Supplementary information

Supplementary table 1: GATC sites partially or fully non-methylated during log-to-stationary phase transition in LB or over the course of ampicillin treatment.

		Site	Position	Coverage^b	Genetic arrangement				Ref.
					Inter-/intragenic	Upstream gene	Downstream gene	Overlapping protein binding site	
Methylation pattern over time	**Methylation increases**	**1**	1031046(+) 1031047(-)	104 ± 24 106 ± 24	intergenic	serT (-)	hyaA (+)	-	-
		2	1218081(+) 1218082(-)	103 ± 24 99 ± 22	intergenic	ycgG (+)	ymgF (+)	-	-
		3	1528561(+) 1528562(-)	97 ± 21 99 ± 21	intergenic	ydcD (+)	yncI (+)	-	-
		4	3740678(+) 3740679(-)	158 ± 35 157 ± 35	intergenic	yiaJ (-)	yiaK(+)	R- Pol*	-
		5	3873329(+) 3873330(-)	155 ± 35 155 ± 34	intergenic	dgoR (+)	yidX (+)	-	-
	Methylation decreases	**6**	1099423(+) 1099424(-)	100 ± 20 101 ± 18	intergenic	ycdY (+)	ycdZ (+)	CRP	[1]
		7	1168222(+) 1168223(-)	103 ± 21 105 ± 23	intergenic	comR (-)	bhsA (+)	CRP	-
		8	2229801(+) 2229802(-)	106 ± 18 108 ± 15	intergenic	yohK (+)	cdd (+)	CRP	[1-3]
		9	3490456(+) 3490457(-)	154 ± 32 150 ± 31	intergenic	ppiA (-)	tgsA (+)	CRP	-
		10	3769994(+) 3769995(-)	158 ± 34 158 ± 34	intergenic	yibL (-)	mltA (+)	CRP	-
	Non-methylated	**11**	1168246(+) 1168247(-)	105 ± 23 106 ± 23	intergenic	comR (-)	bhsA (+)	ComR*	[1]
		12	1653239(+) 1653240(-)	95 ± 20 95 ± 20	intergenic	rpsA (+)	ynfA (+)	Yhdf*	[1,2,4]
		13	2823768(+) 2823769(-)	119 ± 21 121 ± 24	intergenic	mltB (+)	gutA (+)	CRP; GutR*	[1,2,4]
		14	4099564(+) 4099565(-)	150 ± 35 150 ± 35	intergenic	sodA (+)	kdgT (+)	-	-
	Hemimethylated	**15**	3371659(+) 3371660(-)	142 ± 29 142 ± 27	intergenic	-- (+)	-nR (+)	Fis	-
		16	4501643(+) 4501644(-)	145 ± 32 144 ± 32	intergenic	insG (+)	yjhB (+)	-nR*	-
		17	4537968(+) 4537969(-)	152 ± 26 150 ± 25	intergenic	-nC (-)	fimB (+)	-nR*	-
	Other	**18**	621451(+) 621452(-)	113 ± 21 115 ± 23	intergenic	fepD (-)	entS (+)	Fur	-
		19	1840399(+) 1840400(-)	100 ± 22 100 ± 21	intragenic	gdhA (+)	gdhA(+)	-	-

^{a}Base pairs and strand; ^{b}average sequencing coverage ± SD, n = 2–3 replicates; *protein binding sites is within 10 bp of methylation site. Sites are grouped based on methylation pattern over time in untreated *E. coli*.

Supplementary table 2: Statistical analysis of differential methylation in LB versus ampicillin treatment at selected GATC sites from Fig. 2d.

		P-values (undjusted) [b]				P-values (multiplicity adjusted) [c]			
Site	**Position** [a]	**1h**	**2h**	**3h**	**4h**	**1h**	**2h**	**3h**	**4h**
1	1031046(+)	0.491	0.937	0.011	0.219	0.833	1.000	0.887	0.886
	1031047(-)	0.174	0.883	0.273	0.281	0.833	1.000	0.887	0.886
2	1218081(+)	0.008	0.708	0.455	0.455	0.833	0.952	0.887	0.886
	1218082(-)	0.621	0.894	0.492	0.546	0.859	1.000	0.887	0.887
3	1528561(+)	0.570	0.327	0.364	0.492	0.843	0.937	0.887	0.886
	1528562(-)	0.478	0.931	0.636	0.328	0.833	1.000	0.890	0.886
4	3740678(+)	0.980	0.345	0.342	0.193	1.000	0.937	0.887	0.886
	3740679(-)	-	0.423	0.398	0.001	1.000	0.937	0.887	0.886
5	3873329(+)	-	0.630	0.759	0.166	1.000	0.938	0.932	0.886
	3873330(-)	0.423	0.184	0.499	0.064	0.833	0.937	0.887	0.886
6	1099423(+)	0.386	0.832	0.423	0.112	0.833	0.987	0.887	0.886
	1099424(-)	0.999	-	-	0.500	1.000	1.000	1.000	0.886
7	1168222(+)	0.939	0.423	-	0.848	1.000	0.937	1.000	0.980
	1168223(-)	0.594	0.990	0.185	0.971	0.850	1.000	0.887	1.000
8	2229801(+)	0.243	0.500	0.423	0.209	0.833	0.937	0.887	0.886
	2229802(-)	0.412	0.593	0.846	0.346	0.833	0.937	0.961	0.886
9	3490456(+)	0.074	0.645	0.389	0.755	0.833	0.939	0.887	0.948
	3490457(-)	0.387	0.694	0.837	0.003	0.833	0.949	0.957	0.886
10	3769994(+)	0.423	-	-	-	0.833	1.000	1.000	1.000
	3769995(-)	0.542	0.500	0.423	-	0.835	0.937	0.887	1.000
11	1168246(+)	-	0.500	0.423	0.423	1.000	0.937	0.887	0.886
	1168247(-)	0.423	0.864	0.186	0.127	0.833	0.997	0.887	0.886
12	1653239(+)	0.541	-	-	0.500	0.835	1.000	1.000	0.886
	1653240(-)	-	-	-	-	1.000	1.000	1.000	1.000
13	2823768(+)	0.886	0.423	-	-	0.977	0.937	1.000	1.000
	2823769(-)	0.554	-	-	-	0.838	1.000	1.000	1.000
14	4099564(+)	-	-	-	0.500	1.000	1.000	1.000	0.886
	4099565(-)	-	-	-	0.500	1.000	1.000	1.000	0.886
15	3371659(+)	0.838	0.860	0.423	0.423	0.955	0.995	0.887	0.886
	3371660(-)	0.423	0.423	0.500	0.603	0.833	0.937	0.887	0.894
16	4501643(+)	0.199	0.849	0.127	0.638	0.833	0.993	0.887	0.903
	4501644(-)	-	-	-	-	1.000	1.000	1.000	1.000
17	4537968(+)	0.441	0.423	-	0.423	0.833	0.937	1.000	0.886
	4537969(-)	0.423	0.901	0.507	0.508	0.833	1.000	0.887	0.886
18	621451(+)	0.671	0.136	0.191	0.005	0.879	0.937	0.887	0.886
	621452(-)	0.803	0.185	0.013	0.001	0.939	0.937	0.887	0.886
19	1840399(+)	0.873	0.319	0.033	0.106	0.971	0.937	0.887	0.886
	1840400(-)	0.489	0.532	0.000	0.311	0.833	0.937	0.004	0.886

[a] Base pairs and strand; [b] p-value determined using a two-tailed heteroskedastic (unequal variances) t-test; [c] p-value adjusted for multiple hypothesis testing on the whole dataset using Benjamini-Hochberg false discovery rate correction; for sites with no change in frac between LB and ampicillin treatment the t-test cannot be performed and the adjusted p-value is 1 (non-significant); cells in red, $p<0.05$.

Supplementary table 3: Quinolone-resistance conferring mutations in Cipro^R UPEC clinical isolate

Gene	Function	Mutation	Ref.
gyrA	D- gyrase subunit A	S83L	5
		D87N	
parC	D- topoisomerase IV, subunit A	S80I	
parE	D- topoisomerase IV, subunit B	S458A	6
marR	Activator of multidrug efflux system	G103S	
		Y137H	

Supplementary references

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