gamma	this study
low-flow season				
EC	position = 0.00, 50% = 1.91, 75% = 13.09	%	Gamma	this study
SA	position = 0.00, 50% = 0.05, 75% = 0.075	%	Gamma	this study
ST	position = 0.00, 50% = 1.80, 75% = 6.76	%	Gamma	this study

I (water intake)	minimum = 0, most probability value = 16, maximum = 154	mL	Triangular	[2]
life expectancy	mean = 77.93, SD = 7.793	Year	Normal	[3]
age infected	mean = 30, SD = 3	Year	Normal	[4]
Dose-response relationship				
EC				
α	0.155	/	/	[4]
β	24386.19	/	/	[4]
SA				
α	0.3999	/	/	[5]
β	5721.88	/	/	[5]
ST				
α	0.89	/	/	[6]
β	440000	/	/	[6]
DW (disability weight)				
EC	1% = 0.039, 99% = 0.152	/	Normal	[7]
SA	1% = 0.039, 99% = 0.152	/	Normal	[7]
ST				
moderate typhoid fever	minimum = 0.032, most probability value = 0.074, maximum = 0.051	/	Beta (PERT)	[8]
severe typhoid fever	minimum = 0.088, most probability value = 0.133, maximum = 0.19	/	Beta (PERT)	[8]
severe typhoid fever with gastrointestinal bleeding-bleeding	minimum = 0.209, most probability value = 0.325, maximum = 0.462	/	Beta (PERT)	[8]

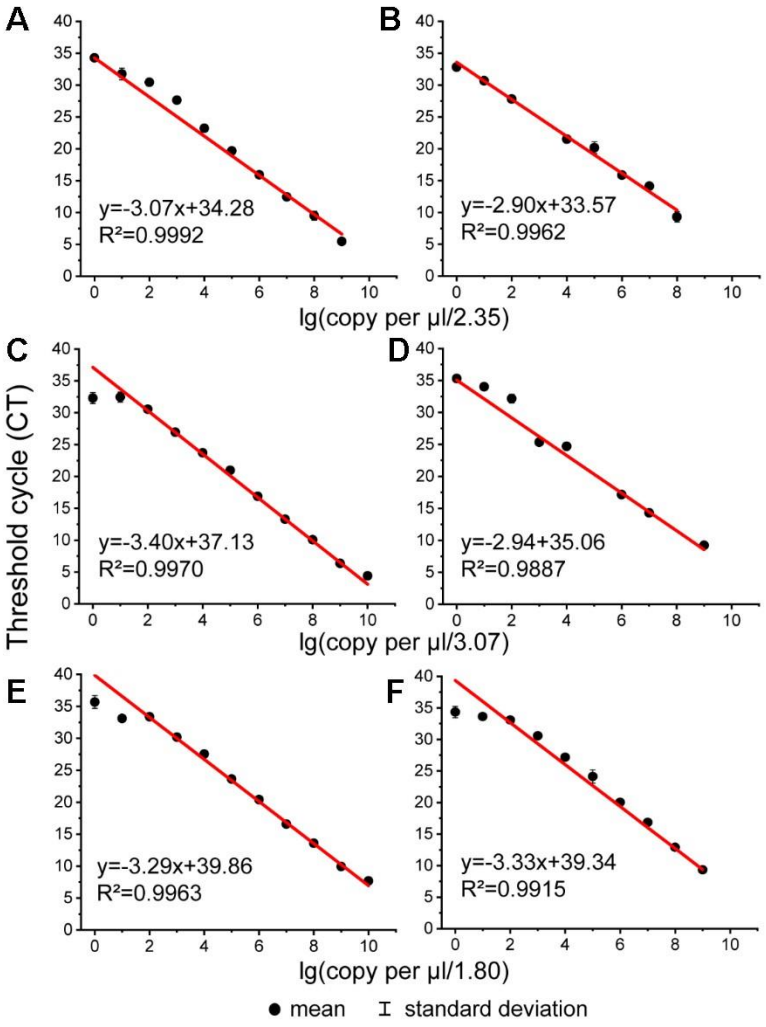
severe typhoid fever with gastrointestinal bleeding-fever	minimum = 0.088, most probability value = 0.133, maximum = 0.19	/	Beta (PERT)	[8]
typhoid fever with other abdominal complications	minimum = 0.22, most probability value = 0.324, maximum = 0.442	/	Beta (PERT)	[8]
P_{lethal} (probability of death)				
EC	minimum = 0, maximum = 0.0008, α = 2, β = 3	%	Beta	[7]
SA	minimum = 0, maximum = 0.0008, α = 2, β = 3	%	Beta	[7]
ST	minimum = 0.6287, maximum = 1.449, α = 2, β = 3	%	Beta	[8]
3CEC (three-generation cephalosporin-resistant *Escherichia coli*)	minimum = 0, maximum = 3.05, α = 2, β = 3	%	Beta	[7]
CREC (carbapenem-resistant *Escherichia coli*)	minimum = 0, maximum = 5.38, α = 2, β = 3	%	Beta	[7]
MRSA (methicillin-resistant *Staphylococcus aureus*)	minimum = 0, maximum = 4.74, α = 2, β = 3	%	Beta	[7]
FRST (fluoroquinolone-resistant *Salmonella typhimurium*)	minimum = 0.6287, maximum = 2.299, α = 2, β = 3	%	Beta	[8]
L_C (course of disease)				
EC	minimum = 0.0082, maximum = 0.0301, α = 2, β = 3	Year	Beta	[7]
SA	minimum = 0.0082, maximum = 0.0301, α = 2, β = 3	Year	Beta	[7]
ST				
moderate typhoid fever	minimum = 0.019, most probability value = 0.038, maximum = 0.058	Year	Beta (PERT)	[8]
severe typhoid fever	minimum = 0.038, most probability value = 0.079, maximum = 0.134	Year	Beta (PERT)	[8]
severe typhoid fever with gastrointestinal bleeding-bleeding	minimum = 0.003, most probability value = 0.005, maximum = 0.010	Year	Beta (PERT)	[8]

severe typhoid fever with gastrointestinal bleeding-fever	minimum = 0.036, most probability value = 0.071, maximum = 0.125	Year	Beta (PERT)	[8]
typhoid fever with other abdominal complications	minimum = 0.038, most probability value = 0.079, maximum = 0.134	Year	Beta (PERT)	[8]
3CEC	minimum = 0.0219, maximum = 0.0575, $\alpha = 2$, $\beta = 3$	Year	Beta	[7]
CREC	minimum = 0.0164, maximum = 0.0740, $\alpha = 2$, $\beta = 3$	Year	Beta	[7]
MRSA	minimum = 0.0219, maximum = 0.0521, $\alpha = 2$, $\beta = 3$	Year	Beta	[7]
FRST				
moderate typhoid fever	minimum = 0.019, most probability value = 0.038, maximum = 0.058	Year	Beta (PERT)	[8, 9]
severe typhoid fever	minimum = 0.038, most probability value = 0.079, maximum = 0.134	Year	Beta (PERT)	[8, 9]
severe typhoid fever with gastrointestinal bleeding-bleeding	minimum = 0.003, most probability value = 0.005, maximum = 0.010	Year	Beta (PERT)	[8, 9]
severe typhoid fever with gastrointestinal bleeding-fever	minimum = 0.055, most probability value = 0.109, maximum = 0.192	Year	Beta (PERT)	[8, 9]
typhoid fever with other abdominal complications	minimum = 0.038, most probability value = 0.079, maximum = 0.134	Year	Beta (PERT)	[8, 9]
L_D (course from onset to death)				
EC	position = 0.0082, mean = 0.0301, SD = 0.00301	Year	Lognormal	[7]
SA	position = 0.0082, mean = 0.0301, SD = 0.00301	Year	Lognormal	[7]
ST				
moderate typhoid fever	position = 0.019, mean = 0.058, SD = 0.0058	Year	Lognormal	[8]
severe typhoid fever	position = 0.038, mean = 0.134, SD = 0.0134	Year	Lognormal	[8]

severe typhoid fever with gastrointestinal bleeding-bleeding	position = 0.003, mean = 0.010, SD = 0.0010	Year	Lognormal	[8]
severe typhoid fever with gastrointestinal bleeding-fever	position = 0.036, mean = 0.125, SD = 0.0125	Year	Lognormal	[8]
typhoid fever with other abdominal complications	position = 0.038, mean = 0.134, SD = 0.0134	Year	Lognormal	[8]
3CEC	position = 0.0219, mean = 0.0575, SD = 0.00575	Year	Lognormal	[7]
CREC	position = 0.0164, mean = 0.0740, SD = 0.00740	Year	Lognormal	[7]
MRSA	position = 0.0219, mean = 0.0521, SD = 0.00521	Year	Lognormal	[7]
ratio				
moderate typhoid fever	0.35	/	/	[8]
severe typhoid fever	0.43	/	/	[8]
severe typhoid fever with gastrointestinal bleeding	0.05	/	/	[8]
typhoid fever with other abdominal complications	0.17	/	/	[8]

Supplementary Table 7. Quantification of general pathogens (conc) and resistant ratio (r) of the samples

Season	Samples	*conc* (copies/mL)			*r* (%)		
		EC	***SA***	***ST***	***EC***	***SA***	***ST***
high-flow season	DZH	0.04	19.98	6.33	0.27	0.00	2.38
	LCG	0.00	25.44	11.19	5.95	4.76	0.00
	LHK	0.08	14.16	20.61	1.20	26.32	1.75
	QYK	0.00	18.61	12.80	0.66	0.00	13.33
	SCK	0.00	20.51	24.35	0.00	1.56	0.00
	SDK	0.30	19.32	6.73	0.62	0.00	2.38
	WSK	1.01	65.14	26.13	2.87	12.82	13.43
	XP	0.37	16.67	0.00	3.59	0.00	0.00
	mean	0.22	24.98	13.52	1.89	5.68	4.16
	median	0.06	19.65	11.99	0.93	0.78	2.07
low-flow season	DZH	5.38	25.49	75.33	1.00	0.00	1.99
	LCG	3.26	23.75	305.16	1.12	0.00	0.47
	LHK	255.52	67.95	2073.95	1.61	0.00	1.16
	QYK	4.13	4.49	314.28	9.51	0.00	6.17
	SCK	1.81	1.12	110.42	2.21	0.00	1.61
	SDK	0.92	4.82	36.49	16.82	0.00	9.52
	WSK	4.43	13.99	112.04	16.67	0.10	7.34
	XP	2.70	1.16	76.67	1.10	0.00	0.73
	mean	34.77	17.85	388.04	6.26	0.01	3.62
	median	3.70	9.40	111.23	1.91	0.00	1.80



Supplementary Figure 1. Standard curves of qPCR for (A) rfbE, (C) rpoB, and (E) invA in high-flow season, and (B) rfbE, (D) rpoB, and (F) invA in low-flow season.

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