

Supplementary Materials

Occurrence of viable but non-culturable (VBNC) pathogenic bacteria in tap water of public places

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“T” and “V” represented total (non-PMA treatment) and viable (PMA treatment) bacterial communities.

Table S1. Linear distance bewteen each sampling points (Unit: km)

Sites	DWTP	Railway station	Campus	Hospital	Shopping mall	Institute
DWTP	0	3.21	1.58	3.16	4.20	3.06
Railway station		0	3.99	3.87	6.95	2.88
Campus			0	2.45	2.98	2.47
Hospital				0	1.52	4.89
Shopping mall					0	5.01
Institute						0

Table S2. Identification results of suspicious colonies growth on each selective medium

Numbers	Strains	Identities
1	<i>Pseudomonas putida</i>	99.93%
2	<i>Pseudomonas</i> sp.	99.93%
3	<i>Pseudomonas putida</i>	99.93%
4	<i>Pseudomonas</i> sp.	99.79%
5	<i>Stenotrophomonas</i> sp.	99.86%
6	<i>Leclercia adecarboxylata</i>	99.93%
7	<i>Pseudomonas putida</i>	99.86%
8	<i>Bacillus flexus</i>	99.93%
9	<i>Pseudomonas putida</i>	99.86%
10	<i>Pseudomonas putida</i>	99.86%
11	<i>Acinetobacter baumannii</i>	99.79%
12	<i>Acinetobacter</i> sp.	97.01%
13	<i>Paenibacillus alvei</i>	99.76%
14	<i>Staphylococcus epidermidis</i>	99.93%
15	<i>Bacterium</i> strain	100%
16	<i>P. aeruginosa</i> (CN medium, May)	99.93%
17	<i>P. aeruginosa</i> (CN medium, May)	99.86%
18	<i>Pseudomonas</i> sp.	99.86%
19	<i>P. aeruginosa</i> (m-TEC medium, May)	100%
20	<i>Staphylococcus hominis</i>	100%
21	<i>Pseudomonas putida</i>	100%
22	<i>Bacillus flexus</i>	100%
23	<i>P. aeruginosa</i> PAO1 (CN medium, June)	99.93%
24	<i>P. aeruginosa</i> (CN medium, June)	99.93%
25	<i>Pseudomonas putida</i>	100%
26	<i>P. aeruginosa</i> (m-TEC medium, June)	100%
27	<i>Pseudomonas putida</i>	100%
28	<i>P. aeruginosa</i> (SS medium, June)	100%
29	<i>Bacillus</i> sp.	100%
30	<i>Bacillus</i> sp.	100%
31	<i>Enterobacter tabaci</i>	100%
32	<i>Staphylococcus epidermidis</i>	100%

Table S3. Standard curves of gene plasmid copy numbers and CT values (Guo et al., 2020).

Bacteria	Standard curve formula	R²	Amplification efficiency
Total bacteria (16S rRNA)	$y=-3.363x+39.055$	0.999	98.31%
<i>E. coli</i>	$y=-2.793x+37.298$	0.997	128.1%
<i>E. faecalis</i>	$y=-3.085x+41.927$	0.990	110.9%
<i>P. aureginosa</i>	$y=-2.962x+42.835$	0.998	117.6%
<i>Salmonella</i> sp.	$y=-3.028x+40.322$	0.996	113.9%
<i>Shigella</i> sp.	$y=-3.030x+39.532$	0.998	113.8%
<i>L. pneumophila</i>	$y=-3.215x+43.782$	0.999	104.7%

Table S4. Standard curves of specific gene copies and viable cell numbers after serial dilutions, by TaqMan-qPCR (Guo et al., 2020).

Bacteria	Standard curve formula	R²
<i>E. coli</i>	$y=0.86x+0.83$	0.999
<i>E. faecalis</i>	$y=0.92x-0.29$	0.999
<i>P. aureginosa</i>	$y=0.89x-0.94$	0.998
<i>Salmonella</i> sp.	$y=0.94x+0.57$	0.999
<i>Shigella</i> sp.	$y=0.99x-0.42$	0.988

Table S5. The frequency of detection of each pathogenic bacteria in all tap water samples

Sampling points	The frequency of detection (FOD) % (total samples n=45)					
	<i>E. coli</i>	<i>E. faecalis</i>	<i>P. aureginosa</i>	<i>Salmonella</i> sp.	<i>Shigella</i> sp.	<i>L. pneumophila</i>
Railway station	17.78	6.67	4.44	6.67	11.11	2.22
Campus	6.67	8.89	6.67	6.67	13.33	0.00
Hospital	11.11	8.89	0.00	6.67	0.00	0.00
Shopping mall	15.56	17.78	4.44	6.67	13.33	11.11
Institution	13.33	4.44	4.44	6.67	2.22	0.00
Total	64.45	46.67	19.99	33.35	39.99	13.33

Table S6. Correlations between target organisms and abiotic parameters in tap water samples collected from different public places

Physiochemical parameters	16S rRNA	R2A	<i>E. coli</i>	<i>E. faecalis</i>	<i>P. aureginosa</i>	<i>Salmonella</i> sp.	<i>Shigella</i> sp.	<i>L. pneumophila</i>
Residual chlorine	$r_s = -0.260$	$r_s = -0.505$	$r_s = -0.145$	$r_s = 0.127$	$r_s = -0.301$	$r_s = -0.076$	$r_s = 0.104$	$r_s = 0.137$
	P = 0.085	P < 0.001	P = 0.342	P = 0.406	P < 0.05	P = 0.622	P = 0.498	P = 0.371
TOC	$r_s = -0.119$	$r_s = -0.390$	$r_s = -0.046$	$r_s = 0.104$	$r_s = 0.286$	$r_s = -0.028$	$r_s = 0.228$	$r_s = -0.287$
	P = 0.437	P < 0.05	P = 0.763	P = 0.496	P = 0.057	P = 0.853	P = 0.132	P = 0.056
Turbidity	$r_s = -0.242$	$r_s = -0.041$	$r_s = -0.168$	$r_s = -0.220$	$r_s = 0.215$	$r_s = -0.007$	$r_s = -0.126$	$r_s = -0.357$
	P = 0.110	P = 0.791	P = 0.270	P = 0.146	P = 0.156	P = 0.965	P = 0.410	P < 0.05
NO ₃ -N	$r_s = 0.420$	$r_s = 0.133$	$r_s = -0.239$	$r_s = -0.161$	$r_s = -0.020$	$r_s = -0.059$	$r_s = 0.014$	$r_s = 0.403$
	P < 0.05	P = 0.382	P = 0.115	P = 0.290	P = 0.898	P = 0.698	P = 0.927	P < 0.05
DO	$r_s = 0.237$	$r_s = -0.177$	$r_s = -0.615$	$r_s = 0.021$	$r_s = -0.113$	$r_s = 0.199$	$r_s = 0.235$	$r_s = -0.021$
	P = 0.117	P = 0.244	P < 0.001	P = 0.893	P = 0.461	P = 0.191	P = 0.121	P = 0.891
pH	$r_s = -0.056$	$r_s = 0.241$	$r_s = -0.090$	$r_s = 0.076$	$r_s = 0.160$	$r_s = -0.214$	$r_s = -0.006$	$r_s = -0.059$
	P = 0.717	P = 0.111	P = 0.557	P = 0.619	P = 0.294	P = 0.158	P = 0.968	P = 0.701
Temperature	$r_s = -0.237$	$r_s = 0.063$	$r_s = 0.619$	$r_s = 0.075$	$r_s = -0.043$	$r_s = -0.314$	$r_s = -0.087$	$r_s = -0.024$
	P = 0.116	P = 0.680	P < 0.001	P = 0.626	P = 0.778	P < 0.05	P = 0.571	P = 0.878

Note: Correlations were analyzed using the nonparametric spearman rank correlation method

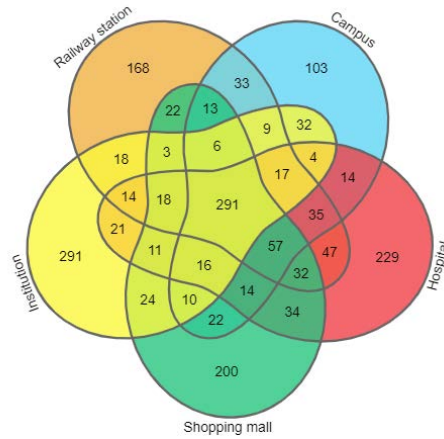


Fig. S1. Venn diagram of detected taxa among each tap water in the five public places.

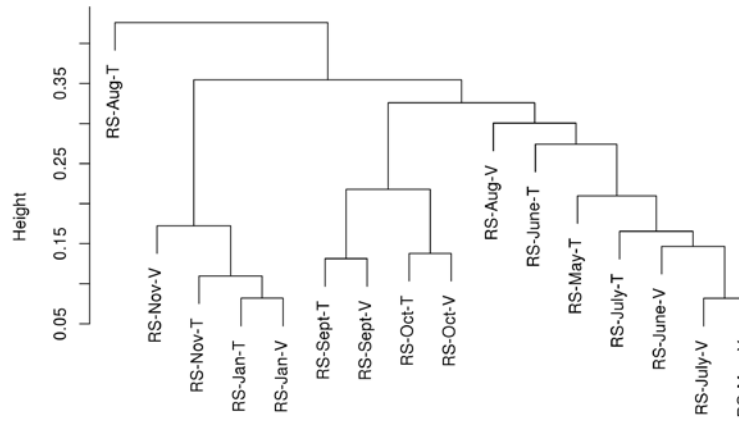


Fig. S2. Hierarchical clustering of the tap water samples for railway station (RS) based on the average linkage clustering method. The branch length describes the difference between the samples. “T” and “V” represented total (non-PMA treatment) and viable (PMA treatment) bacterial communities.

Reference

Guo L, Wan K, Zhu J, Ye C, Chabi K, Yu X (2020). Detection and Distribution of VBNC/Viable Pathogenic Bacteria in Full-Scale Drinking Water Treatment Plants. *Journal of Hazardous Materials*, 124335