SUPPLEMENTARY MATERIAL

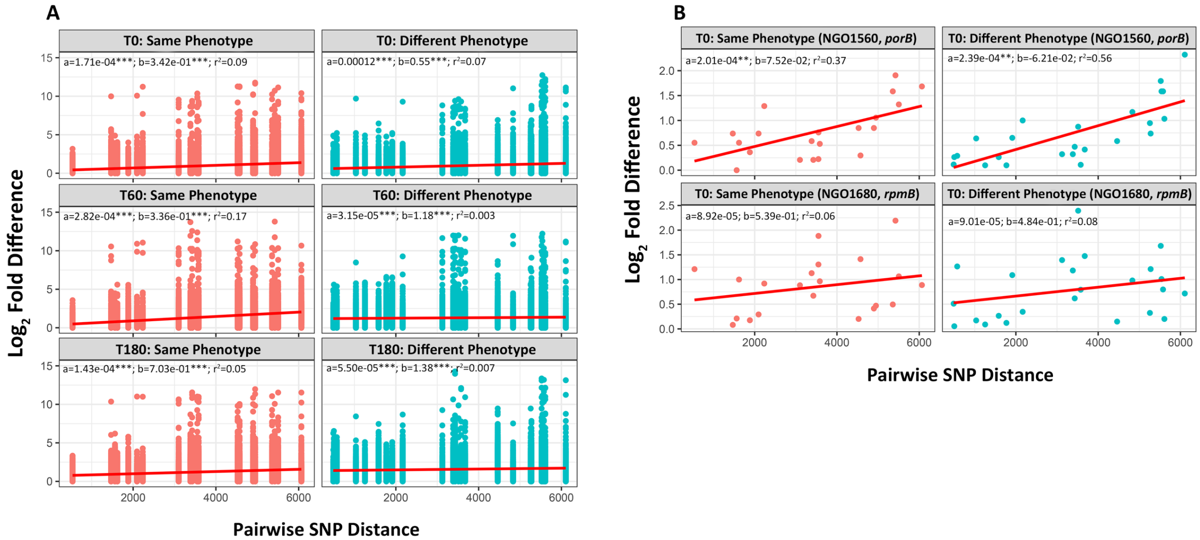

Figure S1. Transcriptome-wide differentially transcriptional regulation as a function of genetic distance in ten phylogenetically diverse isolates. (A) For all ten isolates for which we collected RNA-seq libraries, the number of pairwise SNP differences were compared to the per-transcript pairwise differences in gene expression at baseline (T0, no drug), after 60 minutes of drug exposure (T60), and after 180 minutes of drug exposure (T180). Regression lines were fit to either paired isolate comparisons that belonged to the same (resistant *vs.* resistant or susceptible *vs.* susceptible) or different (resistant *vs.* susceptible) phenotypic classes; and in all cases we observed positive and significant slope values. (B) For the candidate ciprofloxacin diagnostic *porB* (NGO1560) and *rpmB* (NGO1680) (1), there were generally stronger correlations and positive relationships.

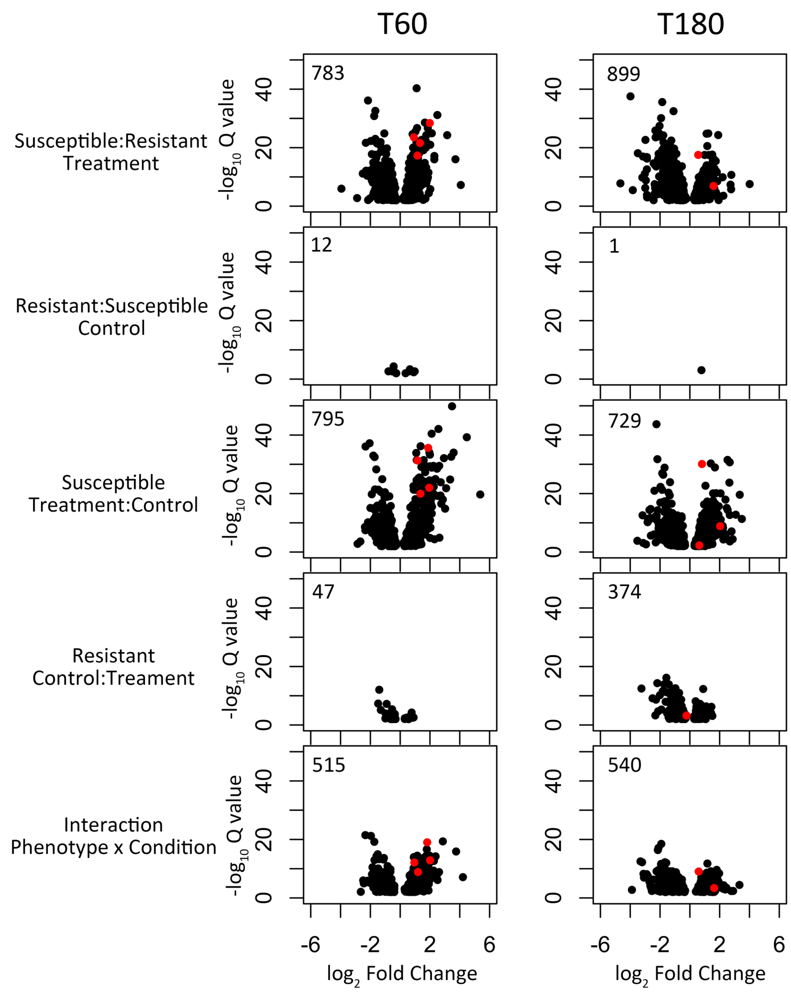

Figure S2. Volcano plots displaying the number of significantly differentially expressed transcripts (FDR cutoff ≤ 0.01) for each contrast of interest between the control to 60-minute and 180-minute azithromycin exposure conditions (black circles). The majority of transcripts were significantly differential expressed between resistant and susceptible isolates in the treatment conditions, and between the control and treatment conditions in susceptible isolates. Many transcripts also had a significant interaction term, suggesting that they had a different response trajectory between resistant and susceptible isolates across conditions. There was a high level of transcriptional response to azithromycin exposure in both the 60-minute and 180-minute treatments. Thus, diagnostic markers were selected from the earlier 60-minute exposure. These markers included: NGO0191, NGO1079, NGO1577, and NGO1829 (red circles) which all had highly significant model terms indicative of differential expression between phenotypes in the treatment condition, expression change across condition in susceptible isolates, and an interaction term.

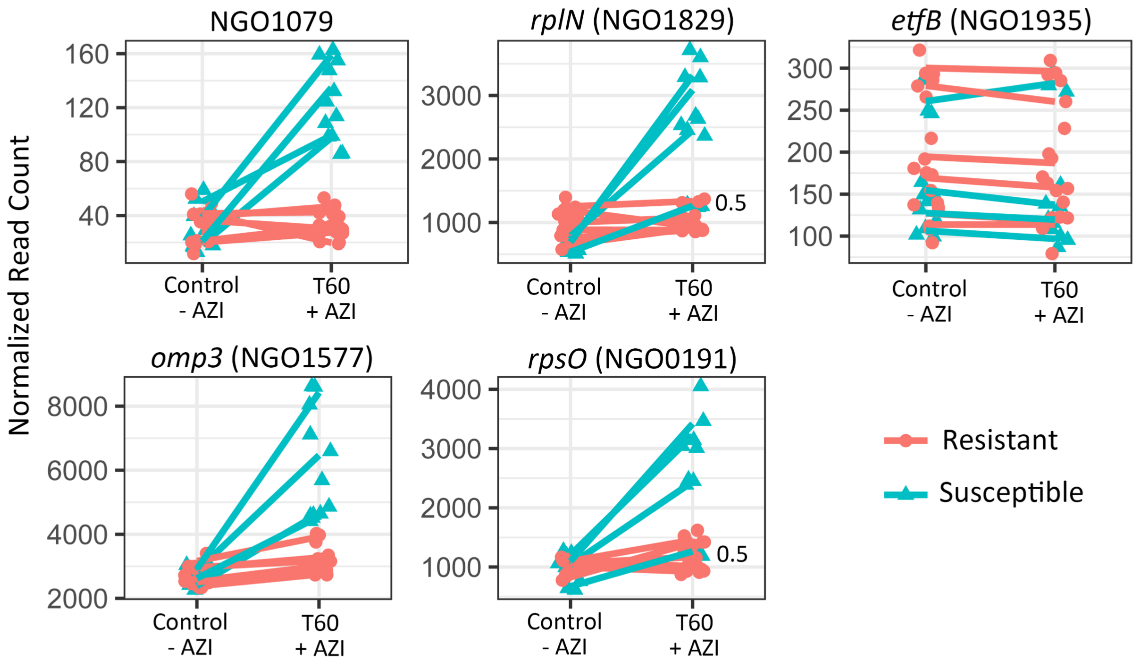

Figure S3. RNA-seq expression patterns for azithromycin diagnostic NGO0191, NGO1079, NGO1577, NGO1829 markers and the control NGO1935 marker. Read counts normalized by library size are plotted against the azithromycin exposure condition. The control condition was sampled prior to the addition of azithromycin to the culture media and the treatment condition was 60-minutes post exposure. Expression profiles across conditions are shown for the four susceptible isolates and six resistant isolates for which we collected RNA-seq data. All diagnostic markers had highly significant model terms indicative of differential expression between phenotypes in the treatment condition, expression change across condition in susceptible isolates, and an interaction term; though we did observe that isolates with intermediate MICS (i.e., 0.5 µg/ml) sometimes were more phenotypically similar to resistant or susceptible isolates dependent on the marker. The control NGO1935 gene was selected by the absence of significance in any of the tested glm contrasts.

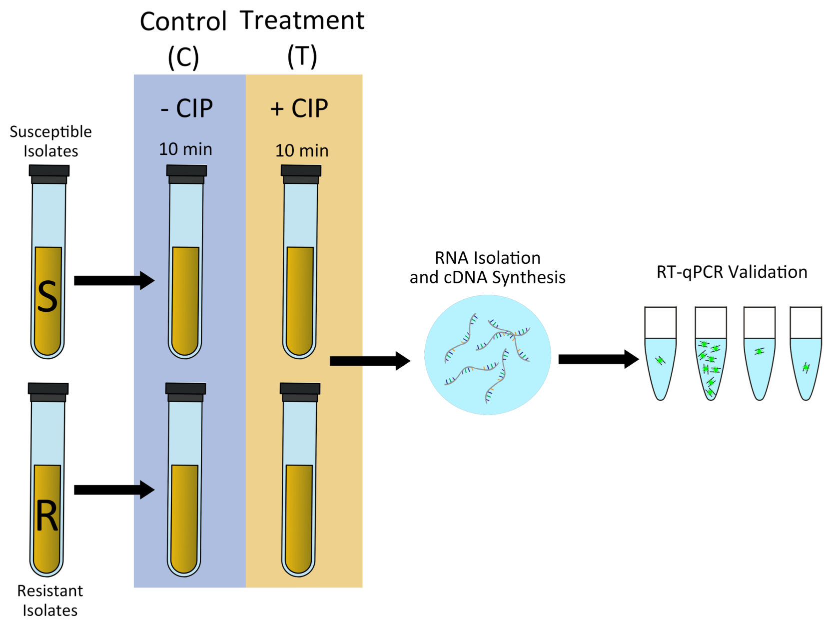

Figure S4. Workflow for validating ciprofloxacin resistance diagnostic markers. Susceptible (MIC < 1 μg/ml) and resistant (MIC ≥ 1 μg/ml) isolates were cultured in liquid GCP supplemented with 1% IsoVitaleX and 0.042% sodium bicarbonate. Paired cultures for each strain were either exposed to 0.5 μg/ml ciprofloxacin or unexposed, and then sampled after 10 minutes. RNA was isolated using the Direct-Zol kit and the SuperScript IV reverse transcriptase was used for first-strand cDNA synthesis. We then tested for differences in fold-change between condition across a panel of isolates using the nominated *porB* and *rpmB* (1) in reference to the control 16s rRNA gene.

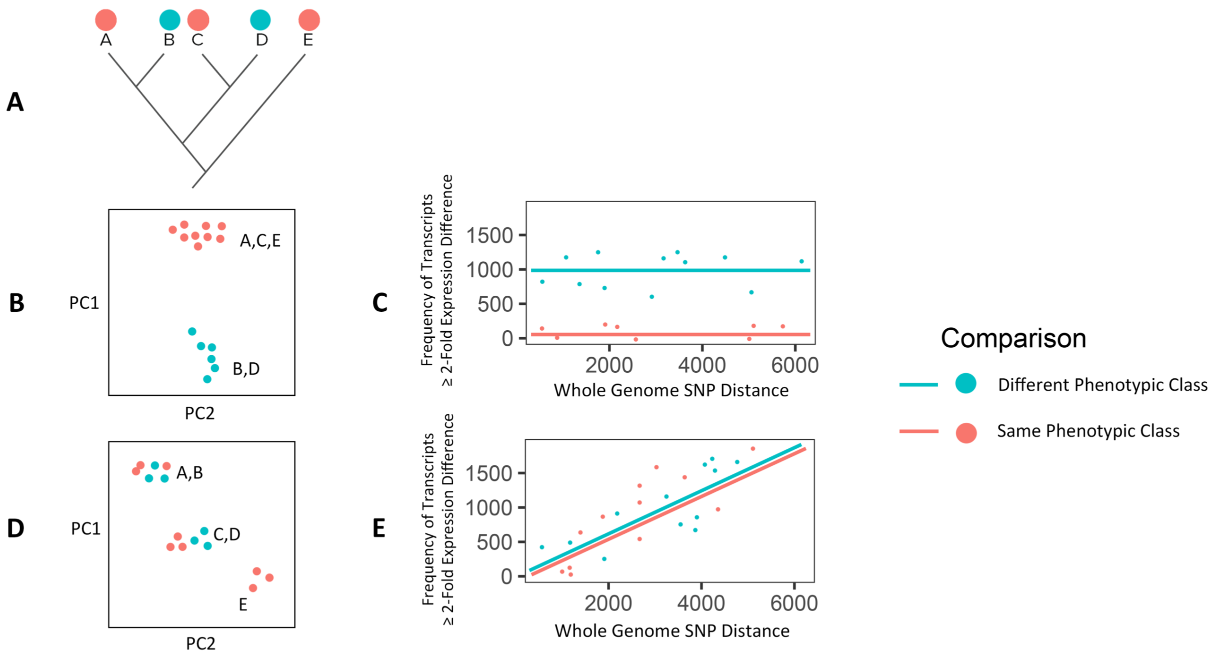

Figure S5. Contrasting the impacts of phenotypic class or genetic distance as the main dimension in driving transcriptional regulation. Here, we hypothesize that there are two major dimensions that may drive the majority of variance in transcriptional regulation between isolates: either phenotypic class (drug resistant *vs*. susceptible), or evolutionary time. (A) Given a phylogeny of a hypothetical population with branches indicating evolutionary distance and colors representing resistant (red) and susceptible isolates (blue): (B) if resistance phenotype is the major component driving transcriptional variation, isolates will transcriptionally cluster by phenotype as they are physiologically responding to the environment in the same way, and (C) regardless of the genetic distance of isolates, paired comparisons of isolates of the same phenotypes will show very little differences in transcriptional regulation, and paired comparisons of isolates of different phenotypes will display greater divergence in regulation of their transcriptomes. (D,E) If genetic distance is the major factor impacting transcriptional regulation, we expect that with increasing evolutionary time isolates will display increasing levels of differential transcriptome regulation.

Table S1: RT-qPCR primers and cycling conditions

Marker	Type	Forward Primer (5'-3')	Reverse Primer (5'-3')	Annealing Temperature	Reference
NGO1935	Control	GCGCAAGGTACATTTGTTTCA	CAGGCAGTTTCAACGCAATC	61	This study
NGO1079	Test	GGAAAACTCCGTATTGCCGC	GTTCCTGACGCGCAAGATTT	61	This study
NGO1577	Test	TGGCTTTAGAGGCTTTCGCA	AACGCCTGAGTCGAACCAAT	61	This study
NGO1829	Test	CGCTCAGTACGCAATTCACG	GTACTGCTAAGGGTGTGCGT	61	This study
NGO0191	Test	AACCCCAAAGACCACCACAG	CAGACCCAAGCGGGTAATCA	61	This study
16s rRNA	Control	ACTGCGTTCTGAACTGGGTG	GGCGGTCAATTTCACGCG	60	Khazaei et al. (2018)
porB	Test	GCTACGATTCTCCCGAATTTGCC	CCGCCKACCAAACGGTGAAC	64	Khazaei et al. (2018)
rpmB	Test	TTGCCCAACTTGCAATCACG	AGCACGCAAATCAGCCAATAC	60	Khazaei et al. (2018)

Table S2. Average 2-△△CT values (n=3-4 replicates) for marker loci normalized to NGO1935

Locus	Average ddCt	Strain	MIC	Phenotype	Drug Exposure
NGO1560	1.83	GCGS1019	16	R	ciprofloxacin
NGO1560	0.77	GCGS0850	1	R	ciprofloxacin
NGO1560	0.61	GCGS0481	32	R	ciprofloxacin
NGO1560	1.04	GCGS0562	4	R	ciprofloxacin
NGO1560	1.25	GCGS0099	16	R	ciprofloxacin
NGO1560	6.1	GCGS0432	0.03	S	ciprofloxacin
NGO1560	19.27	GCGS0641	0.03	S	ciprofloxacin
NGO1560	1.42	GCGS0791	0.03	S	ciprofloxacin
NGO1560	1.27	GCGS0709	0.06	S	ciprofloxacin
NGO1560	3.91	GCGS0575	0.03	S	ciprofloxacin
NGO1560	2.87	GCGS0870	0.25	S	ciprofloxacin
NGO1560	1.4	GCGS1029	0.125	S	ciprofloxacin
NGO1560	1.54	GCGS0997	16	R	ciprofloxacin
NGO1560	1.84	GCGS0922	8	R	ciprofloxacin
NGO1560	1.86	GCGS1007	16	R	ciprofloxacin
NGO1560	0.36	GCGS0862	8	R	ciprofloxacin
NGO1560	1.23	GCGS0864	8	R	ciprofloxacin
NGO1560	1.04	GCGS0985	16	R	ciprofloxacin
NGO1560	5.2	GCGS0550	0.015	S	ciprofloxacin
NGO1560	5.67	GCGS0338	0.015	S	ciprofloxacin
NGO1560	3.24	GCGS0354	0.015	S	ciprofloxacin
NGO1560	2.79	GCGS0574	0.03	S	ciprofloxacin
NGO1560	13.84	GCGS0745	0.015	S	ciprofloxacin
NGO1680	3.8	GCGS1019	16	R	ciprofloxacin
NGO1680	14.16	GCGS0850	1	R	ciprofloxacin
NGO1680	2.2	GCGS0481	32	R	ciprofloxacin
NGO1680	15.68	GCGS0562	4	R	ciprofloxacin
NGO1680	3.63	GCGS0099	16	R	ciprofloxacin
NGO1680	2.26	GCGS0432	0.03	S	ciprofloxacin
NGO1680	1.35	GCGS0641	0.03	S	ciprofloxacin
NGO1680	0.17	GCGS0791	0.03	S	ciprofloxacin
NGO1680	1.33	GCGS0709	0.06	S	ciprofloxacin
NGO1680	5.62	GCGS0575	0.03	S	ciprofloxacin
NGO1680	16.61	GCGS0870	0.25	S	ciprofloxacin
NGO1680	0.16	GCGS1029	0.125	S	ciprofloxacin
NGO1680	0.44	GCGS0997	16	R	ciprofloxacin
NGO1680	2.53	GCGS0922	8	R	ciprofloxacin
NGO1680	1.14	GCGS1007	16	R	ciprofloxacin
NGO1680	0.33	GCGS0862	8	R	ciprofloxacin
NGO1680	1.7	GCGS0864	8	R	ciprofloxacin
NGO1680	2.36	GCGS0985	16	R	ciprofloxacin
NGO1680	2.98	GCGS0550	0.015	S	ciprofloxacin
NGO1680	7.4	GCGS0338	0.015	S	ciprofloxacin

NGO1680	10.34	GCGS0354	0.015	S	ciprofloxacin
NGO1680	4.43	GCGS0574	0.03	S	ciprofloxacin
NGO1680	10.32	GCGS0745	0.015	S	ciprofloxacin
NGO0191	3.55	GCGS0524	0.25	S	azithromycin
NGO0191	0.25	GCGS0550	8	R	azithromycin
NGO0191	1.04	GCGS0354	8	R	azithromycin
NGO0191	1.13	GCGS0745	256	R	azithromycin
NGO0191	10.18	GCGS0353	0.03	S	azithromycin
NGO0191	0.74	GCGS0363	4	R	azithromycin
NGO0191	1.60	GCGS0276	1	R	azithromycin
NGO0191	1.29	GCGS0834	2	R	azithromycin
NGO0191	3.83	GCGS0641	0.25	S	azithromycin
NGO0191	3.63	GCGS0996	0.5	S	azithromycin
NGO0191	0.62	GCGS0525	2	R	azithromycin
NGO0191	4.55	GCGS0313	0.06	S	azithromycin
NGO0191	0.72	GCGS1026	16	R	azithromycin
NGO0191	2.93	GCGS1035	0.5	S	azithromycin
NGO0191	2.68	GCGS0605	0.25	S	azithromycin
NGO0191	3.92	GCGS0926	0.125	S	azithromycin
NGO0191	1.48	GCGS0436	2	R	azithromycin
NGO0191	3.47	GCGS0465	0.06	S	azithromycin
NGO0191	1.01	GCGS0481	2	R	azithromycin
NGO0191	0.50	GCGS0298	2	R	azithromycin
NGO1079	2.36	GCGS0524	0.25	S	azithromycin
NGO1079	0.17	GCGS0550	8	R	azithromycin
NGO1079	1.31	GCGS0354	8	R	azithromycin
NGO1079	0.50	GCGS0745	256	R	azithromycin
NGO1079	9.65	GCGS0353	0.03	S	azithromycin
NGO1079	1.04	GCGS0363	4	R	azithromycin
NGO1079	1.24	GCGS0276	1	R	azithromycin
NGO1079	0.75	GCGS0834	2	R	azithromycin
NGO1079	5.60	GCGS0641	0.25	S	azithromycin
NGO1079	1.36	GCGS0996	0.5	S	azithromycin
NGO1079	0.63	GCGS0525	2	R	azithromycin
NGO1079	3.49	GCGS0313	0.06	S	azithromycin
NGO1079	0.82	GCGS1026	16	R	azithromycin
NGO1079	2.58	GCGS1035	0.5	S	azithromycin
NGO1079	1.77	GCGS0605	0.25	S	azithromycin
NGO1079	2.22	GCGS0926	0.125	S	azithromycin
NGO1079	1.07	GCGS0436	2	R	azithromycin
NGO1079	1.93	GCGS0465	0.06	S	azithromycin
NGO1079	1.08	GCGS0481	2	R	azithromycin
NGO1079	0.81	GCGS0298	2	R	azithromycin
NGO1577	3.85	GCGS0524	0.25	S	azithromycin
NGO1577	0.30	GCGS0550	8	R	azithromycin

NGO1577	0.90	GCGS0354	8	R	azithromycin
NGO1577	1.74	GCGS0745	256	R	azithromycin
NGO1577	3.64	GCGS0353	0.03	S	azithromycin
NGO1577	1.52	GCGS0363	4	R	azithromycin
NGO1577	1.45	GCGS0276	1	R	azithromycin
NGO1577	2.35	GCGS0834	2	R	azithromycin
NGO1577	7.21	GCGS0641	0.25	S	azithromycin
NGO1577	2.44	GCGS0996	0.5	S	azithromycin
NGO1577	1.44	GCGS0525	2	R	azithromycin
NGO1577	7.82	GCGS0313	0.06	S	azithromycin
NGO1577	0.92	GCGS1026	16	R	azithromycin
NGO1577	2.03	GCGS1035	0.5	S	azithromycin
NGO1577	5.44	GCGS0605	0.25	S	azithromycin
NGO1577	6.80	GCGS0926	0.125	S	azithromycin
NGO1577	0.71	GCGS0436	2	R	azithromycin
NGO1577	3.56	GCGS0465	0.06	S	azithromycin
NGO1577	0.46	GCGS0481	2	R	azithromycin
NGO1577	0.84	GCGS0298	2	R	azithromycin
NGO1829	5.38	GCGS0524	0.25	S	azithromycin
NGO1829	0.96	GCGS0550	8	R	azithromycin
NGO1829	1.07	GCGS0354	8	R	azithromycin
NGO1829	1.85	GCGS0745	256	R	azithromycin
NGO1829	23.01	GCGS0353	0.03	S	azithromycin
NGO1829	1.99	GCGS0363	4	R	azithromycin
NGO1829	2.66	GCGS0276	1	R	azithromycin
NGO1829	1.62	GCGS0834	2	R	azithromycin
NGO1829	6.03	GCGS0641	0.25	S	azithromycin
NGO1829	1.49	GCGS0996	0.5	S	azithromycin
NGO1829	0.81	GCGS0525	2	R	azithromycin
NGO1829	3.72	GCGS0313	0.06	S	azithromycin
NGO1829	0.47	GCGS1026	16	R	azithromycin
NGO1829	3.53	GCGS1035	0.5	S	azithromycin
NGO1829	2.11	GCGS0605	0.25	S	azithromycin
NGO1829	3.45	GCGS0926	0.125	S	azithromycin
NGO1829	0.96	GCGS0436	2	R	azithromycin
NGO1829	2.70	GCGS0465	0.06	S	azithromycin
NGO1829	0.77	GCGS0481	2	R	azithromycin
NGO1829	1.25	GCGS0298	2	R	azithromycin

REFERENCES

1. Khazaei T, Barlow JT, Schoepp NG, Ismagilov RF. 2018. RNA markers enable phenotypic test of antibiotic susceptibility in *Neisseria gonorrhoeae* after 10 minutes of ciprofloxacin exposure. Sci Rep 8:11606.