Supplementary Materials

Pharmaceutical Exposure Changed Antibiotic Resistance Genes and Bacterial Communities in Soil-Surface- and Overhead-Irrigated Greenhouse Lettuce

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15 pages, 4 figures and 4 tables

Supplementary Methods

Raw results of ARGs and MGEs were extracted from the WaferGen qPCR software (Version 2.8.1.23). Raw data spreadsheet with cycle numbers (Ct) were imported into R Studio (version 1.1.383) interface in R (Version 3.4.2). Genes were removed if they were only detected once in technical triplicate measurements of a sample. The cutoff threshold of $C_T < 30$ was selected and genes that had no detection or a $C_T > 30$ were then removed. Next, the C_T values from at least two measurements of each sample were averaged. Afterwards if a gene was only detected once in the triplicate pharmaceutical treated samples of the same treatment, the gene was then removed from further analyses. Average C_T values for the genes detected in at least two treatment replicates were computed, which could eliminate potential false positive gene detection. Copy number of genes was calculated via Gene Copy Number = $10^{(30-C_T)/(10/3)}$ (Stedtfeld et al., 2008). Relative abundance of detected genes was computed by dividing the estimated gene copy number with the gene copy number of 16S rRNA. R packages 'tidyr', 'tidyverse', 'dplyr' and a R workflow were used to perform the above data preprocessing. Relative abundance heatmap of genes was plotted using "pheatmap" and "RColorBrewer" packages. Relative abundance heatmap can visualize the abundance and distribution of genes in samples, including ARGs and MGEs. Chord diagram was plotted using "circlize" package and can be used to visualize the most abundant ARGs and MGEs in each sample. UpSet plot of gene intersections of shoots, roots, and soils samples was plotted using "UpSetR" package, which shows the sharing of ARGs and MGEs among all samples. Ordination of ARGs and MGEs was plotted using "Vegan" package based on Bray-Curties distance, indicating the degree of similarity in ARGs and MGEs between different samples.

Bacteria community analysis was first preprocessed using the MacQIIME pipelines v. 1.9. following online tutorial for operational taxonomic unit (OTU) picking (Caporaso et al., 2010). A total of 14,884 bacteria genus were picked up using close reference OTU picking based on a 97% similarity threshold with default uclust to cluster to Greengenes reference database (Caporaso et al., 2010; DeSantis et al., 2006). Bacteria detected at least twice in the triplicates measurements with OTU greater than 0 for total 27 samples in the Illumina MiSeq among triplicates were averaged. Next, bacteria belonging to mitochondria and chloroplast were removed because small subunit ribosomal RNA genes of plant organelles (mitochondria and chloroplast) are easily amplified by PCR and thus contaminate bacterial gene pool. This is because those genes are originated from endosymbiotic bacteria (Lamond, 2002; Sakai and Ikenaga, 2013; Smith and Keeling, 2015). A total 6519 taxa were picked for downstream analysis. The OTU table, taxa table, sample composition table, and tree table were placed into a "phyloseq" dataframe, followed by the downstream analysis using "phyloseq", "vegan", "ggplot2", and "ape" packages (Paradis et al., 2004; Paul and Susan, 2013). Top 10 phyla and families were selected to plot the composition of bacterial communities. Top ten phyla were selected for ordination analysis with singleton (OTU = 1) and doubleton (OTU = 2) removed based on Bray-Curtis distance. We first use multiple constrained and unconstrained ordination methods to analyze the beta diversity of our samples, including detrended correspondence analysis, canonical correspondence analysis, redundancy analysis, detrended principle coordinates analysis, non-metric multidimensional scaling, multidimensional scaling, and principal coordinates analysis (PCoA) (Supplementary Figure S5). PCoA was then selected because it revealed the best position among our samples and made less assumptions in

calculating distance. Alpha diversity was calculated by the Chao1 estimator (Chao, 1984; Hughes et al., 2001) and plotted using "phyloseq" package (Paul and Susan, 2013).

Network analysis among antibiotics concentrations, ARGs/MGEs relative abundance, and percentages of family-level bacterial communities were conducted based on correlation test. First, correlations between antibiotics concentrations and the relative abundance of ARGs/MGEs or bacterial families were performed for the lettuce and soil samples with pharmaceutical exposure (7 averaged measurements respectively). Then, correlations between the relative abundance of ARGs/MGEs and bacterial communities were performed for all samples (13 averaged measurements respectively). We selected ARGs and MGEs detected in more than half samples in the correlation tests to eliminate false positive correlations. Correlation coefficient greater than 0.6 and less than −0.6 with p-value < 0.05 were selected (Supplementary Table S4). The network was plotted using Gephi v0.9.1 software. The 8 antibiotics were included in the network analysis. As acetaminophen and caffeine only inhibited certain bacteria at non-environmentally relevant high concentrations of 756–1516 mg/L and 300–10,000 mg/L, respectively (Al-Janabi, 2010; Al-Janabi, 2011; Verma et al., 2018), they were not considered to be related to microbiomes and ARGs. It was recently reported that at an environmentally relevant concentration (50 μg/L) carbamazepine enhanced horizontal transfer of several plasmid-borne ARGs (Wang et al., 2019). However, our preliminary test found minimal interactions of carbamazepine with ARGs in this study (i.e., negative correlation with only one *mexF* gene). To simplify the network analysis, we only included 8 antibiotics.

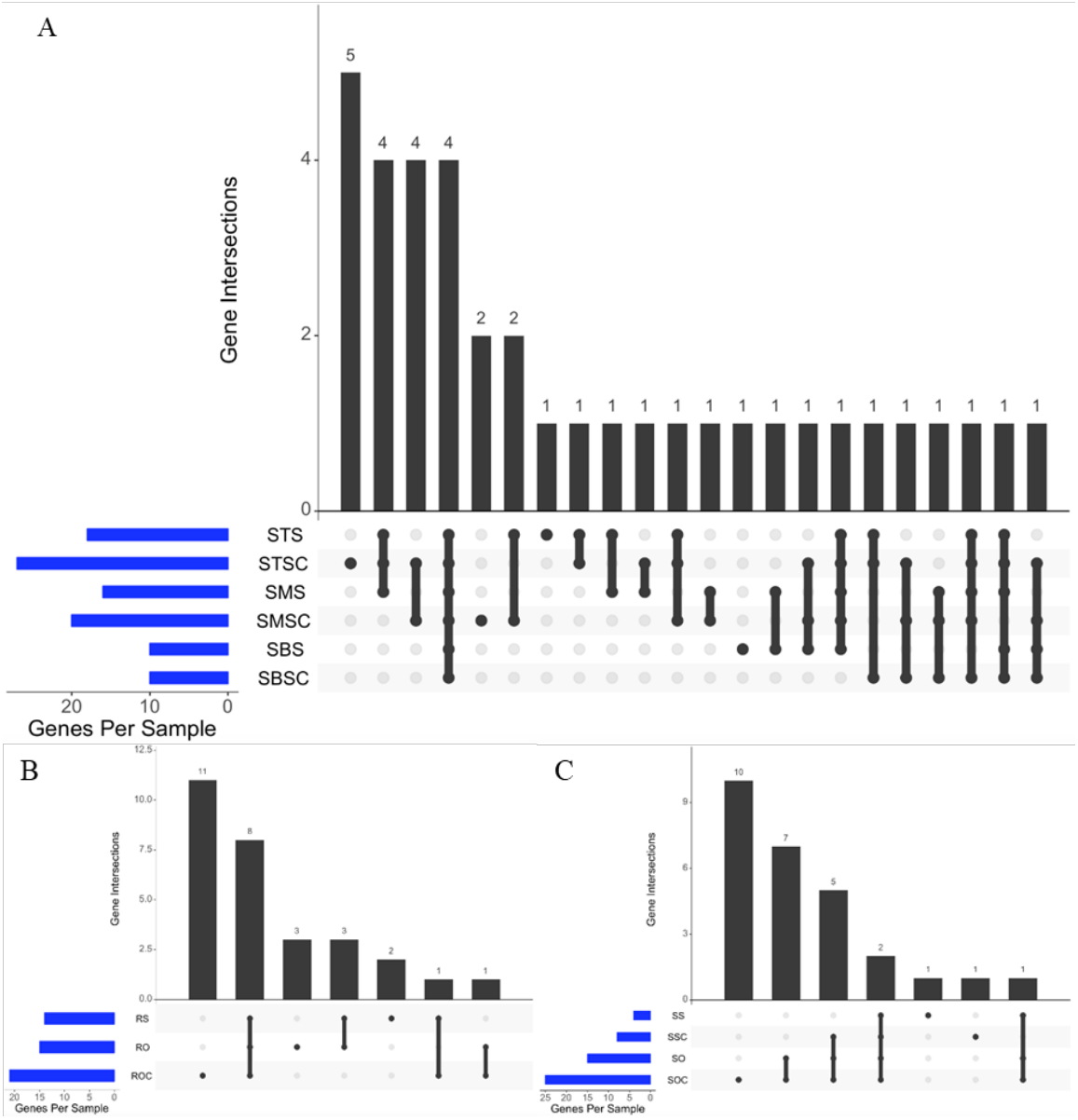

Figure S1. Intersections of antibiotic resistance genes (ARGs) and mobile genetic elements (MGEs) in soil, lettuce root and lettuce shoot samples. Left blue bar charts represent the total count of ARGs in each sample. The right black bar charts represent gene intersection (1-11 genes). The dark black dot highlights the samples of soils, lettuce shoots, and lettuce roots that share certain genes. For example, the three black dots connected in the second column in Figure S1A indicate that there were four commonly shared genes among STS, STSC, and SMS (See Table S1 for sample naming convention).

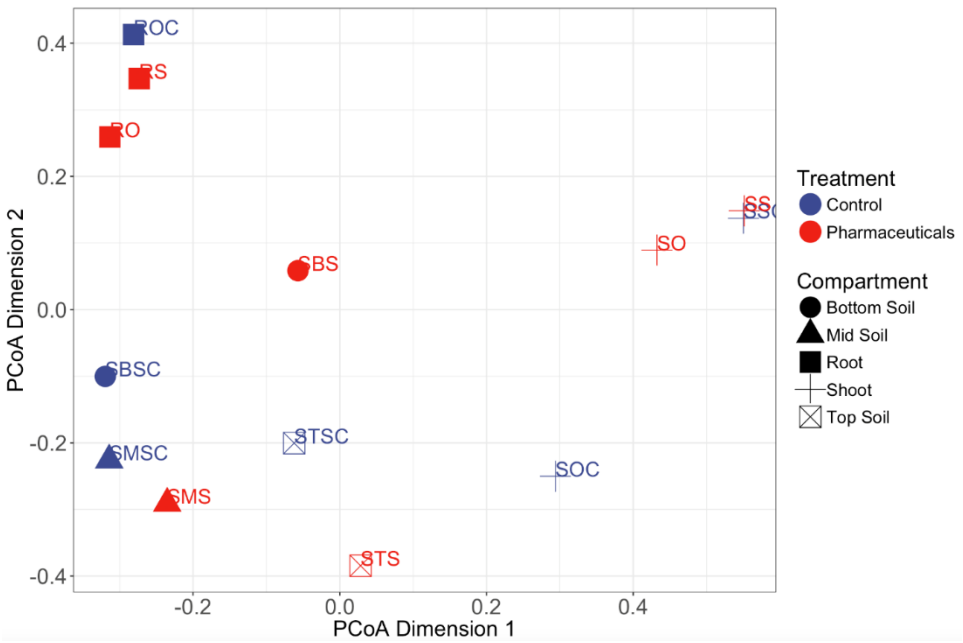

Figure S2. Principal coordinates analysis of antibiotic resistance genes (ARGs) and mobile genetic elements MGEs in the soil, lettuce root and shoot samples based on Bray-Curtis distance.

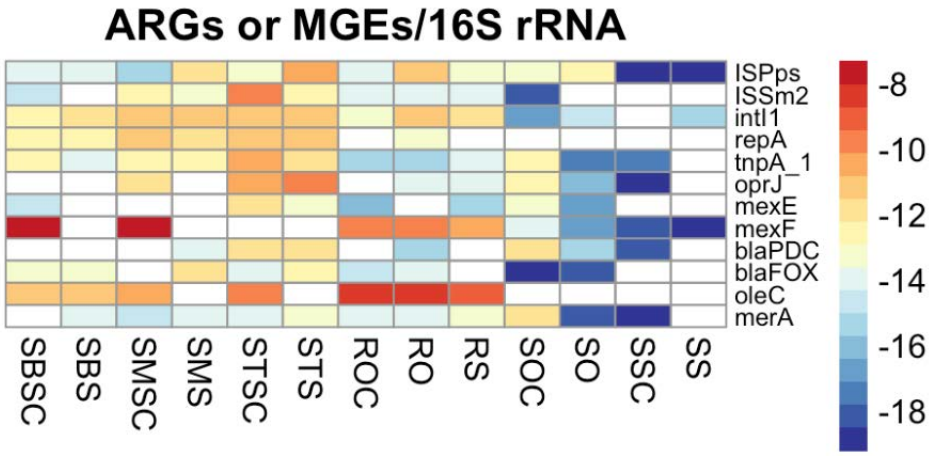

Figure S3. Relative abundance (gene copy number/16s rRNA gene copy number) of antibiotic resistance genes (ARGs) and mobile genetic elements (MGEs) in the soil, lettuce root and shoot samples. Sample naming convention is provided in Table S1. Data were Log 2 transformed. Blank cells represent genes that were either not detected or below detection limit. Color bar on the right means relative abundance from low (blue) to high (red) levels. Top 12 genes were selected based on more than half detection in all samples (> 7/13).

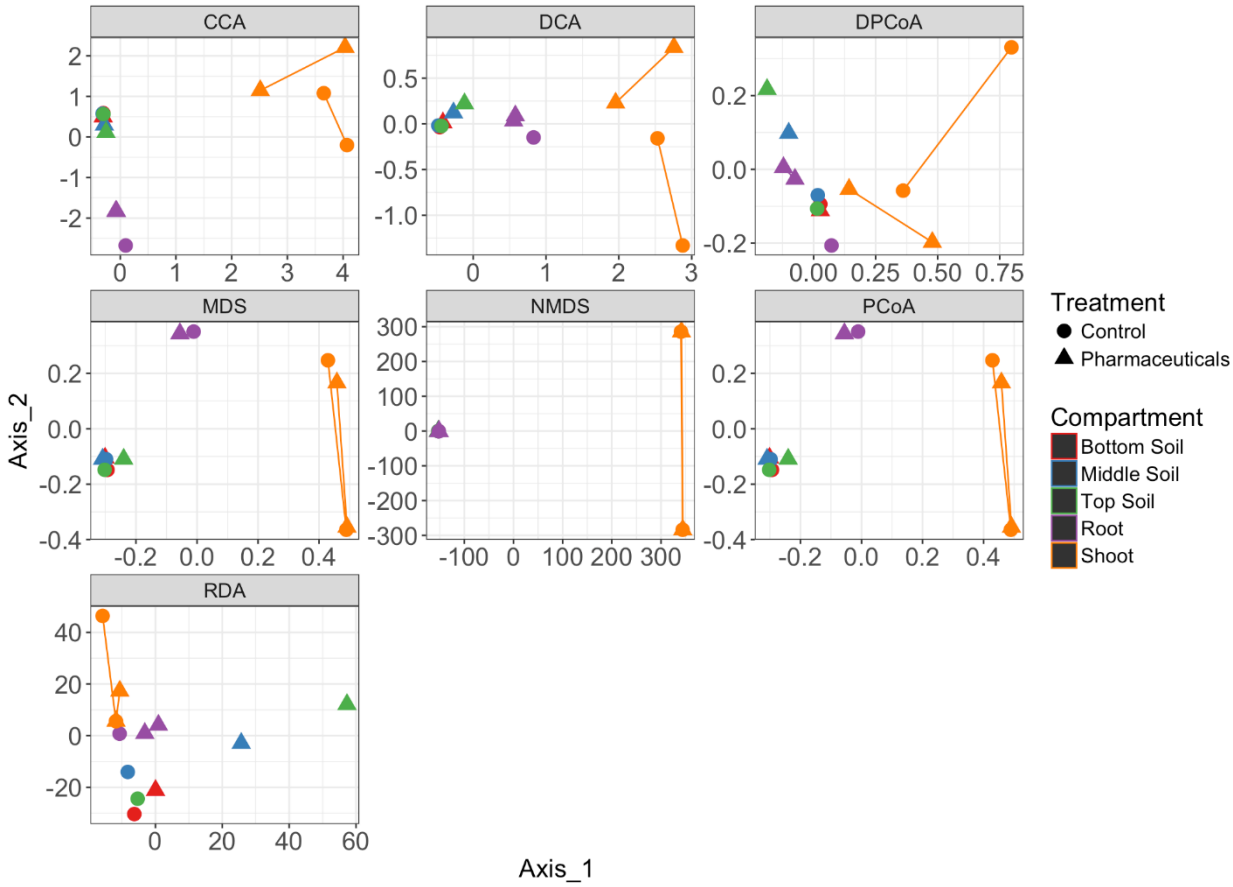

Figure S4. Multivariate analysis of bacterial community based on Bray-Curties distance.

Table S1: Experimental treatment and sample name abbreviation

Sample name	Sample Triplicates	Experimental treatment
STSC	No replicate	Top soil layer for soil-surface irrigation without pharmaceuticals (control treatment)
SMSC	No replicate	Middle soil layer for soil-surface irrigation without pharmaceuticals (control treatment)
SBSC	No replicate	Bottom soil layer for soil-surface irrigation without pharmaceuticals (control treatment)
STS	STS1	Top soil layer for soil-surface irrigation with pharmaceuticals
	STS2	
	STS3	
SMS	SMS1	Middle soil layer for soil-surface irrigation with pharmaceuticals
	SMS2	
	SMS3	
SBS	SBS1	Bottom soil layer for soil-surface irrigation with pharmaceuticals
	SBS2	
	SBS3	
ROC	No replicate	Lettuce root receiving overhead irrigation without pharmaceuticals (control treatment)
RO	RO1	Lettuce root receiving overhead irrigation with pharmaceuticals
	RO2	
	RO3	
RS	RS1	Lettuce shoot receiving soil-surface irrigation with pharmaceuticals
	RS2	
	RS3	
SOC	No replicate	Lettuce shoot receiving overhead irrigation without pharmaceuticals (control treatment)
SSC	No replicate	Lettuce shoot receiving soil-surface irrigation without pharmaceuticals (control treatment)
SO	SO1	Lettuce shoot receiving overhead irrigation with pharmaceuticals
	SO2	
	SO3	
SS	SS1	Lettuce shoot receiving soil-surface irrigation with pharmaceuticals
	SS2	
	SS3	

Table S2. Primer set for 16S rRNA, antibiotic resistance genes (ARGs) and mobile genetic elements (MGEs).

Assay	Name	Forward Primer	Reverse Primer	Target
1	16S rRNA	GGGTTGCGCTCGTTGC	ATGGYTGTCGTCAGCTCGTG	16S rRNA
33	ampC	TCCGGTGACGCGACAGA	CAGCACGCCGGTGAAAGT	Beta Lactam
36	blaPDC	CGCCGTACAACCGGTGAT	GAAGTAATGCGGTTCTCCTTTCA	Beta Lactam
39	bla1	GCAAGTTGAAGCGAAAGAAAAGA	TACCAGTATCAATCGCATATACACCTAA	Beta Lactam
46	cphA	GCGAGCTGCACAAGCTGAT	CGGCCCAGTCGCTCTTC	Beta Lactam
48	blaL1	CACCGGGTTACCAGCTGAAG	GCGAAGCTGCGCTTGTAGTC	Beta Lactam
56	floR	ATTGTCTTCACGGTGTCCGTTA	CCGCGATGTCGTCGAACT	Amphenicol
64	emrD	CTCAGCAGTATGGTGGTAAGCATT	ACCAGGCGCCGAAGAAC	MDR[a]
72	vanC	AAATCAATACTATGCCGGGCTTT	CCGACCGCTGCCATCA	Vancomycin
89	mexA	AGGACAACGCTATGCAACGAA	CCGGAAAGGGCCGAAAT	MDR[a]
93	aac3-VI	CGTCACTTATTCGATGCCCTTAC	GTCGGGCGCGGCATA	Aminoglycoside
111	blaCMY	GCGAGCAGCCTGAAGCA	CGGATGGGCTTGTCCTCTT	Beta Lactam
113	blaFOX	GGTTTGCCGCTGCAGTTC	GCGGCCAGGTGACCAA	Beta Lactam
121	blaSFO	CCGCCGCCATCCAGTA	GGGCCGCCAAGATGCT	Beta Lactam
125	qacH	CATCGTGCTTGTGGCAGCTA	TGAACGCCCAGAAGTCTAGTTTT	MDR[a]
132	rarD	TGACGCATCGCGTGATCT	AAATTTTCTGTGGCGTCTGAATC	Amphenicol
140	mphA	CTGACGCGCTCCGTGTT	GGTGGTGCATGGCGATCT	MLSB
156	emrB/qacA	CTTTTCTCTAACCGTACATTATCTACGATAAA	AGAACGTAGCGACTGATAAAATGCT	MDR[a]
157	bacA	CGGCTTCGTGACCTCGTT	ACAATGCGATACCAGGCAAAT	other
177	strB	GCTCGGTCGTGAGAACAATCT	CAATTTCGGTCGCCTGGTAGT	Aminoglycoside
202	tnpA_1	CCGATCACGGAAAGCTCAAG	GGCTCGCATGACTTCGAATC	Transposase
203	tnpA_2	GGGCGGGTCGATTGAAA	GTGGGCGGGATCTGCTT	Transposase
210	vanA	AAAAGGCTCTGAAAACGCAGTTAT	CGGCCGTTATCTTGTAAAAACAT	Vancomycin
215	vanHB	GAGGTTTCCGAGGCGACAA	CTCTCGGCGGCAGTCGTAT	Vancomycin
217	vanRA_2	CCACTCCGGCCTTGTCATT	GCTAACCACATTCCCCTTGTTTT	Vancomycin
229	pncA	GCAATCGAGGCGGTGTTC	TTGCCGCAGCCAATTCA	MLSB
234	oprD	ATGAAGTGGAGCGCCATTG	GGCCACGGCGAACTGA	MDR[a]
235	oprJ	ACGAGAGTGGCGTCGACAA	AAGGCGATCTCGTTGAGGAA	MDR[a]
243	ttgA	ACGCCAATGCCAAACGATT	GTCACGGCGCAGCTTGA	MDR[a]
244	ttgB	TCGCCCTGGATGTACACCTT	ACCATTGCCGACATCAACAAC	MDR[a]
245	mepA	ATCGGTCGCTCTTCGTTCAC	ATAAATAGGATCGAGCTGCTGGAT	MDR[a]
246	mexE	GGTCAGCACCGACAAGGTCTAC	AGCTCGACGTACTTGAGGAACAC	MDR[a]
247	mexF	CCGCGAGAAGGCCAAGA	TTGAGTTCGGCGGTGATGA	MDR[a]
256	acrA_1	TACTTTGCGCGCCATCTTC	CGTGCGCGAACGAACAT	MDR[a]
257	acrA_2	CGTGCGCGAACGAACA	ACTTTGCGCGCCATCTTC	MDR[a]

276	msrA	AACGAAATCAAGCGCAACAA	CAACCGTGCCTTTTTCTTTTG	MLSB
285	oleC	CCCGGAGTCGATGTTCGA	GCCGAAGACGTACACGAACAG	MLSB
290	pikR2	TCGTGGGCCAGGTGAAGA	TTCCCCTTGCCGGTGAA	MLSB
292	tetPB	ACACCTGGACACGCTGATTTT	ACCGTCTAGAACGCGGAATG	Tetracycline
299	tolC_1	CAGGCAGAGAACCTGATGCA	CGCAATTCCGGGTTGCT	MDR[a]
300	tolC_2	GCCAGGCAGAGAACCTGATG	CGCAATTCCGGGTTGCT	MDR[a]
310	vanSB	GCGCGGCAAATGACAAC	TTTGCCATTTTATTCGCACTGT	Vancomycin
331	merA	GTGCCGTCCAAGATCATG	GGTGGAAGTCCAGTAGGGTGA	Murcury
332	sul2	TCCGATGGAGGCCGGTATCTGG	CGGGAATGCCATCTGCCTTGAG	Sulfonamide
342	IncP_oriT	CAGCCTCGCAGAGCAGGAT	CAGCCGGGCAGGATAGGTGAAGT	plasmid incompatibility
350	acrR	TGCAACACGCGCTTTCTC	ACGATTGCGGGCAGGTT	MDR
359	intI1	CGAACGAGTGGCGGAGGGTG	TACCCGAGAGCTTGGCACCCA	Integrase
366	orf39-IS26	GCGCGTCGAGCATCAATAG	CAGTTGTGCTGCTGGTGGTC	Insertional sequence
369	ISPps	CACACTGCAAAAACGCATCCT	TGTCTTTGGCGTCACAGTTCTC	Insertional sequence
370	ISSm2	TGGATCGACCGGTTCCAT	GCTGACCGAGCTGTCCATGT	Insertional sequence
374	mexB	CTGGAGATCGACGACGAGAAG	GAAATCGTTGACGTAGCTGGAA	MDR[a]
378	repA	CCCCCAGGACTTGCGAGCG	GAGGCATGCACGCCGACCA	plasmid replication
380	pAKD1	GGTAAGATTACCGATAAACT	GTTCGTGAAGAAGATGTA	plasmid replication

[a] MDR is multidrug resistance.

Table S3: Bacterial community composition for top 20 families. Number represents percentage (%) of each bacteria in each sample.

	SBSC	SBS	SMSC	SMS	STSC	STS	ROC	RO	RS	SOC	SO	SSC	SS
Methylophilaceae	0.1	6.6	0.2	23.5	0.4	28.1	0.5	17.5	12.5	0.3	16.5	0.0	0.0
Chthoniobacteraceae	3.6	3.8	4.8	5.6	4.4	7.1	0.7	0.5	0.3	0.2	0.0	0.0	0.0
Chitinophagaceae	10.0	7.2	9.9	6.7	8.7	6.3	7.4	5.2	3.2	2.4	0.3	0.0	0.0
Sphingomonadaceae	13.9	18.9	11.6	11.4	15.4	9.9	6.3	5.0	5.8	1.3	0.6	0.0	15.3
Pirellulaceae	5.3	6.1	9.0	4.5	5.8	3.1	2.1	0.6	0.5	0.0	0.0	0.0	0.0
Xanthomonadaceae	3.0	2.9	2.0	1.3	3.0	1.6	2.9	3.4	2.1	15.6	14.2	33.3	0.0
Pseudomonadaceae	0.3	0.2	0.0	0.2	0.1	0.1	0.9	0.3	0.3	50.6	37.9	8.3	6.1
Ellin6075	1.5	2.2	3.0	3.4	4.9	9.1	0.3	0.8	0.7	0.0	0.0	0.0	0.0
Bradyrhizobiaceae	5.4	3.3	4.7	4.5	4.2	4.3	2.2	5.3	4.5	0.3	0.7	0.0	0.0
Micromonosporaceae	2.8	3.5	5.4	2.8	4.2	1.4	6.2	3.8	5.2	0.0	0.0	8.3	0.0
Bacillaceae	4.5	3.8	5.7	2.6	6.3	0.9	0.5	0.5	0.4	0.0	0.0	0.0	0.0
Micrococcaceae	3.8	2.2	4.2	2.4	5.6	1.5	0.5	0.4	0.5	8.8	1.8	16.7	0.0
Nocardioidaceae	4.4	4.2	4.6	3.2	5.1	1.9	2.2	2.6	4.0	0.9	0.0	8.3	0.0
Methylobacteriaceae	0.4	1.3	0.4	4.3	0.5	7.5	0.1	7.6	7.0	0.1	14.6	0.0	78.6
Cytophagaceae	11.7	7.0	8.9	5.9	5.9	3.3	7.5	4.3	4.6	0.3	0.2	0.0	0.0
Comamonadaceae	4.7	3.3	4.8	5.4	5.1	5.3	27.4	7.1	8.0	12.9	8.0	16.7	0.0
Hyphomicrobiaceae	10.1	8.3	8.9	7.1	6.1	6.1	5.9	7.2	7.1	1.3	0.0	0.0	0.0
Sphingobacteriaceae	5.0	8.9	3.7	1.4	3.3	0.6	2.5	1.8	1.4	2.6	4.9	0.0	0.0
Streptomycetaceae	2.4	2.0	2.8	1.0	2.7	0.6	14.7	18.6	18.1	0.0	0.0	8.3	0.0
Oxalobacteraceae	7.4	4.3	5.5	3.2	8.5	1.7	9.0	7.7	14.1	2.5	0.3	0.0	0.0

Table S4: Correlation tests in the network analysis.

	Variable 1	Variable 2	ρ	p value
1	Antibiotics_Con	ISPps	0.79	0.04
2	Antibiotics_Con	Lincomycin	0.82	0.02
3	blaFOX	Oxytetracycline	-0.90	0.04
4	mexF	Oxytetracycline	-0.90	0.04
5	oprJ	Sulfadiazine	1.00	0.00
6	intI1	Sulfadiazine	0.82	0.02
7	ISPps	Sulfadiazine	0.89	0.01
8	Antibiotics_Con	Sulfamethoxazole	0.79	0.04
9	oprJ	Sulfamethoxazole	1.00	0.00
10	ISPps	Sulfamethoxazole	0.86	0.01
11	Carbadox	Sulfamethoxazole	0.86	0.01
12	Sulfadiazine	Sulfamethoxazole	0.96	0.00
13	tnpA_1	Trimethoprim	-0.81	0.05
14	Antibiotics_Con	Tylosin	0.79	0.04
15	blaPDC	Tylosin	1.00	0.00
16	ISPps	Tylosin	0.86	0.01
17	ISSm2	Tylosin	1.00	0.00
18	Antibiotics_Con	Methylophilaceae	0.82	0.02
19	Tylosin	Methylophilaceae	0.86	0.01
20	Oxytetracycline	Chitinophagaceae	-0.93	0.00
21	Trimethoprim	Sphingomonadaceae	-0.88	0.01
22	Oxytetracycline	Pirellulaceae	-0.90	0.01
23	Sulfadiazine	Bradyrhizobiaceae	0.89	0.01
24	Sulfamethoxazole	Bradyrhizobiaceae	0.82	0.02
25	Trimethoprim	Comamonadaceae	0.85	0.02
26	Oxytetracycline	Hyphomicrobiaceae	-0.76	0.05
27	ARGscon	Chitinophagaceae	0.75	0.00
28	Chitinophagaceae	Pirellulaceae	0.80	0.00
29	Sphingomonadaceae	Pirellulaceae	0.62	0.03
30	Chitinophagaceae	Bradyrhizobiaceae	0.69	0.01
31	Pirellulaceae	Bradyrhizobiaceae	0.78	0.00
32	Chitinophagaceae	Cytophagaceae	0.82	0.00
33	Pirellulaceae	Cytophagaceae	0.90	0.00
34	Bradyrhizobiaceae	Cytophagaceae	0.77	0.00
35	Sphingomonadaceae	Comamonadaceae	-0.90	0.00
36	Chitinophagaceae	Hyphomicrobiaceae	0.82	0.00
37	Pirellulaceae	Hyphomicrobiaceae	0.91	0.00
38	Bradyrhizobiaceae	Hyphomicrobiaceae	0.86	0.00
39	Cytophagaceae	Hyphomicrobiaceae	0.94	0.00
40	Chitinophagaceae	blaPDC	0.93	0.00
41	Sphingomonadaceae	blaFOX	0.70	0.04
42	Pirellulaceae	blaFOX	0.68	0.04

43	Comamonadaceae	blaFOX	-0.85	0.00
44	ARGscon	tnpA_1	0.66	0.02
45	Chitinophagaceae	tnpA_1	0.71	0.01
46	Sphingomonadaceae	tnpA_1	0.60	0.04
47	blaPDC	tnpA_1	0.79	0.04
48	Chitinophagaceae	oprJ	0.83	0.01
49	Sphingomonadaceae	oprJ	0.83	0.01
50	Pirellulaceae	oprJ	0.76	0.03
51	Comamonadaceae	oprJ	-0.79	0.02
52	tnpA_1	oprJ	0.90	0.00
53	ARGscon	mexE	0.79	0.04
54	blaPDC	mexE	1.00	0.00
55	tnpA_1	mexE	0.96	0.00
56	ARGscon	mexF	0.72	0.02
57	Chitinophagaceae	mexF	0.80	0.01
58	Pirellulaceae	mexF	0.92	0.00
59	Bradyrhizobiaceae	mexF	0.89	0.00
60	Cytophagaceae	mexF	0.86	0.00
61	Hyphomicrobiaceae	mexF	0.93	0.00
62	blaFOX	mexF	0.94	0.00
63	tnpA_1	mexF	0.68	0.04
64	oprJ	mexF	0.83	0.04
65	Sphingomonadaceae	oleC	-0.86	0.01
66	Cytophagaceae	oleC	-0.86	0.01
67	Comamonadaceae	oleC	0.89	0.01
68	Hyphomicrobiaceae	oleC	-0.79	0.04
69	Streptomycetaceae	oleC	0.96	0.00
70	blaFOX	oleC	-0.90	0.04
71	Pirellulaceae	intI1	0.66	0.02
72	Bradyrhizobiaceae	intI1	0.73	0.01
73	blaFOX	intI1	0.75	0.02
74	mexF	intI1	0.80	0.01
75	Methylophilaceae	ISPps	0.86	0.00
76	intI1	ISSm2	0.77	0.02
77	tnpA_1	repA	0.93	0.00
78	oprJ	repA	1.00	0.00
79	ISSm2	repA	0.89	0.02

References

Al-Janabi, A.A.H.S., 2010. In vitro antibacterial activity of Ibuprofen and acetaminophen. J. Glob. Infect. Dis. 2: 105-108.

Al-Janabi, A.A.H.S., 2011. Potential activity of the purine compounds caffeine and aminophylline on bacteria. J. Glob. Infect. Dis. 3:133-137.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Pena, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Tumbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R., 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods.7:335-336.

Chao, A., 1984. Nonparametric-estimation of the number of classes in a population. Scand. J. Stat. 11:265-270.

DeSantis, T.Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E.L., Keller, K., Huber, T., Dalevi, D., Hu, P., Andersen, G.L., 2006. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl. Environ. Microbiol. 72:5069-5072.

Hughes, J.B., Hellmann, J.J., Ricketts, T.H., Bohannan, B.J.M., 2001. Counting the uncountable: Statistical approaches to estimating microbial diversity. Appl. Environ. Microbiol. 67:4399-4406.

Lamond, A.I., 2002. Molecular biology of the cell, 4th edition. Nature. 417:383-383.

Paradis, E., Claude, J., Strimmer, K., 2004. APE: analyses of phylogenetics and evolution in R language. Bioinformatics. 20:289-290.

Paul, J.M., Susan, H., 2013. phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One. 8:e61217.

Sakai, M., Ikenaga, M., 2013. Application of peptide nucleic acid (PNA)-PCR clamping technique to investigate the community structures of rhizobacteria associated with plant roots. J. Microbiol. Meth. 92:281-288.

Smith, D.R., Keeling, P.J., 2015. Mitochondrial and plastid genome architecture: Reoccurring themes, but significant differences at the extremes. P. Natl. Acad. Sci. USA. 112:10177-10184.

Stedtfeld, R.D., Baushke, S.W., Tourlousse, D.M., Miller, S.M., Stedtfeld, T.M., Gulari, E., Tiedje, J.M., Hashsham, S.A., 2008. Development and experimental validation of a predictive threshold cycle equation for quantification of virulence and marker genes by high-throughput nanoliter-volume PCR on the OpenArray platform. Appl. Environ. Microbiol. 74:3831-3838.

Verma, T., Bhaskarla, C., Sadhir, I., Sreedharan, S., Nandi, D., 2018. Non-steroidal anti-inflammatory drugs, acetaminophen and ibuprofen, induce phenotypic antibiotic resistance in Escherichia coli: roles of marA and acrB. FEMS Microbiol. Lett. 365.

Wang, Y., Lu, J., Mao, L., Li, J., Yuan, Z., Bond, P.L., Guo, J., 2019. Antiepileptic drug carbamazepine promotes horizontal transfer of plasmid-borne multi-antibiotic resistance genes within and across bacterial genera. ISME J. 13: 509-522.