Removal of antibiotic resistance genes and co-selectors in a full-scale sewage treatment plant during drought and flood.

Supplementary Information

Text S1: In between samples, the sampling equipment was wiped with 70% ethanol for disinfection. Water quality parameters (pH, DO, conductivity) were measured using HQ40d Portable Water Quality Lab Package (Hach, USA) *in situ*. Before use, all glassware were washed thrice with soap and water, rinsed thrice with DI water, and submerged in a 10% nitric acid water bath overnight. Finally, they were rinsed thrice with DI water and then baked at 250 °C overnight.

Text S2: Solid phase extraction lines were prewashed with nanopure water and LC grade methanol. The metal frit ends of the solid phase extraction lines were submerged first in the 500 mL amber bottle containing methanol. Vacuum was applied to allow ~250 mL methanol to wash through the solid phase extraction lines and cartridges. Then, metal frits from methanol bottle were removed and inserted into the bottle containing nanopure water ~250 mL to flush through the lines. Conditioning was done with 6 mL of acetonitrile, followed by 6 mL nanopure water.

Text S3: LCMS Method

Acetonitrile (ACN) and methanol, both LCMS grade and ammonium acetate were purchased from Merck (Darmstadt, Germany). Formic acid (LC–MS grade) and disodium ethylenediamine tetraacetate (Na2EDTA) ACS reagent were purchased from Sigma. The mobile phase used for Ciprofloxacin analysis was aqueous 0.1% formic acid in LCMS grade water (A) and methanol (B). The mobile phase used for triclosan was Aqueous 0.1% Ammonium acetate in LCMS grade water (A) and Acetonitrile (B). Standards for antibiotics were purchased from Sigma. Stock solutions were prepared in wastewater as matrix. Wastewater sample was used as a matrix blank and matrix spike was carried out evaluated to matrix interference.

Quality Assurance

Antibiotic standards were prepared using the starting mobile phase of the LC-MS/MS method with the solvent. An initial solution of 100 ppb (μ g/L) was made and then it was serially diluted to obtain mixtures with concentrations of 75, 50, 25, 10, 5, 3, and 1 ppb. These standards were analysed by LC-MS/MS, with replicates for each concentration, to obtain the areas of both the quantifying and qualifying ions. The ion ratios were calculated by dividing the area of the quantifying ion by the area of the qualifying ion for each analyte. The average ion ratio and the deviations from the average value for the ion ratios were calculated at all concentrations for each compound. The ions selected for Ciprofloxacin and Triclosan were 332.10 < 314.10 and 287<35; 289<37 respectively.

Limits of Detection and Limits of Quantification values

Antimicrobials LOD LOQ

Ciprofloxacin 0.95 ng/L 2.90 ng/L

Triclosan 7.44 ng/L 22.57 ng/L

S/N value > 10

Table S1: List of primers used in the study.

Gene target	Forward Primer	Reverse Primer		
16S rRNA gene (1369F -	CGGTGAATACGTTCYCGG	GGWTACCTTGTTACGACTT		
1492R) ¹				
gyrA ²	GGTACACCGTCGCGTACTTT	CAACGAAATCGACCGTCTCT		
parC ²	GCCTTGCGCTACATGAATTT	ACCATCAACCAGCGGATAAC		
acrA ³	CTCTCAGGCAGCTTAGCCCTA	TGCAGAGGTTCAGTTTTGACT		
	A	GTT		
acrB ³	GGTCGATTCCGTTCTCCGTTA	CTACCTGGAAGTAAACGTCAT		
		TGGT		
qepA ⁴	GCAGGTCCAGCAGCGGGTAG	CTTCCTGCCCGAGTATCGTG		
<i>erm</i> B ⁵	GATACCGTTTACGAAATTGG	GAATCGAGACTTGAGTGTGC		
<i>van</i> A (36F -	TTGCTCAGAGGAGCATGACG	TCGGGAAGTGCAATACCTGC		
992R) ⁶				
FAB RJH101	CATATGTTAAATCTTGAAAAC	GGATCCTTATTTAATTGCGTG		
-1027	AAAACGTATGTCAT	GAATCCGCTATC		
tetA ⁸	GCGCGATCTGGTTCACTCG	AGTCGACAGYRGCGCCGGC		
<i>bla</i> _{TEM} ⁸	ATGAGTATTCAACATTTCCG	CCAATGCTTAATCAGTGAGG		
tetQ ⁹	AGAATCTGCTGTTTGCCAGTG	CGGAGTGTCAATGATATTGCA		
<i>tcr</i> B ¹⁰	GGAAAGGCAACTGAATATCC	GCCGTCTTGATGTCACTTTC		
fabV ¹¹	GATCACCCACGACATCTTCTG	GCCGATGGAACCGTTCCAGAA		
	GAACGGTTCCATCGGC	GATGTCGTGGGTGATC		
czcA ¹²	GTTTGAACGTATCATTAGTTT	GTAGCCATCCGAAATATTCG		

	С	
chrA ¹³	CTTATACGCTACGCCAACTG	GTAATGGCATTCAGTCGCTTG
chrB ¹³	GTCGTTAGCTTGCCAACATC	CGGAAAGCAAGATGTCGATCG
<i>sul</i> 1 ¹⁴	CGCACCGGAAACATCGCTGCA	TGAAGTTCCGCCGCAAGGCTC
	С	G
mecA ¹⁵	CGCAACGTTCAATTTAATTTT	CCACTTCATATCTTGTAACG
	GTTAA	
tetW ⁹	GAGAGCCTGCTATATGCCAGC	GGGCGTATCCACAATGTTAAC
tetO ⁹	ACGGARAGTTTATTGTATACC	TGGCGTATCTATAATGTTGAC
<i>erm</i> F ⁵	CGACACAGCTTTGGTTGAAC	GGACCTACCTCATAGACAAG
<i>int</i> I1 ¹⁴	GGGTCAAGGATCTGGATTTCG	ACATGCGTGTAAATCATCGTC
		G
<i>mcr</i> 1 ^{16,17}	TCTTGTGGCGAGTGTTGCCGT	CCAATGATACGCATGATAAAC
		GCTG
<i>mcr</i> 5 ¹⁷	GTGAAACAGGTGATCGTGACT	CGTGCTTTACACCGATCATGT
	TACCG	GCT
<i>ycc</i> T ¹⁸	GCATGCTGACCACCTTGA	CAGCGTGGTGGCAAAA
<i>mex</i> F ¹⁹	TGTACGCGAACGACTTCAAC	GAGGTGTCGCTGACCTTGAT
$bla_{\rm OXA-1}^{20}$	CAAGCCAAAGGCACGATAGT	ACGATTGCCTCCTCTTGAA
$bla_{\rm OXA-7}^{21,22}$	GAAGCCGTCAATGGTGTTTT	ATGCCCTCACTTGCCATGAT
qnrS ²³	CAATCATACATATCGGCACC	TCAGGATAAACAACAATACCC
<i>sul</i> 2 ¹⁴	TCCGGTGGAGGCCGGTATCTG	CGGGAATGCCATCTGCCTTGA
	G	G
Total E. coli ²⁴	TGGGAAGCGAAAATCCTG	CAGTACAGGTAGACTTCTG

Gene	LOQ (gene copy numbers/µL)	% Var
16S rRNA	102	24.1%
уссТ	102	10.1%
sul1	101	15.5%
sul2	101	13.2%
parC	10 ²	11.9%
bla _{OXA-1}	103	21.0%
tetW	101	8%
ermF	103	6%
mcr5	101	24.7%
intI1	10 ²	14.7%

Table S2: LOQ for each gene present in the samples.

Table S3: Details of qPCR for all genes except intI1 and bla_{OXA-1}

Step	Temperature	Time
Initial hold	50° C	120 secs
Initial denaturation	95° C	120 secs
Denaturation	95° C	15 secs
Annealing and Extension	60° C	60 secs
Repeat	Go to step 'Denaturation' step	Repeat 39 times
Denaturing gradient	60° C - 95° C gradient	15 mins

Step	Temperature	Time
Initial hold	50° C	120 s
Initial denaturation	95° C	120 s
Denaturation	95° C	15 s
Annealing	60° C	60 s
Extension	72°C	60 s
Repeat	Go to step 'Denaturation' step	Repeat 39 times
Denaturing gradient	60° C - 95° C gradient	15 mins

Table S5: qPCR assay preparation

All	the	targeted	gene	5µL SYBR Green, 1µL Forward Primer (5mM), 1µL Reverse
marke	ers			Primer (5mM), 2µL DNA free water and 1µL DNA insert of
				the target gene.
bla _{OX}	A-1			5µL SYBR Green, 0.8µL Forward Primer (5mM), 0.8µL
				Reverse Primer (5mM), 2.4 μ L DNA free water and 1 μ L
				DNA insert of the target gene.

PCR type	Assay
PCR for	5µL Hotstar master mix (Qiagen®, Hilden, Germany), 0.8µL Forward
presence/absence	Primer (5mM), 0.8µL Reverse Primer (5mM), 2.4µL DNA free water
	and 1µL DNA insert of the target gene.
Gradient PCR	5µL Hotstar master mix (Qiagen®, Hilden, Germany), 0.8µL Forward
	Primer (5mM), 0.8µl Reverse Primer (5mM), 1.4µL DNA free water
	and 2µL DNA insert of the target gene.
M13 PCR	5µL Hotstar master mix (Qiagen®, Hilden, Germany), 1µL M13
	Forward Primer (5mM), 1µL M13 Reverse Primer (5mM), 2.4µL
	DNA free water and 1µL DNA insert of the target gene.

Table S7: Median values of the levels of 16S rRNA gene copies (gene copy/L or gene copy/g), relative levels of the ARGs on log10 scale (copy number/16S rRNA gene copies), and co-selectors (ppb) during the study period at different sampling locations within the targeted STP: IN – Grit chamber outlet, ATIN – Inlet of aeration tank i.e., sample collected post primary treatment, ATOUT – Outlet of aeration tank, MPIN – Inlet of maturation pond, and MPOUT – Outlet of maturation pond.

Gene	Inlet	ATIN	ATOUT	MPIN	MPOUT	FS
log 16SrRNA	9.51E+00	8.60E+00	9.82E+00	8.63E+00	8.56E+00	1.20E+01
ycc T	-7.16E-01	-2.02E-01	-9.93E-01	-1.19E+00	-1.03E+00	-2.86E+00
int I1	7.66E-02	1.76E-01	2.93E-01	6.58E-01	5.95E-01	-1.37E+00
<i>sul</i> 1	3.45E-01	4.65E-01	3.54E-01	4.86E-01	5.08E-01	-4.17E+00
sul 2	-2.33E+00	-2.32E+00	-2.64E+00	-2.39E+00	-2.38E+00	-7.21E+00
par C	-2.52E+00	-2.13E+00	-2.41E+00	-1.94E+00	-2.41E+00	-5.59E+00
bla _{OXA-1}	-3.75E+00	-1.63E+00	-4.42E+00	-6.74E+00	-1.65E+00	-2.22E+00
tet W	-7.25E-01	-3.92E-01	-1.07E+00	-8.71E-01	-7.36E-01	5.75E-01
<i>erm</i> F	2.03E-01	-2.06E-02	-9.38E-02	-3.04E-01	-1.68E-01	1.37E+00
mcr 5	-1.18E+00	-2.21E+00	-2.38E+00	-2.26E+00	-1.83E+00	-1.56E+00
Ciprofloxacin	102.27	87.49	87.21	74.37	44.37	27.17
Triclosan	172.88	135.80	109.46	113.06	104.38	0.00
Copper	0.80	0.72	0.29	0.53	0.14	0.00
Lead	0.31	0.29	0.21	0.11	0.00	0.00
Chromium	4.14	3.57	3.88	2.07	2.13	0.00

Table S8: Median values of the levels of 16S rRNA gene copies (gene copy/L or gene copy/g) and ARGs on log10 scale, and co-selectors (ppb) during the study period different sampling locations within the targeted STP: IN – Grit chamber outlet, ATIN – Inlet of aeration tank i.e., sample collected post primary treatment, ATOUT – Outlet of aeration tank, MPIN – Inlet of maturation pond, and MPOUT – Outlet of maturation pond in each season.

Gene	Season	Inlet	ATIN	ATOUT	MPIN	MPOUT
16SrRNA	Summer	3.41E+10	2.06E+08	2.22E+10	7.43E+09	2.10E+09
	Monsoon	1.52E+09	5.96E+08	6.35E+09	1.90E+08	3.52E+08
	Winter	1.67E+10	1.66E+08	3.89E+09	2.84E+08	1.87E+09
уссТ	Summer	-1.08E+00	-1.45E+00	-7.91E-01	-2.63E+00	-3.13E+00
	Monsoon	-7.16E-01	-2.02E-01	-9.93E-01	-1.19E+00	-1.03E+00
	Winter	-9.05E-01	-4.75E-01	-6.95E-01	3.47E-01	-1.40E+00
intI1	Summer	-2.39E+00	-2.22E+00	-2.22E+00	-1.87E+00	-2.37E+00
	Monsoon	1.19E+00	7.45E-01	6.01E-01	8.20E-01	1.04E+00
	Winter	-1.74E-01	-2.33E-01	8.29E-01	7.65E-01	2.61E-01
sul1	Summer	5.54E-01	4.15E-01	3.54E-01	2.92E-01	5.25E-01
	Monsoon	5.91E-01	7.53E-01	-4.58E-02	6.10E-01	2.17E-01
	Winter	3.23E-01	2.08E-01	8.80E-01	7.04E-01	6.64E-01
sul2	Summer	-2.56E+00	-2.32E+00	-2.75E+00	-2.46E+00	-2.41E+00
	Monsoon	-2.26E+00	-2.37E+00	-2.50E+00	-2.03E+00	-2.40E+00
	Winter	-1.95E+00	-1.67E+00	-1.79E+00	-4.38E-01	-1.44E+00
parC	Summer	-7.92E-01	-3.09E-01	1.96E-01	-2.60E-01	-3.15E-01
	Monsoon	-2.45E+00	-2.70E+00	-3.26E+00	-2.62E+00	-2.46E+00
	Winter	-3.95E+00	-1.92E+00	-2.50E+00	-1.52E+00	-2.98E+00
bla _{OXA-1}	Summer	-3.25E+00	-2.71E+00	-5.13E+00	-3.79E+00	-2.16E+00
	Monsoon	-8.21E+00	-3.02E+00	-4.00E+00	-5.09E+00	-1.64E+00
	Winter	-6.68E+00	-1.41E+00	-5.75E+00	-7.64E+00	-2.03E+00
tetW	Summer	-1.82E+00	-6.69E-01	-1.07E+00	-8.71E-01	-3.27E+00

	Monsoon	7.09E-02	2.95E-02	-9.23E-01	-6.78E-01	-5.21E-01
	Winter	-1.04E+00	-4.45E-01	-5.73E-01	-6.91E-01	-1.73E+00
mcr5	Summer	-3.01E+00	-3.70E+00	-3.20E+00	-3.76E+00	-2.66E+00
	Monsoon	-5.55E-01	-6.81E-01	-2.46E+00	-9.37E-01	-9.20E-01
	Winter	-2.34E+00	-2.37E+00	-2.24E+00	-2.48E+00	-2.41E+00
ermF	Summer	-5.39E-01	-5.76E-01	-4.61E-01	-7.16E-02	-2.74E+00
	Monsoon	6.99E-01	3.48E-01	1.19E-01	-1.27E-01	3.83E-01
	Winter	-3.72E-01	-6.19E-01	-2.85E-01	-4.60E-01	-1.51E+00
Ciprofloxacin	Summer	125.62	91.76	105.19	92.22	31.98
	Monsoon	103.04	90.32	89.43	78.48	46.04
	Winter	100.13	85.39	74.66	72.97	40.68
Triclosan	Summer	204.23	184.20	170.52	141.21	109.63
	Monsoon	188.77	154.25	128.39	134.81	110.64
	Winter	143.87	125.06	95.43	103.49	91.01
Copper	Summer	0.07	0.00	0.00	0.32	0.14
	Monsoon	0.83	0.73	0.40	0.32	0.00
	Winter	0.79	0.75	0.42	0.78	0.18
Lead	Summer	0.00	0.00	0.15	0.00	0.00
	Monsoon	0.37	0.25	0.22	0.09	0.00
	Winter	0.36	0.36	0.24	0.20	0.00
Chromium	Summer	0.00	1.27	0.41	0.00	0.00
	Monsoon	3.20	4.18	3.90	2.52	2.44
	Winter	4.37	3.86	4.11	3.80	3.77

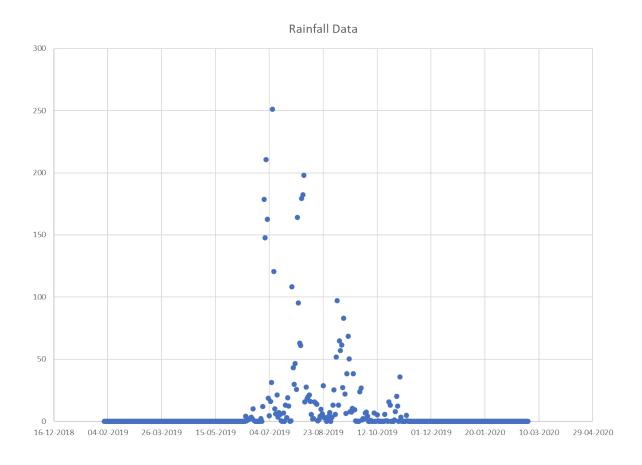


Figure S1: Average daily rainfall in mm during the sampling period in the city Chennai. Data was obtained from IMD website^{25,26}.

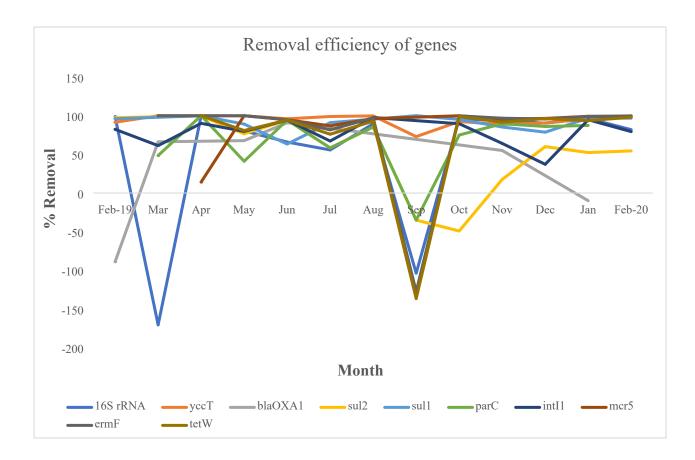


Figure S2: Removal efficiencies of the targeted genes in the STP from Feb 2019 (Feb19) all through the remaining months in 2019 till Feb 2020 (Feb 20).

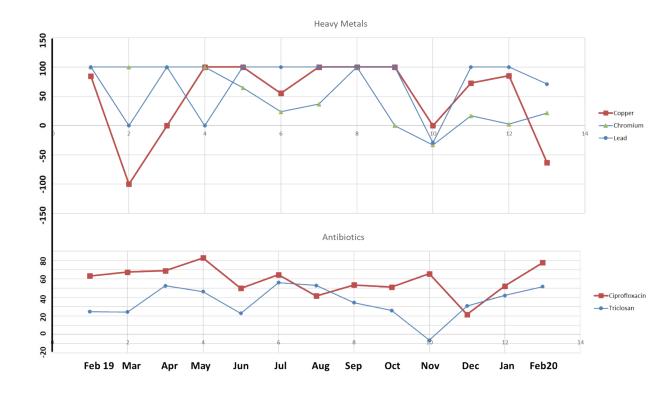


Figure S3: Removal efficiencies of the co-selectors from the STP from Feb 2019 (Feb19) all through the remaining months in 2019 till Feb 2020 (Feb 20).

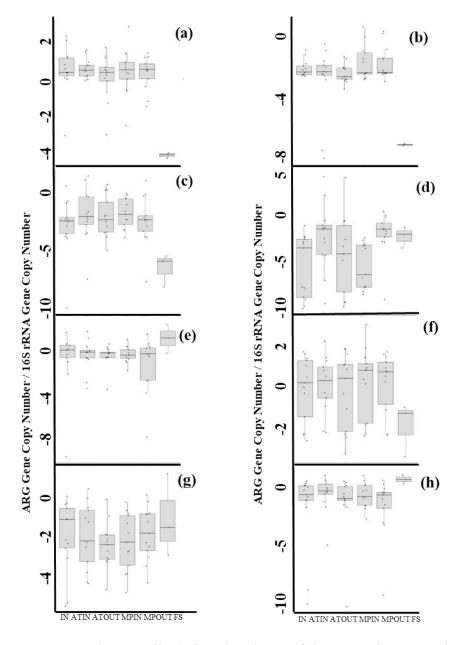


Figure S4: The overall relative abundance of the respective ARGs in the STP over the sampling period: a) *sul*1, b) *sul*2, c) *par*C, d) *bla*_{OXA-1}, e) *erm*F, f) *int*I1, g) *mcr*5, and h) *tet*W The x-axis represents different sampling locations within the targeted STP: IN – Grit chamber outlet (n=13), ATIN – Inlet of aeration tank i.e., sample collected post primary treatment (n=13), ATOUT – Outlet of aeration tank (n=13), MPIN – Inlet of maturation pond (n=13), and MPOUT – Outlet of maturation pond and (n=13), FS - Final sludge collected after the drying of the anaerobically digested sludge (n=3).

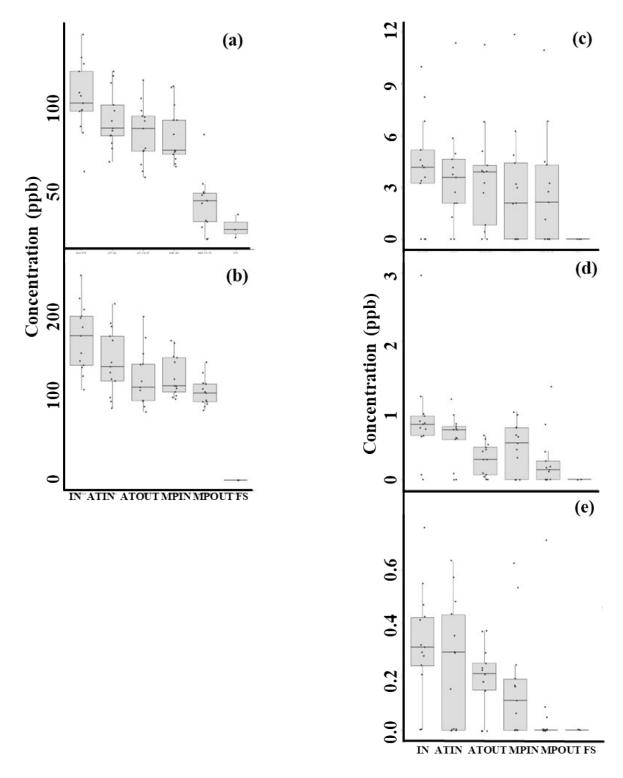


Figure S5: The y-axis represents the levels of co-selective agents over the sampling period: **a**. Ciprofloxacin, **b**. Triclosan, **c**. Chromium, **d**. Copper, and **e**. Lead in ppb. The x-axis represents different sampling locations within the targeted STP: IN – Grit chamber outlet (n=13), ATIN – Inlet of aeration tank i.e., sample collected post primary treatment (n=13),

ATOUT – Outlet of aeration tank (n=13), MPIN – Inlet of maturation pond (n=13), and MPOUT – Outlet of maturation pond and (n=13), FS - Final sludge collected after the drying of the anaerobically digested sludge (n=3).

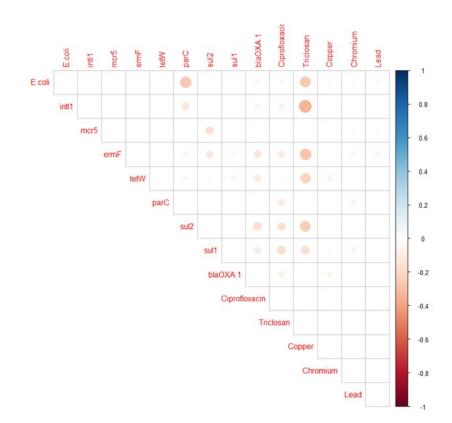


Figure S6: The correlogram indicating Spearman correlation coefficient (when p-value <0.05) between the relative levels of the ARGs and the co-selective agents (having the absolute values)

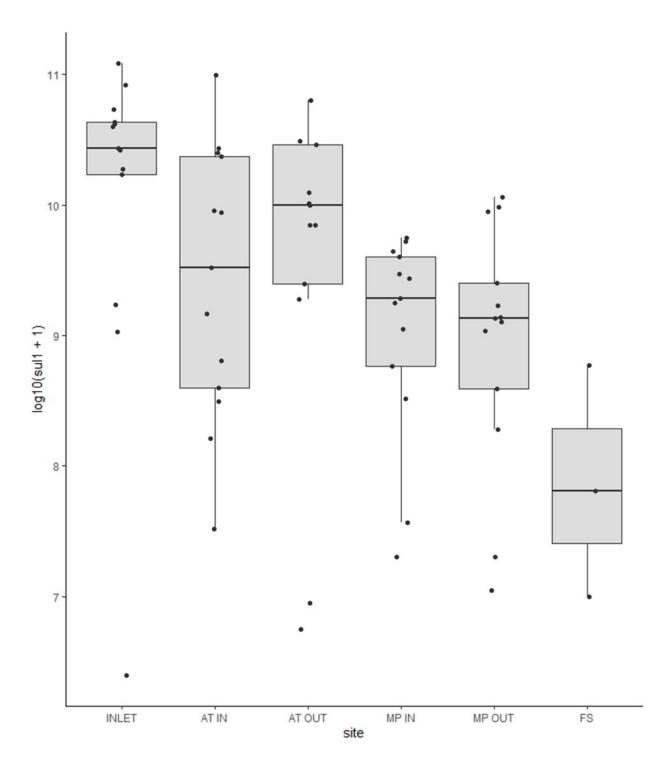


Figure S7: The y-axis represents the absolute abundance of *sul*1 at different sites in the STP over the sampling period. The x-axis represents different sampling locations within the targeted STP: IN - Grit chamber outlet (n=13), ATIN – Inlet of aeration tank i.e., sample collected post primary treatment (n=13), ATOUT – Outlet of aeration tank (n=13), MPIN –

Inlet of maturation pond (n=13), and MPOUT – Outlet of maturation pond and (n=13), FS -Final sludge collected after the drying of the anaerobically digested sludge (n=3).

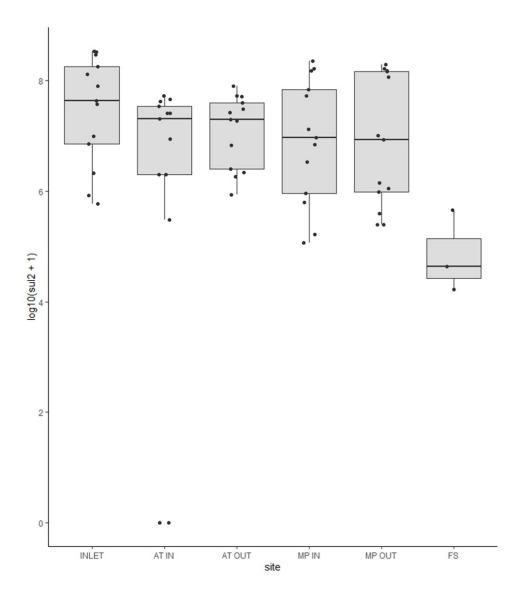


Figure S8: The y-axis represents the absolute abundance of *sul*2 at different sites in the STP over the sampling period. The x-axis represents different sampling locations within the targeted STP: IN – Grit chamber outlet (n=13), ATIN – Inlet of aeration tank i.e., sample collected post primary treatment (n=13), ATOUT – Outlet of aeration tank (n=13), MPIN – Inlet of maturation pond (n=13), and MPOUT – Outlet of maturation pond and (n=13), FS – Final sludge collected after the drying of the anaerobically digested sludge (n=3).

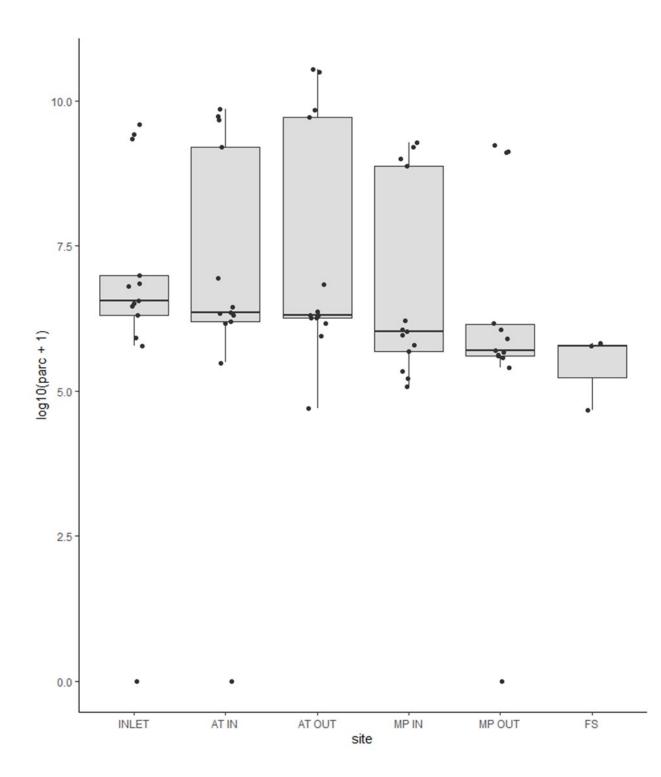


Figure S9: The y-axis represents the absolute abundance of *par*C at different sites in the STP over the sampling period. The x-axis represents different sampling locations within the targeted STP: IN - Grit chamber outlet (n=13), ATIN – Inlet of aeration tank i.e., sample collected post primary treatment (n=13), ATOUT – Outlet of aeration tank (n=13), MPIN –

Inlet of maturation pond (n=13), and MPOUT – Outlet of maturation pond and (n=13), FS - Final sludge collected after the drying of the anaerobically digested sludge (n=3).

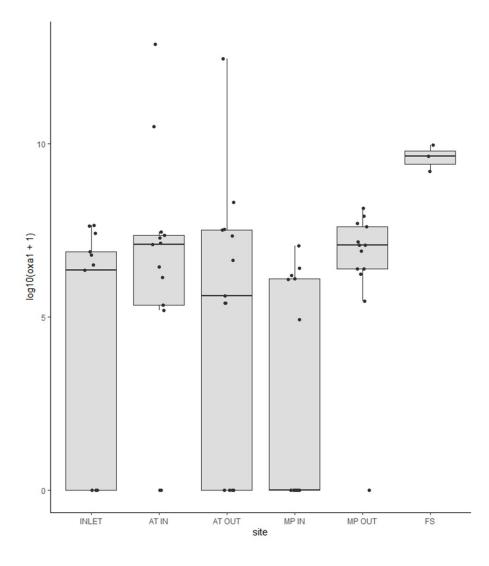


Figure S10: The y-axis represents the absolute abundance of bla_{OXA-1} at different sites in the STP over the sampling period. The x-axis represents different sampling locations within the targeted STP: IN – Grit chamber outlet (n=13), ATIN – Inlet of aeration tank i.e., sample collected post primary treatment (n=13), ATOUT – Outlet of aeration tank (n=13), MPIN – Inlet of maturation pond (n=13), and MPOUT – Outlet of maturation pond and (n=13), FS – Final sludge collected after the drying of the anaerobically digested sludge (n=3).

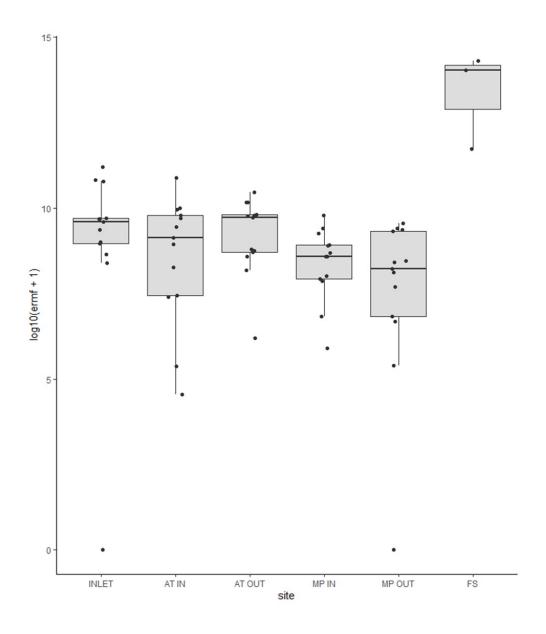


Figure S11: The y-axis represents the absolute abundance of *erm*F at different sites in the STP over the sampling period. The x-axis represents different sampling locations within the targeted STP: IN - Grit chamber outlet (n=13), ATIN – Inlet of aeration tank i.e., sample collected post primary treatment (n=13), ATOUT – Outlet of aeration tank (n=13), MPIN – Inlet of maturation pond (n=13), and MPOUT – Outlet of maturation pond and (n=13), FS - Final sludge collected after the drying of the anaerobically digested sludge (n=3).

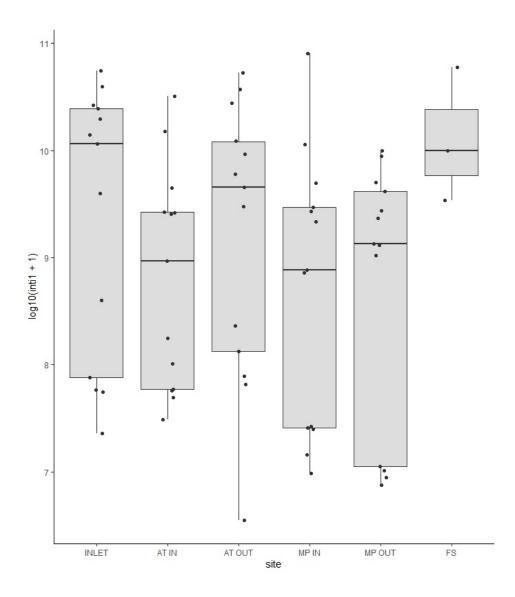


Figure S12: The y-axis represents the absolute abundance of *int*I1 at different sites in the STP over the sampling period. The x-axis represents different sampling locations within the targeted STP: IN - Grit chamber outlet (n=13), ATIN – Inlet of aeration tank i.e., sample collected post primary treatment (n=13), ATOUT – Outlet of aeration tank (n=13), MPIN – Inlet of maturation pond (n=13), and MPOUT – Outlet of maturation pond and (n=13), FS - Final sludge collected after the drying of the anaerobically digested sludge (n=3).

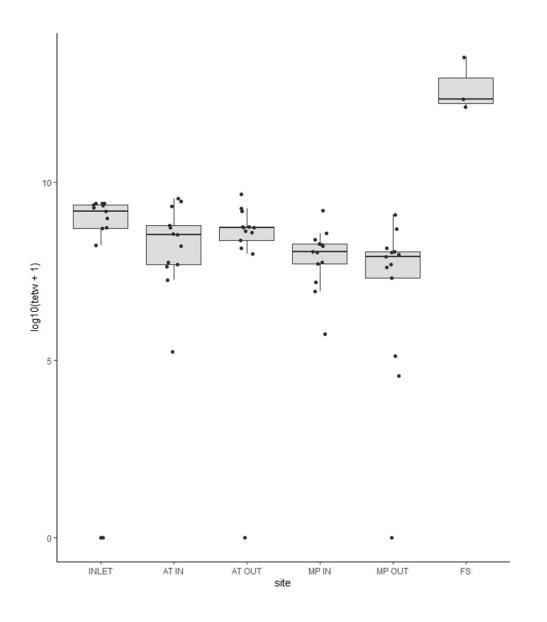


Figure S13: The y-axis represents the absolute abundance of *tet*W at different sites in the STP over the sampling period. The x-axis represents different sampling locations within the targeted STP: IN - Grit chamber outlet (n=13), ATIN – Inlet of aeration tank i.e., sample collected post primary treatment (n=13), ATOUT – Outlet of aeration tank (n=13), MPIN – Inlet of maturation pond (n=13), and MPOUT – Outlet of maturation pond and (n=13), FS - Final sludge collected after the drying of the anaerobically digested sludge (n=3).

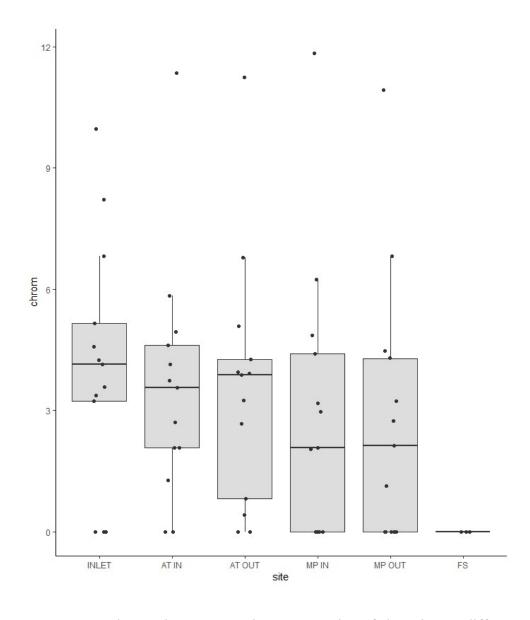


Figure S14: The y-axis represents the concentration of chromium at different sites in the STP over the sampling period. The x-axis represents different sampling locations within the targeted STP: IN – Grit chamber outlet (n=13), ATIN – Inlet of aeration tank i.e., sample collected post primary treatment (n=13), ATOUT – Outlet of aeration tank (n=13), MPIN – Inlet of maturation pond (n=13), and MPOUT – Outlet of maturation pond and (n=13), FS - Final sludge collected after the drying of the anaerobically digested sludge (n=3).

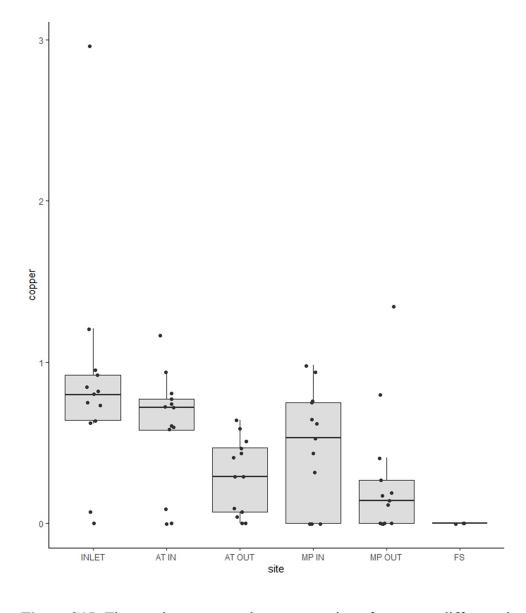


Figure S15: The y-axis represents the concentration of copper at different sites in the STP over the sampling period. The x-axis represents different sampling locations within the targeted STP: IN – Grit chamber outlet (n=13), ATIN – Inlet of aeration tank i.e., sample collected post primary treatment (n=13), ATOUT – Outlet of aeration tank (n=13), MPIN – Inlet of maturation pond (n=13), and MPOUT – Outlet of maturation pond and (n=13), FS - Final sludge collected after the drying of the anaerobically digested sludge (n=3).

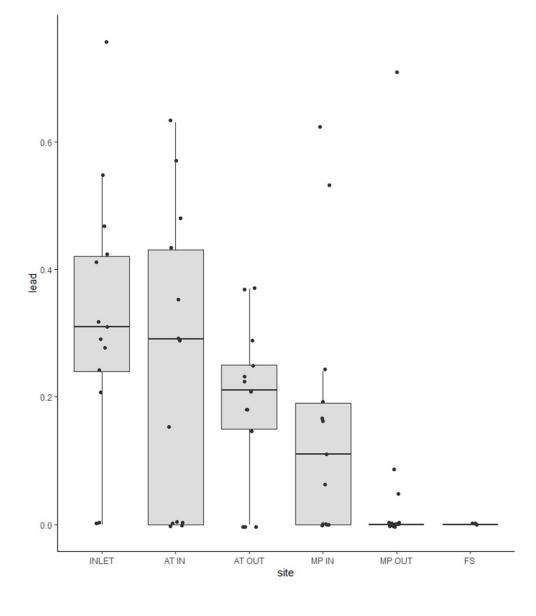


Figure S16: The y-axis represents the concentration of lead at different sites in the STP over the sampling period. The x-axis represents different sampling locations within the targeted STP: IN – Grit chamber outlet (n=13), ATIN – Inlet of aeration tank i.e., sample collected post primary treatment (n=13), ATOUT – Outlet of aeration tank (n=13), MPIN – Inlet of maturation pond (n=13), and MPOUT – Outlet of maturation pond and (n=13), FS - Final sludge collected after the drying of the anaerobically digested sludge (n=3).

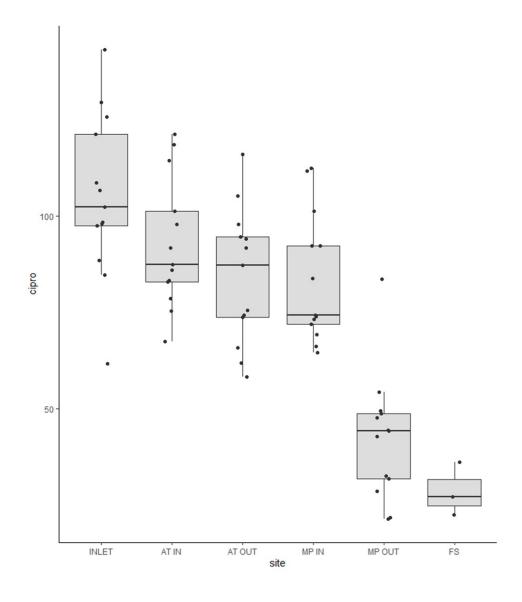


Figure S17: The y-axis represents the concentration of ciprofloxacin at different sites in the STP over the sampling period. The x-axis represents different sampling locations within the targeted STP: IN - Grit chamber outlet (n=13), ATIN – Inlet of aeration tank i.e., sample collected post primary treatment (n=13), ATOUT – Outlet of aeration tank (n=13), MPIN – Inlet of maturation pond (n=13), and MPOUT – Outlet of maturation pond and (n=13), FS - Final sludge collected after the drying of the anaerobically digested sludge (n=3).

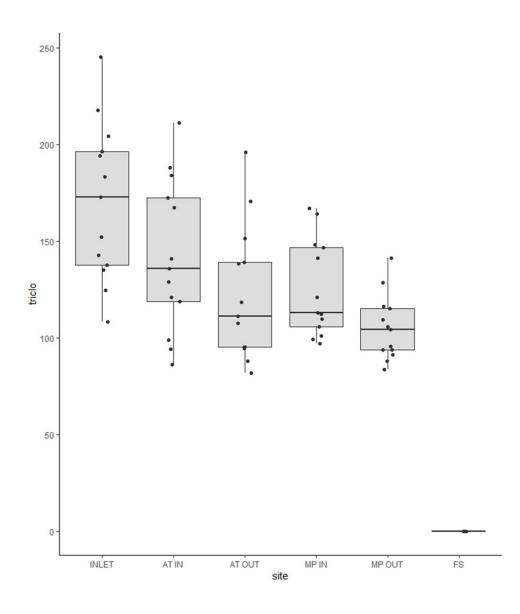


Figure S18: The y-axis represents the concentration of triclosan at different sites in the STP over the sampling period. The x-axis represents different sampling locations within the targeted STP: IN – Grit chamber outlet (n=13), ATIN – Inlet of aeration tank i.e., sample collected post primary treatment (n=13), ATOUT – Outlet of aeration tank (n=13), MPIN – Inlet of maturation pond (n=13), and MPOUT – Outlet of maturation pond and (n=13), FS – Final sludge collected after the drying of the anaerobically digested sludge (n=3).

References

- 1. Suzuki, M. T., Taylor, L. T. & DeLong, E. F. Quantitative analysis of small-subunit rRNA genes in mixed microbial populations via 5'-nuclease assays. *Appl Environ Microbiol* **66**, 4605–4614 (2000).
- Johnning, A., Kristiansson, E., Fick, J., Weijdegård, B. & Larsson, D. G. J. Resistance mutations in gyrA and parC are common in Escherichia communities of both fluoroquinolone-polluted and uncontaminated aquatic environments. *Front Microbiol* 6, 160210 (2015).
- Swick, M. C., Morgan-Linnell, S. K., Carlson, K. M. & Zechiedrich, L. Expression of Multidrug Efflux Pump Genes acrAB-tolC, mdfA, and norE in Escherichia coli Clinical Isolates as a Function of Fluoroquinolone and Multidrug Resistance. *Antimicrob Agents Chemother* 55, 921 (2010).
- 4. Yamane, K., Wachino, J. I., Suzuki, S. & Arakawa, Y. Plasmid-mediated qepA gene among Escherichia coli clinical isolates from Japan. *Antimicrob Agents Chemother* **52**, 1564–1566 (2008).
- 5. Chen, J., Yu, Z., Michel, F. C., Wittum, T. & Morrison, M. Development and Application of Real-Time PCR Assays for Quantification of erm Genes Conferring Resistance to Macrolides-Lincosamides-Streptogramin B in Livestock Manure and Manure Management Systems. *Appl Environ Microbiol* **73**, 4407 (2007).
- 6. Klein, G. *et al.* Exclusion of vanA, vanB and vanC type glycopeptide resistance in strains of Lactobacillus reuteri and Lactobacillus rhamnosus used as probiotics by polymerase chain reaction and hybridization methods. *J Appl Microbiol* **89**, 815–824 (2000).
- 7. Heath, R. J., Li, J., Roland, G. E. & Rock, C. O. Inhibition of the Staphylococcus aureus NADPH-dependent enoyl-acyl carrier protein reductase by triclosan and hexachlorophene. *J Biol Chem* **275**, 4654–4659 (2000).
- 8. Gharajalar, S. N. & Sofiani, V. H. Patterns of Efflux Pump Genes Among Tetracycline Resistance Uropathogenic Escherichia coli Isolates Obtained From Human Urinary Infections. *Jundishapur Journal of Microbiology 2017 10:2* **10**, (2017).
- 9. Aminov, R. I., Garrigues-Jeanjean, N. & Mackie, R. I. Molecular ecology of tetracycline resistance: development and validation of primers for detection of tetracycline resistance genes encoding ribosomal protection proteins. *Appl Environ Microbiol* **67**, 22–32 (2001).
- 10. Hasman, H. & Aarestrup, F. M. tcrb, a gene conferring transferable copper resistance in Enterococcus faecium: Occurrence, transferability, and linkage to macrolide and glycopeptide resistance. *Antimicrob Agents Chemother* **46**, 1410–1416 (2002).
- Zhu, L., Lin, J., Ma, J., Cronan, J. E. & Wang, H. Triclosan Resistance of *Pseudomonas aeruginosa* PAO1 Is Due to FabV, a Triclosan-Resistant Enoyl-Acyl Carrier Protein Reductase. *Antimicrob Agents Chemother* 54, 689–698 (2010).

- 12. Luo, Y. *et al.* Cadmium resistance, microbial biosorptive performance and mechanisms of a novel biocontrol bacterium Paenibacillus sp. LYX-1. *Environmental Science and Pollution Research* **29**, 68692–68706 (2022).
- Adekanmbi, A. O., Adelowo, O. O., Okoh, A. I. & Fagade, O. E. Metal-resistance encoding gene-fingerprints in some bacteria isolated from wastewaters of selected printeries in Ibadan, South-western Nigeria. *Journal of Taibah University for Science* 13, 266–273 (2019).
- Lee, M. F., Peng, C. F., Hsu, H. J. & Toh, H. S. Use of Inverse PCR for Analysis of Class 1 Integrons Carrying an Unusual 3' Conserved Segment Structure. *Antimicrob Agents Chemother* 55, 943 (2010).
- 15. Stegger, M. *et al.* Rapid detection, differentiation and typing of methicillin-resistant Staphylococcus aureus harbouring either mecA or the new mecA homologue mecALGA251. *Clinical Microbiology and Infection* **18**, 395–400 (2012).
- 16. Escherichia coli strain IHIT37248 phosphoethanolamine--lipid A transfe Nucleotide NCBI. https://www.ncbi.nlm.nih.gov/nucleotide/2565984364.
- Göpel, L. *et al.* Occurrence of Mobile Colistin Resistance Genes mcr-1–mcr-10 including Novel mcr Gene Variants in Different Pathotypes of Porcine Escherichia coli Isolates Collected in Germany from 2000 to 2021. *Appl Microbiol* 4, 70–84 (2024).
- Hembach, N. *et al.* Occurrence of the mcr-1 Colistin Resistance Gene and other Clinically Relevant Antibiotic Resistance Genes in Microbial Populations at Different Municipal Wastewater Treatment Plants in Germany. *Front Microbiol* 8, (2017).
- 19. Köhler, T. *et al.* Characterization of MexE-MexF-OprN, a positively regulated multidrug efflux system of Pseudomonas aeruginosa. *Mol Microbiol* **23**, 345–354 (1997).
- 20. Wajid, M., Saleemi, M. K., Sarwar, Y. & Ali, A. Detection and characterization of multidrug-resistant Salmonella enterica serovar Infantis as an emerging threat in poultry farms of Faisalabad, Pakistan. *J Appl Microbiol* **127**, 248–261 (2019).
- 21. Scoulica, E., Aransay, A. & Tselentis, Y. Molecular characterization of the OXA-7 βlactamase gene. *Antimicrob Agents Chemother* **39**, 1379–1382 (1995).
- Bert, F., Branger, C. & Lambert-Zechovsky, N. Identification of PSE and OXA βlactamase genes in Pseudomonas aeruginosa using PCR–restriction fragment length polymorphism. *Journal of Antimicrobial Chemotherapy* 50, 11–18 (2002).
- Wu, J. J., Ko, W. C., Tsai, S. H. & Yan, J. J. Prevalence of Plasmid-Mediated Quinolone Resistance Determinants QnrA, QnrB, and QnrS among Clinical Isolates of Enterobacter cloacae in a Taiwanese Hospital. *Antimicrob Agents Chemother* 51, 1223 (2007).
- 24. Goodman, R. N., Tansirichaiya, S. & Roberts, A. P. Development of pBACpAK entrapment vector derivatives to detect intracellular transfer of mobile genetic elements within chloramphenicol resistant bacterial isolates. *J Microbiol Methods* **213**, 106813 (2023).

- 25. Climate Monitoring and Prediction Group. https://www.imdpune.gov.in/cmpg/Griddata/Rainfall_25_NetCDF.html.
- 26. https://www.imdpune.gov.in/cmpg/Griddata/ref_paper_MAUSAM.pdf.