Supplementary Materials

Effects of antibiotics on the bacterial community，metabolic functions and antibiotic resistance genes in mariculture sediments during enrichment culturing

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Supplementary Tables

Table S1 The information of antibiotics used in this study.

Antibiotic name	Class	CAS NO.	Inhibited pathway	Mainly usage
Zinc bacitracin	Peptides	1405-89-6	Cell wall synthesis	veterinary
Ciprofloxacin	Fluoroquinolone	85721-33-1	DNA synthesis	veterinary and human
Ampicillin sodium	β-lactams	69-57-8	Cell wall synthesis	veterinary and human
Chloramphenicol	Chloramphenicols	56-75-7	Protein synthesis	veterinary and human
Tylosin	Macrolides	1401-69-0	Protein synthesis	veterinary
Tetracycline	Tetracyclines	60-54-8	Aminoacyl-tRNA access	veterinary and human

Table S2 The dominant phyla (average abundance > 1.0%) in each treatment

Treatment	Dominant phyla
Control （A）	Proteobacteria (43.01%), Bacteroidetes(18.67%), Fusobacteria (12.91%), Firmicutes (11.74%), Chloroflexi (1.80%), Spirochaetae (1.64%), Planctomycetes (1.48%), Cloacimonetes (1.34%), Latescibacteria (1.03%)
P	Proteobacteria(50.24%), Bacteroidetes(19.79%), Fusobacteria (12.73%), Firmicutes (8.54%), Spirochaetae(2.92%), Synergistetes (1.98%),
Q	Proteobacteria (40.61%), Bacteroidetes (22.39%), Fusobacteria (12.37%), Firmicutes (11.79%), Spirochaetae (6.41%), unclassified_k__norank_d__Bacteria (1.75%), Fibrobacteres (1.17%)
R	Proteobacteria (37.55%), Bacteroidetes (18.28%), Fusobacteria (15.93%), Spirochaetae (12.96%), Firmicutes (7.18%), Tenericutes (3.96%), unclassified_k__norank_d__Bacteria (1.39%)
S	Proteobacteria (43.63%), Firmicutes (28.43%), Bacteroidetes (12.12%), Fusobacteria (8.13%), Spirochaetae (1.49%), Cloacimonetes (1.26%), Tenericutes (1.00%)
T	Proteobacteria (40.22%), Firmicutes (29.22%), Bacteroidetes (10.79%), Fusobacteria (5.47%), Spirochaetae (4.81%), unclassified_k__norank_d__Bacteria (2.41%), Lentisphaerae (2.30%), Planctomycetes (1.16%), Chloroflexi (1.11%)
U	Proteobacteria (47.85%), Bacteroidetes (19.25%), Firmicutes (13.19%), Fusobacteria (12.90%), Spirochaetae (2.31%), unclassified_k__norank_d__Bacteria (1.26%), Synergistetes (1.15%)

Table S3 The dominant genera (average abundance > 1.0%) in each treatment.

Treatment	Genus	Family	Phylum	Class	order	Average abundance
A	Desulfobacter	Desulfobacteraceae	Proteobacteriaia	Deltaproteobacteria	Desulfobacterales	20.93%
	Fusobacterium	Fusobacteriaceae	Fusobacteria	Fusobacteriia	Fusobacteriales	7.04%
	Vibrio	Vibrionaceae	Proteobacteria	Gammaproteobacteria	Vibrionales	6.94%
	Marinifilum	Marinilabiaceae	Bacteroidetes	Bacteroidia	Bacteroidales	6.24%
	Propionigenium	Fusobacteriaceae	Fusobacteria	Fusobacteriia	Fusobacteriales	4.60%
	Dethiosulfatibacter	Clostridiales_Incertae_Sedis_o__Clostridiales	Firmicutes	Clostridia	Clostridiales	3.64%
	Desulfovibrio	Desulfovibrionaceae	Proteobacteria	Deltaproteobacteria	Desulfovibrionales	2.93%
	norank_f__JTB215	JTB215	Firmicutes	Clostridia	Clostridiales	2.72%
	uncultured_f__Marinilabiaceae	Marinilabiaceae	Bacteroidetes	Bacteroidia	Bacteroidales	1.88%
	Ilyobacter	Fusobacteriaceae	Fusobacteria	Fusobacteriia	Fusobacteriales	1.22%
	Bacteroides	Bacteroidaceae	Bacteroidetes	Bacteroidia	Bacteroidales	1.18
	Draconibacterium	Draconibacteriaceae	Bacteroidetes	Bacteroidia	Bacteroidia_Incertae_Sedis_c__Bacteroidia	1.03%
	norank_p__Latescibacteria	norank_p__Latescibacteria	Latescibacteria	norank_p__Latescibacteria	norank_p__Latescibacteria	1.01%
P	Desulfobacter	Desulfobacteraceae	Proteobacteria	Deltaproteobacteria	Desulfobacterales	37.40%
	Marinifilum	Marinilabiaceae	Bacteroidetes	Bacteroidia	Bacteroidales	11.28%

	Fusobacterium	Fusobacteriaceae	Fusobacteria	Fusobacteriia	Fusobacteriales	7.76%
	Vibrio	Vibrionaceae	Proteobacteria	Gammaproteobacteria	Vibrionales	5.22%
	Propionigenium	Fusobacteriaceae	Fusobacteria	Fusobacteriia	Fusobacteriales	4.34%
	norank_f__JTB215	JTB215	Firmicutes	Clostridia	Clostridiales	3.71%
	Desulfovibrio	Desulfovibrionaceae	Proteobacteria	Deltaproteobacteria	Desulfovibrionales	2.69%
	Spirochaeta_2	Spirochaetaceae	Spirochaetae	Spirochaetes	Spirochaetales	2.30%
	unclassified_f__Synergistaceae	Synergistaceae	Synergistetes	Synergistia	Synergistales	1.80%
	Bacteroides	Bacteroidaceae	Bacteroidetes	Bacteroidia	Bacteroidales	1.48%
	Sunxiuqinia	Cytophagaceae	Bacteroidetes	Cytophagia	Cytophagales	1.38%
	uncultured_f__Marinilabiaceae	Marinilabiaceae	Bacteroidetes	Bacteroidia	Bacteroidales	1.31%
	Clostridium_sensu_stricto	Clostridiaceae_4	Firmicutes	Clostridia	Clostridiales	1.31%
Q	Desulfobacter	Desulfobacteraceae	Proteobacteria	Deltaproteobacteria	Desulfobacterales	32.62%
	Marinifilum	Marinilabiaceae	Bacteroidetes	Bacteroidia	Bacteroidales	17.54%
	Psychrilyobacter	Fusobacteriaceae	Fusobacteria	Fusobacteriia	Fusobacteriales	7.80%
	Sphaerochaeta	Spirochaetaceae	Spirochaetae	Spirochaetes	Spirochaetales	5.38%
	Propionigenium	Fusobacteriaceae	Fusobacteria	Fusobacteriia	Fusobacteriales	3.82%
	Vibrio	Vibrionaceae	Proteobacteria	Gammaproteobacteria	Vibrionales	3.49%
	uncultured_f__Family_XI	Family_XI	Firmicutes	Clostridia	Clostridiales	3.17%
	Fusibacter	Family_XII	Firmicutes	Clostridia	Clostridiales	1.89%
	Sunxiuqinia	Cytophagaceae	Bacteroidetes	Cytophagia	Cytophagales	1.86%
	unclassified_k__norank_d__Bacteria	unclassified_k__norank_d__Bacteria	unclassified_k__norank_d__Bacteria	unclassified_k__norank_d__Bacteria	unclassified_k__norank_d__Bacteria	1.75%
	norank_o__possible_order_07	norank_o__possible_order_07	Fibrobacteres	Fibrobacteria	possible_order_07	1.17%
R	Desulfobacter	Desulfobacteraceae	Proteobacteria	Deltaproteobacteria	Desulfobacterales	17.07%

	Fusobacterium	Fusobacteriaceae	Fusobacteria	Fusobacteriia	Fusobacteriales	14.54%
	Vibrio	Vibrionaceae	Proteobacteria	Gammaproteobacteria	Vibrionales	14.29%
	Sphaerochaeta	Spirochaetaceae	Spirochaetae	Spirochaetes	Spirochaetales	12.56%
	Marinifilum	Marinilabiaceae	Bacteroidetes	Bacteroidia	Bacteroidales	12.27%
	unclassified_f__Peptostreptococcaceae	Peptostreptococcaceae	Firmicutes	Clostridia	Clostridiales	5.41%
	norank_o__NB1-n	norank_o__NB1-n	Tenericutes	Mollicutes	NB1-n	3.81%
	Desulfovibrio	Desulfovibrionaceae	Proteobacteria	Deltaproteobacteria	Desulfovibrionales	1.63%
	Sunxiuqinia	Cytophagaceae	Bacteroidetes	Cytophagia	Cytophagales	1.45%
	unclassified_k__norank_d__Bacteria	unclassified_k__norank_d__Bacteria	unclassified_k__norank_d__Bacteria	unclassified_k__norank_d__Bacteria	unclassified_k__norank_d__Bacteria	1.39%
	Propionigenium	Fusobacteriaceae	Fusobacteria	Fusobacteriia	Fusobacteriales	1.37%
	uncultured_f__Marinilabiaceae	Marinilabiaceae	Bacteroidetes	Bacteroidia	Bacteroidales	1.11%
S	Desulfobacter	Desulfobacteraceae	Proteobacteria	Deltaproteobacteria	Desulfobacterales	21.21%
	norank_f__JTB215	JTB215	Firmicutes	Clostridia	Clostridiales	15.15%
	Vibrio	Vibrionaceae	Proteobacteria	Gammaproteobacteria	Vibrionales	10.35%
	Fusobacterium	Fusobacteriaceae	Fusobacteria	Fusobacteriia	Fusobacteriales	6.44%
	Marinifilum	Marinilabiaceae	Bacteroidetes	Bacteroidia	Bacteroidales	4.95%
	Desulfovibrio	Desulfovibrionaceae	Proteobacteria	Deltaproteobacteria	Desulfovibrionales	3.25%
	Dethiosulfatibacter	Clostridiales_Incertae_Sedis_o__Clostridiales	Firmicutes	Clostridia	Clostridiales	2.73%
	norank_f__livecontrolB21	livecontrolB21	Firmicutes	Clostridia	Clostridiales	2.59%
	unclassified_f__Erysipelotrichaceae	Erysipelotrichaceae	Firmicutes	Erysipelotrichia	Erysipelotrichales	2.55%
	Bacteroides	Bacteroidaceae	Bacteroidetes	Bacteroidia	Bacteroidales	2.16%

	Shewanella	Shewanellaceae	Proteobacteria	Gammaproteobacteria	Alteromonadales	1.97%
	Propionigenium	Fusobacteriaceae	Fusobacteria	Fusobacteriia	Fusobacteriales	1.60%
	Tyzzerella	Lachnospiraceae	Firmicutes	Clostridia	Clostridiales	1.49%
	Photobacterium	Vibrionaceae	Proteobacteria	Gammaproteobacteria	Vibrionales	1.42%
	unclassified_f__Desulfobacteraceae	Desulfobacteraceae	Proteobacteria	Deltaproteobacteria	Desulfobacterales	1.21%
T	Desulfobacter	Desulfobacteraceae	Proteobacteria	Deltaproteobacteria	Desulfobacterales	24.43%
	uncultured_f__Family_XI	Family_XI	Firmicutes	Clostridia	Clostridiales	21.59%
	Proteiniphilum	Porphyromonadaceae	Bacteroidetes	Bacteroidia	Bacteroidales	6.41%
	Propionigenium	Fusobacteriaceae	Fusobacteria	Fusobacteriia	Fusobacteriales	5.03%
	Vibrio	Vibrionaceae	Proteobacteria	Gammaproteobacteria	Vibrionales	4.81%
	Sphaerochaeta	Spirochaetaceae	Spirochaetae	Spirochaetes	Spirochaetales	4.46%
	Dethiosulfatibacter	Clostridiales_Incertae_Sedis_o__Clostridiales	Firmicutes	Clostridia	Clostridiales	3.71%
	Desulfovibrio	Desulfovibrionaceae	Proteobacteria	Deltaproteobacteria	Desulfovibrionales	2.91%
	unclassified_k__norank_d__Bacteria	unclassified_k__norank_d__Bacteria	unclassified_k__norank_d__Bacteria	unclassified_k__norank_d__Bacteria	unclassified_k__norank_d__Bacteria	2.41%
	Sunxiuqinia	Cytophagaceae	Bacteroidetes	Cytophagia	Cytophagales	1.91%
	unclassified_o__Oligosphaerales	unclassified_o__Oligosphaerales	Lentisphaerae	Oligosphaeria	Oligosphaerales	1.80%
	Sedimentibacter	Family_XI	Firmicutes	Clostridia	Clostridiales	1.12%
	Proteiniclasticum	Clostridiaceae_1	Firmicutes	Clostridia	Clostridiales	1.02%
U	Desulfobacter	Desulfobacteraceae	Proteobacteria	Deltaproteobacteria	Desulfobacterales	36.29%
	Marinifilum	Marinilabiaceae	Bacteroidetes	Bacteroidia	Bacteroidales	12.53%
	Fusobacterium	Fusobacteriaceae	Fusobacteria	Fusobacteriia	Fusobacteriales	9.87%

uncultured_f__Family_XI	Family_XI	Firmicutes	Clostridia	Clostridiales	5.47%
Vibrio	Vibrionaceae	Proteobacteria	Gammaproteobacteria	Vibrionales	4.91%
Draconibacterium	Draconibacteriaceae	Bacteroidetes	Bacteroidia	Bacteroidia_Incertae_Sedis_c__Bacteroidia	2.77%
Desulfovibrio	Desulfovibrionaceae	Proteobacteria	Deltaproteobacteria	Desulfovibrionales	2.06%
Sphaerochaeta	Spirochaetaceae	Spirochaetae	Spirochaetes	Spirochaetales	2.04%
Propionigenium	Fusobacteriaceae	Fusobacteria	Fusobacteriia	Fusobacteriales	1.65%
Sedimentibacter	Family_XI	Firmicutes	Clostridia	Clostridiales	1.64%
Dethiosulfatibacter	Clostridiales_Incertae_Sedis_o__Clostridiales	Firmicutes	Clostridia	Clostridiales	1.58%
norank_f__JTB215	JTB215	Firmicutes	Clostridia	Clostridiales	1.56%
unclassified_k__norank_d__Bacteria	unclassified_k__norank_d__Bacteria	unclassified_k__norank_d__Bacteria	unclassified_k__norank_d__Bacteria	unclassified_k__norank_d__Bacteria	1.26%
Psychrilyobacter	Fusobacteriaceae	Fusobacteria	Fusobacteriia	Fusobacteriales	1.18%
Mangrovibacterium	Cytophagaceae	Bacteroidetes	Cytophagia	Cytophagales	1.18%
Tyzzerella	Lachnospiraceae	Firmicutes	Clostridia	Clostridiales	1.02%

Table S4 SIMPER analysis of the bacterial community in antibiotic-treated group and Control group at the OTU level.

Comparison	OTU	Taxon			Average abundance	Average abundance	Dissimilarity contribution (%)
		Family	**Gunus**	**Species**			
A&P					A	P	
	OTU2543	Desulfobacteraceae	*Desulfobacter*	unclassified	6158.6	9937.83	12.88
	OTU1450	Desulfobacteraceae	*Desulfobacter*	uncultured	3171	5670.83	7.75
	OTU1652	Marinilabiaceae	*Marinifilum*	unclassified	3172.2	2211.33	5.68
A&Q					A	Q	
	OTU2543	Desulfobacteraceae	*Desulfobacter*	unclassified	6158.6	9890.83	11.65
	OTU1652	Marinilabiaceae	*Marinifilum*	unclassified	3171	7331.5	9.28
	OTU1450	Desulfobacteraceae	*Desulfobacter*	uncultured	3172.2	1497.33	9.21
	OTU1706	Fusobacteriaceae	*Psychrilyobacter*	uncultured	2309	8211.17	5.62
A&R					A	R	
	OTU2543	Desulfobacteraceae	*Desulfobacter*	unclassified	6158.6	5148.5	8.6
	OTU2126	Fusobacteriaceae	*Fusobacterium*	unclassified	3171	3061	7.56
	OTU2034	Vibrionaceae	*Vibrio*	unclassified	3172.2	6584.5	7.28
A&S					A	S	
	OTU1295	JTB215	unclassified	unclassified	6158.6	5585.17	10.45
	OTU2543	Desulfobacteraceae	*Desulfobacter*	unclassified	3171	3445.83	8.65
	OTU2034	Vibrionaceae	*Vibrio*	unclassified	3172.2	4794.33	7.07
A&T					A	T	
	OTU2556	Family_XI	uncultured	unclassified	6158.6	6839.67	14.39
	OTU2543	Desulfobacteraceae	*Desulfobacter*	unclassified	3171	4956.33	7.87

	OTU1450	Desulfobacteraceae	*Desulfobacter*	uncultured	3172.2	2016.83	5.49
	OTU2034	Vibrionaceae	*Vibrio*	unclassified	2309	0.83	5.15
A&U					A	U	
	OTU2543	Desulfobacteraceae	*Desulfobacter*	unclassified	6158.6	10557.5	14.03
	OTU1450	Desulfobacteraceae	*Desulfobacter*	uncultured	3171	7591.83	10.89
	OTU2126	Fusobacteriaceae	*Fusobacterium*	unclassified	3172.2	2237.83	5.2

Only taxa whose dissimilarity contribution ≥5% were listed.

Table S5 SIMPER analysis of the bacterial community in groups of different enrichment time at the OTU level.

Comparison	OTU	Taxon			Average abundance	Average abundance	Dissimilarity contribution (%)
		Family	Gunus	Species			
0d & 5d					0d	5d	
	OTU2034	Vibrionaceae	*Vibrio*	unclassified	83	319.71	11.6
	OTU1652	Marinilabiaceae	*Marinifilum*	unclassified	54	181.29	5.96
	OTU2259	Fusobacteriaceae	*Propionigenium*	uncultured	876	9492.71	5.77
	OTU2126	Fusobacteriaceae	*Fusobacterium*	unclassified	45	4392.43	5.04
0d & 12d					0d	12d	
	OTU2543	Desulfobacteraceae	*Desulfobacter*	unclassified	83	5566.29	7.44
	OTU1652	Marinilabiaceae	*Marinifilum*	unclassified	54	3425.29	5.87
	OTU2126	Fusobacteriaceae	*Fusobacterium*	unclassified	876	2105.43	5.8
0d & 21d					0d	21d	
	OTU2543	Desulfobacteraceae	*Desulfobacter*	unclassified	83	10906.85	12.79
	OTU1450	Desulfobacteraceae	*Desulfobacter*	uncultured	54	7312.46	8.57
0d & 30d					0d	30d	
	OTU2543	Desulfobacteraceae	*Desulfobacter*	unclassified	83	10421.23	12.79
	OTU1450	Desulfobacteraceae	*Desulfobacter*	uncultured	54	6756.69	8.28

Only taxa whose dissimilarity contribution ≥5% were listed.

Supplementary Figures

Figure S1 The number of shared and unique OTUs in the samples of the same group. (a) zinc bacitracin-treated group; (b) ciprofloxacin-treated group; (c) ampicillin sodium-treated group; (d)chloramphenicol-treated group; (e) tylosin-treated group; (f) tetracycline-treated group.

Figure S2 Composition of microbial community at the class level.

Figure S3 Hierarchical clustering tree on OTU level. A, control group; P, zinc bacitracin-treated group; Q, ciprofloxacin-treated group; R, ampicillin sodium-treated group; S, chloramphenicol-treated group; T, tylosin-treated group; U, tetracycline-treated group.

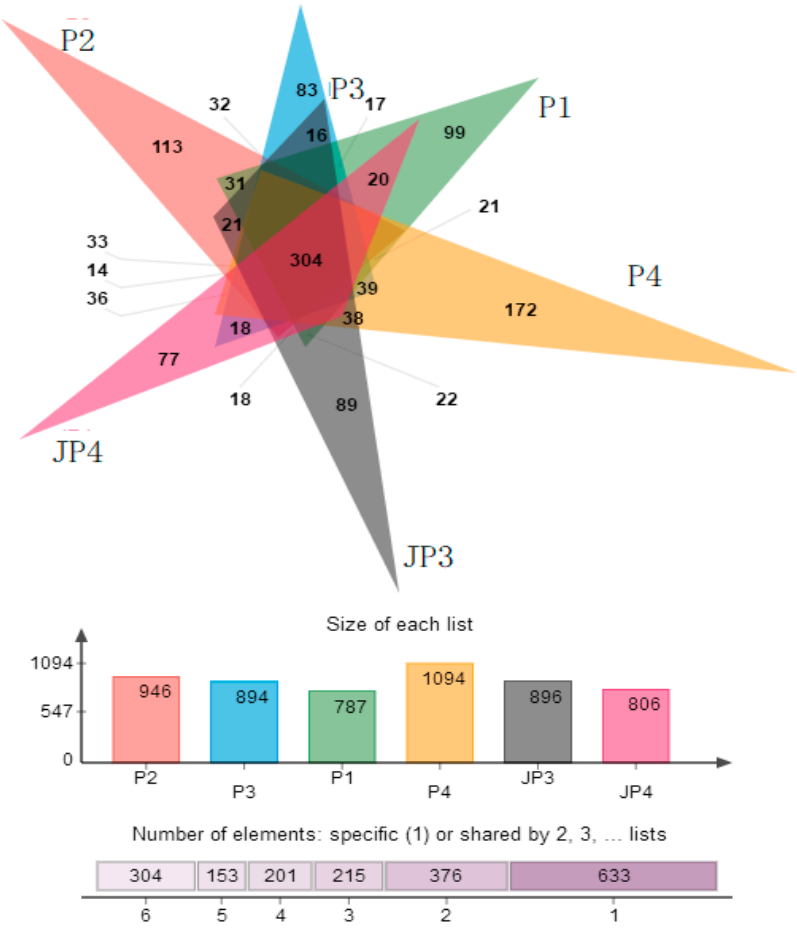
Figure S4 NMDS (Abund-jaccard distance) plot based on OTUs abundance showing the microbial composition variations among different samples. A, control group; P, zinc bacitracin-treated group; Q, ciprofloxacin-treated group; R, ampicillin sodium-treated group; S, chloramphenicol-treated group; T, tylosin-treated group; U, tetracycline-treated group.

Figure S5 Comparison of control group and different antibiotic-treated groups on phylum level (one-way ANOVA). * $0.01 < P \leq 0.05$, ** $0.001 < P \leq 0.01$, *** $P \leq 0.001$. A, control group; P, zinc bacitracin-treated group; Q, ciprofloxacin-treated group; R, ampicillin sodium-treated group; S, chloramphenicol-treated group; T, tylosin-treated group; U, tetracycline-treated group.

Figure S6 Comparison of control group and different antibiotic-treated groups on genus level (one-way ANOVA). * $0.01 < P \leq 0.05$, ** $0.001 < P \leq 0.01$, *** $P \leq 0.001$. A, control group; P, zinc bacitracin-treated group; Q, ciprofloxacin-treated group; R, ampicillin sodium-treated group; S, chloramphenicol-treated group; T, tylosin-treated group; U, tetracycline-treated group.

Figure S7 Comparison of samples of different enrichment time on phylum level (one-way ANOVA). * $0.01 < P \leq 0.05$, ** $0.001 < P \leq 0.01$, *** $P \leq 0.001$. 1, the samples without enrichment; 2, the samples incubating for 5 days; 3, the samples incubating for 12 days; 4, the samples incubating for 21 days; 5, the samples incubating for 30 days.

Figure S8 Comparison of samples of different enrichment time on genus level (one-way ANOVA). * $0.01 < P \leq 0.05$, ** $0.001 < P \leq 0.01$, *** $P \leq 0.001$. 1, the samples without enrichment; 2, the samples incubating for 5 days; 3, the samples incubating for 12 days; 4, the samples incubating for 21 days; 5, the samples incubating for 30 days.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99
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20
21
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36
304
39
172
18
38
77
18
89
22
Size of each list
1094
547
0
946
894
787
1094
896
806
P2
P3
P1
P4
JP3
JP4
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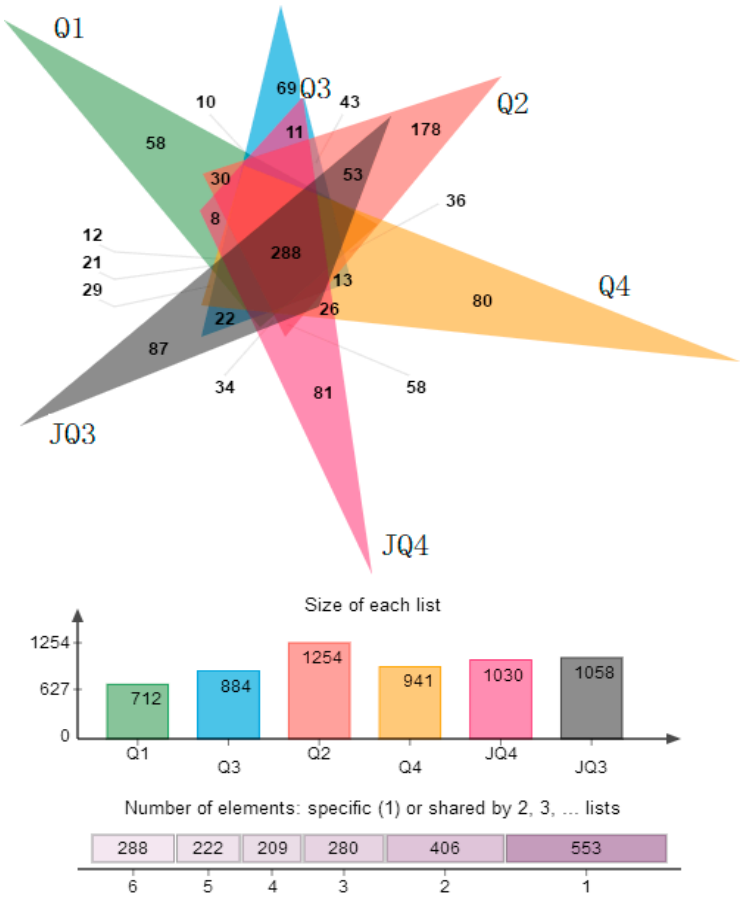
1

b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13
29
80
22
26
87
34
81
58
Size of each list
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884
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941
1030
1058
Q1
Q3
Q2
Q4
JQ4
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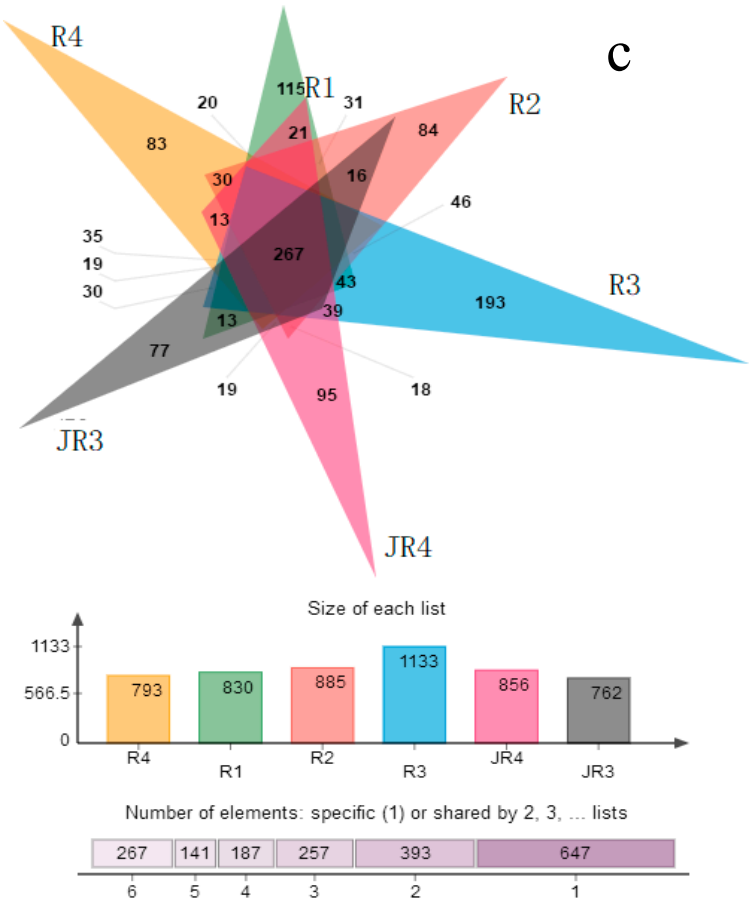
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39
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19
95
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Size of each list
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R4
R1
R2
R3
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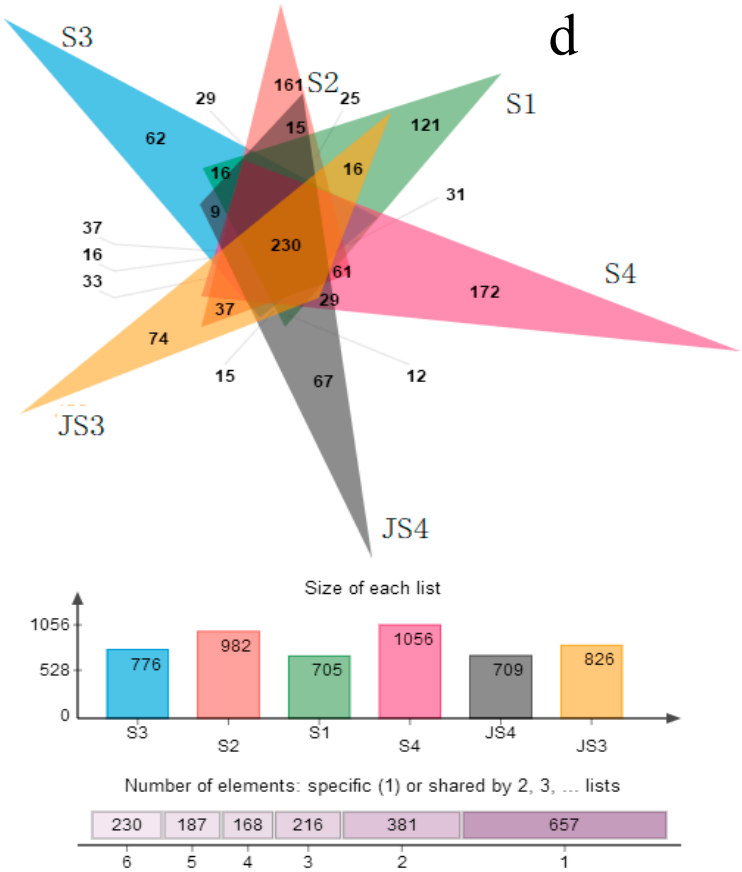
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15
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16
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33
230
61
172
29
37
74
15
67
12
Size of each list
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528
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776
982
705
1056
709
826
S3
S2
S1
S4
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3
2
1

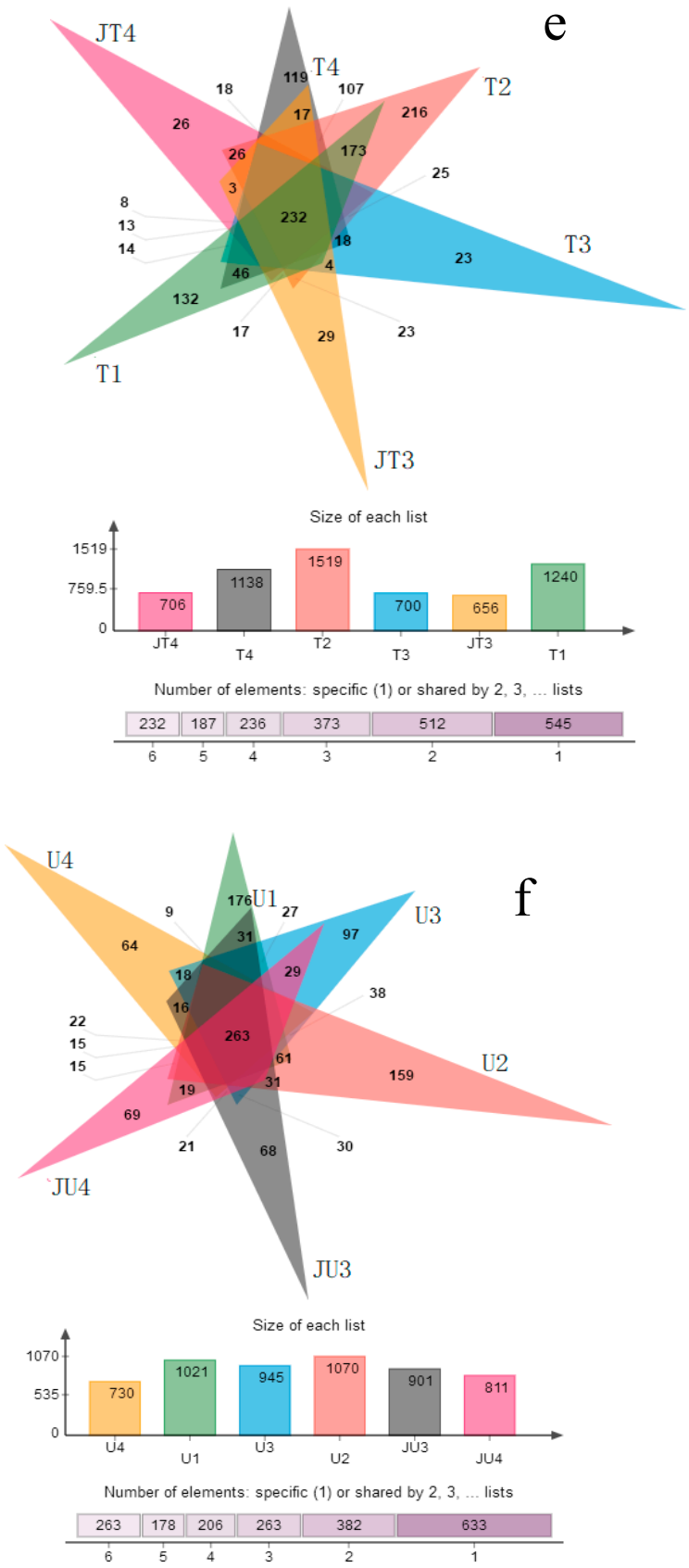

Figure S1 The number of shared and unique OTUs in the samples of the same group.

(a) zinc bacitracin-treated group; (b) ciprofloxacin-treated group; (c) ampicillin sodium-treated group; (d)chloramphenicol-treated group; (e) tylosin-treated group; (f) tetracycline-treated group.

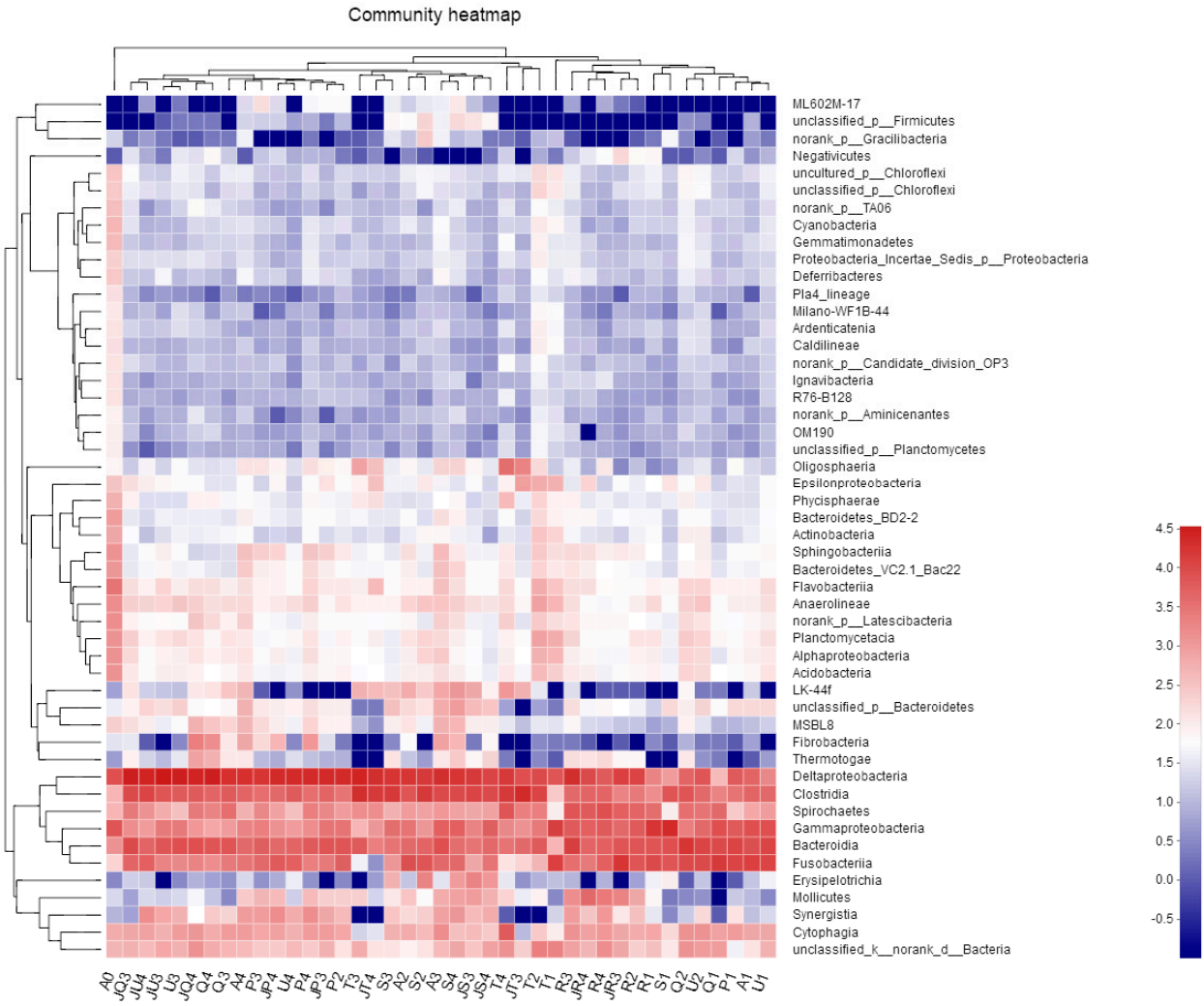

Figure S2 Composition of microbial community at the class level.

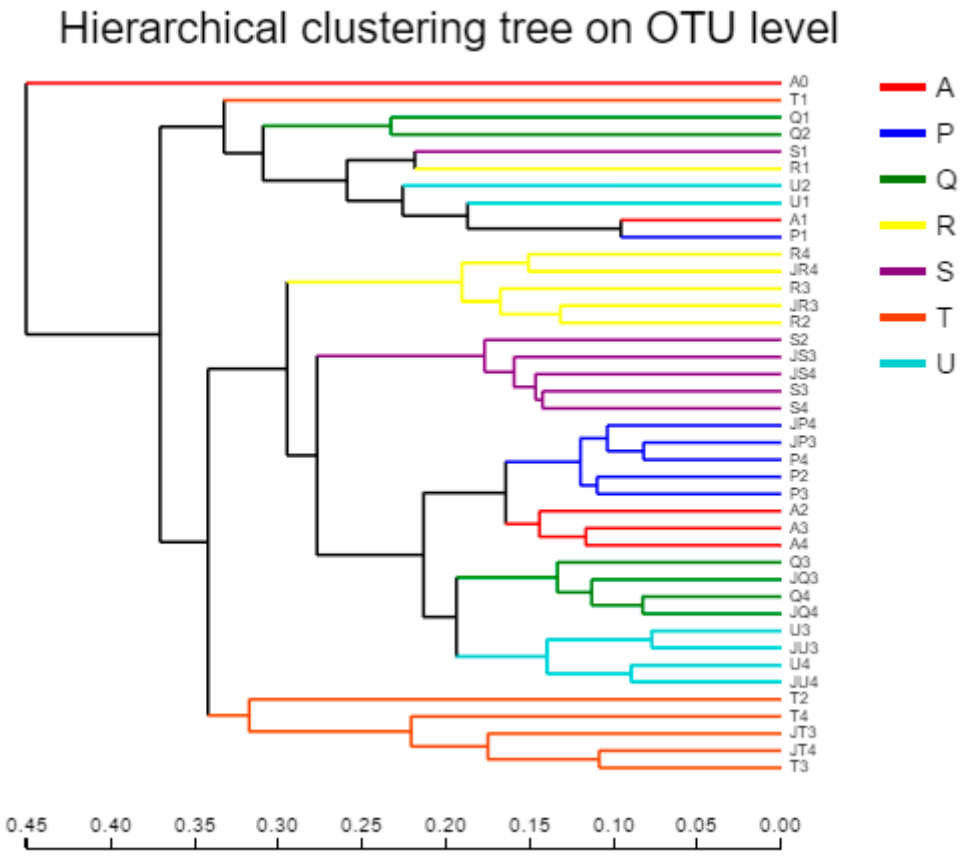

Figure S3 Hierarchical clustering tree at OTU level. A, control group; P, zinc bacitracin-treated group; Q, ciprofloxacin-treated group; R, ampicillin sodium-treated group; S, chloramphenicol-treated group; T, tylosin-treated group; U, tetracycline-treated group.

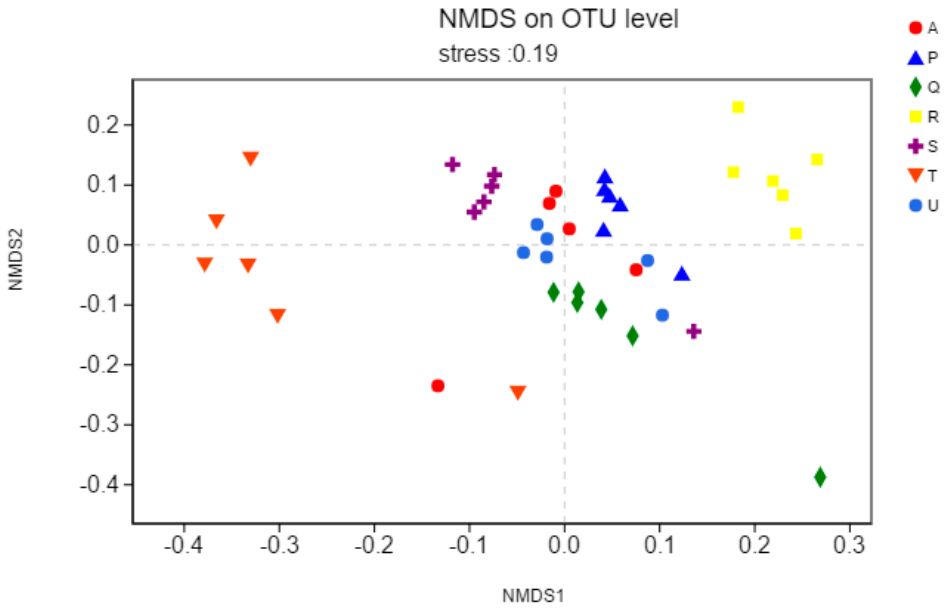

Figure S4 NMDS (Abund-jaccard distance) plot based on OTUs abundance showing the microbial composition variations among different samples. A, control group; P, zinc bacitracin-treated group; Q, ciprofloxacin-treated group; R, ampicillin sodium-treated group; S, chloramphenicol-treated group; T, tylosin-treated group; U, tetracycline-treated group.

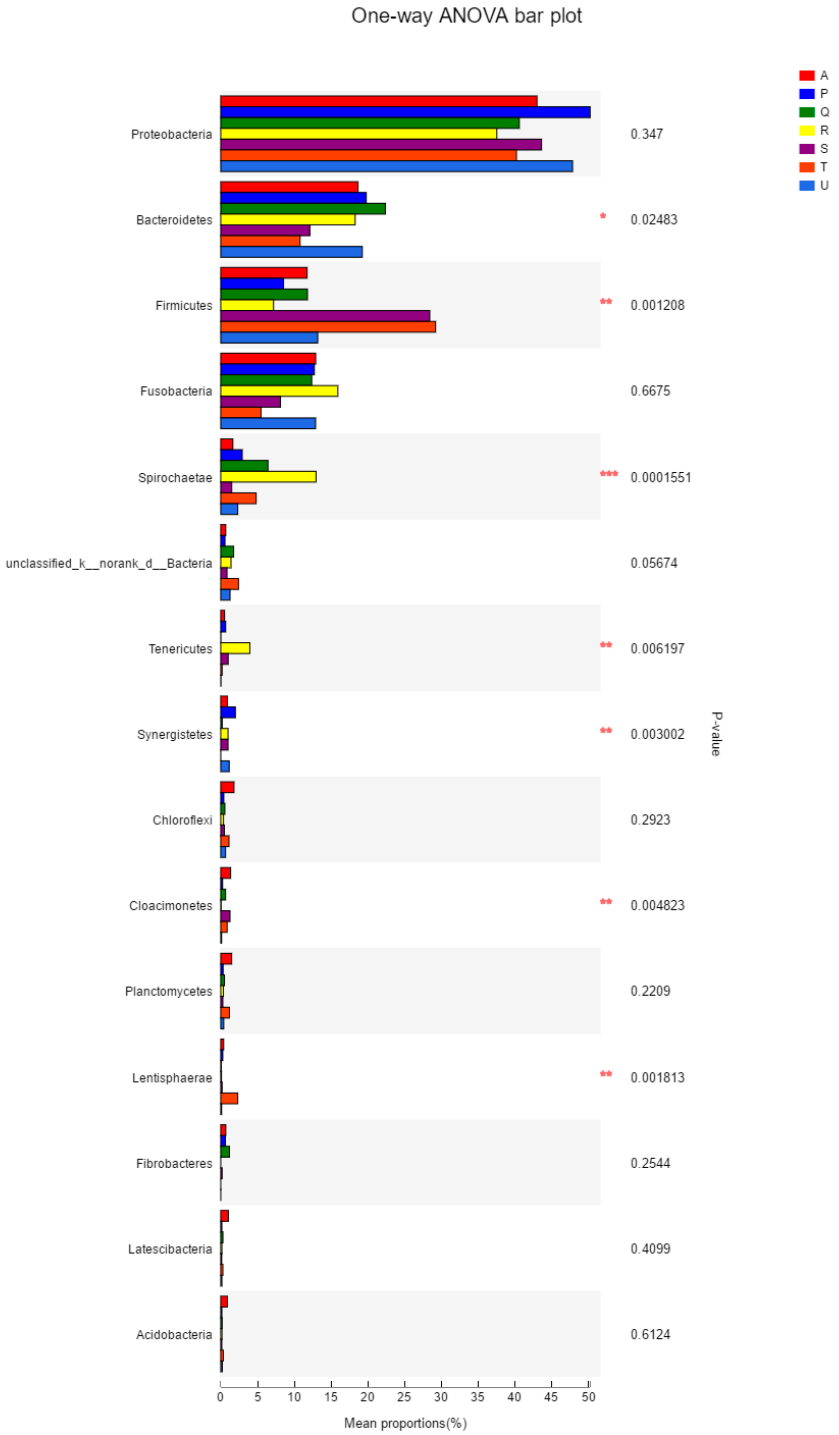

Figure S5 Comparison of control group and different antibiotic-treated groups at phylum level (one-way ANOVA). * $0.01 < P \leq 0.05$, ** $0.001 < P \leq 0.01$, *** $P \leq 0.001$. A, control group; P, zinc bacitracin-treated group; Q, ciprofloxacin-treated group; R, ampicillin sodium-treated group; S, chloramphenicol-treated group; T, tylosin-treated group; U, tetracycline-treated group.

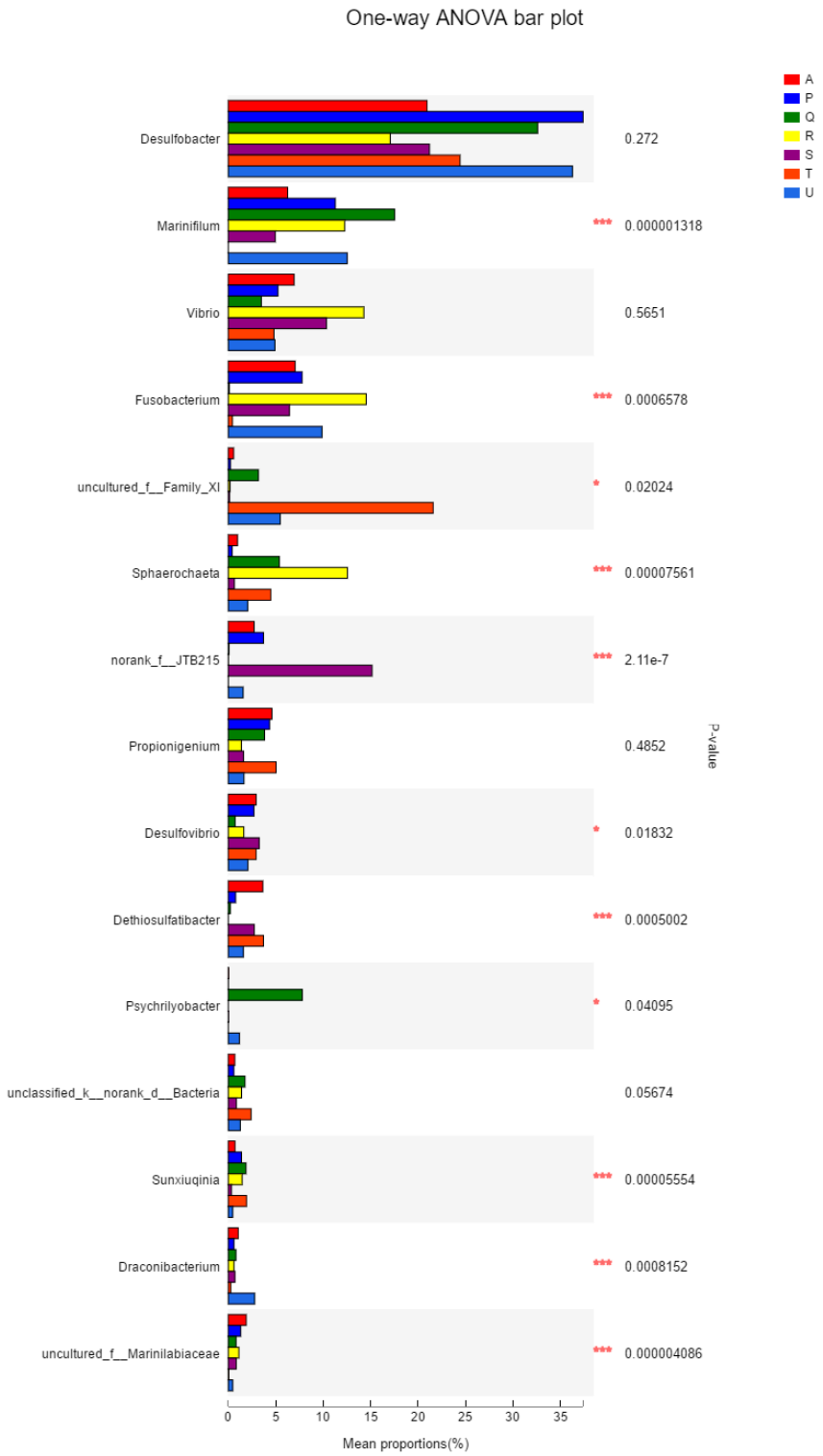

Figure S6 Comparison of control group and different antibiotic-treated groups at genus level (one-way ANOVA). * $0.01 < P \leq 0.05$, ** $0.001 < P \leq 0.01$, *** $P \leq 0.001$. A, control group; P, zinc bacitracin-treated group; Q, ciprofloxacin-treated group; R, ampicillin sodium-treated group; S, chloramphenicol-treated group; T, tylosin-treated group; U, tetracycline-treated group.

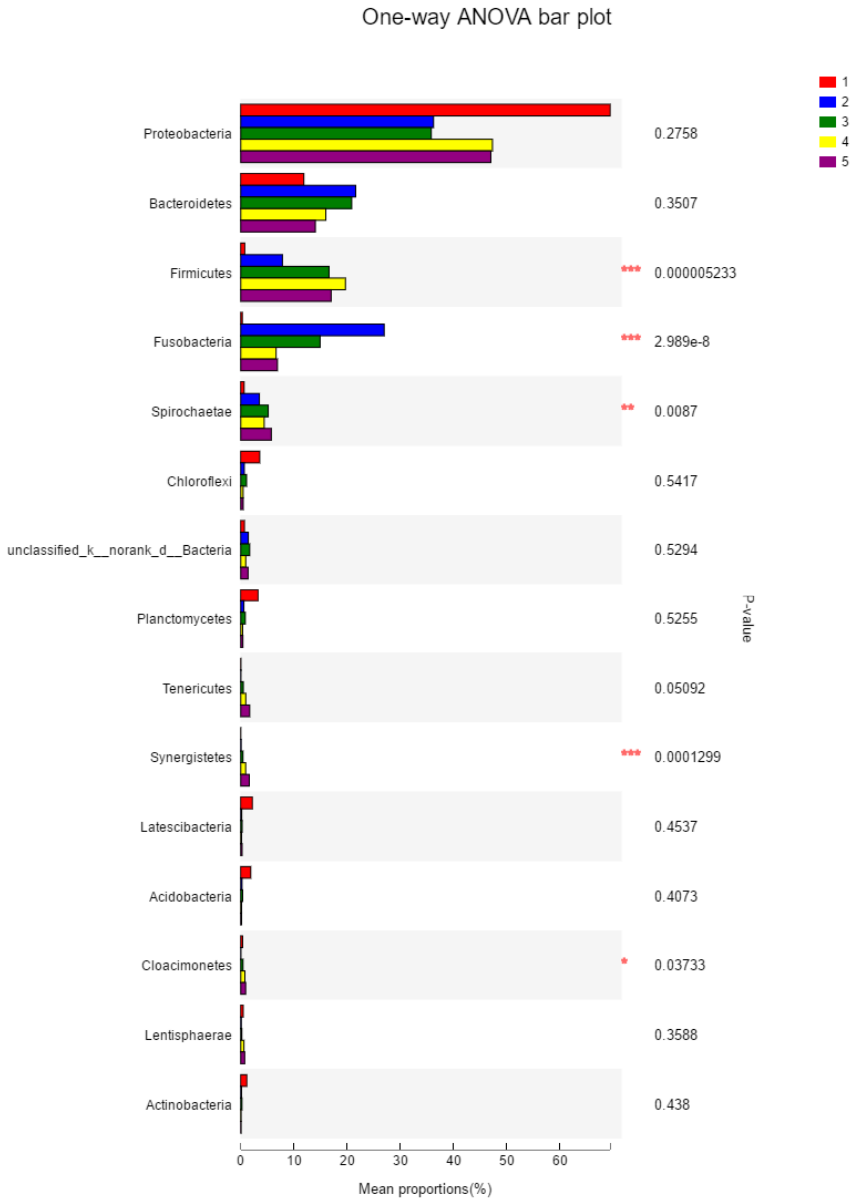

Figure S7 Comparison of samples of different enrichment time at phylum level (one-way ANOVA). * $0.01 < P \leq 0.05$, ** $0.001 < P \leq 0.01$, *** $P \leq 0.001$. 1, the samples without enrichment; 2, the samples incubating for 5 days; 3, the samples incubating for 12 days; 4, the samples incubating for 21 days; 5, the samples incubating for 30 days.

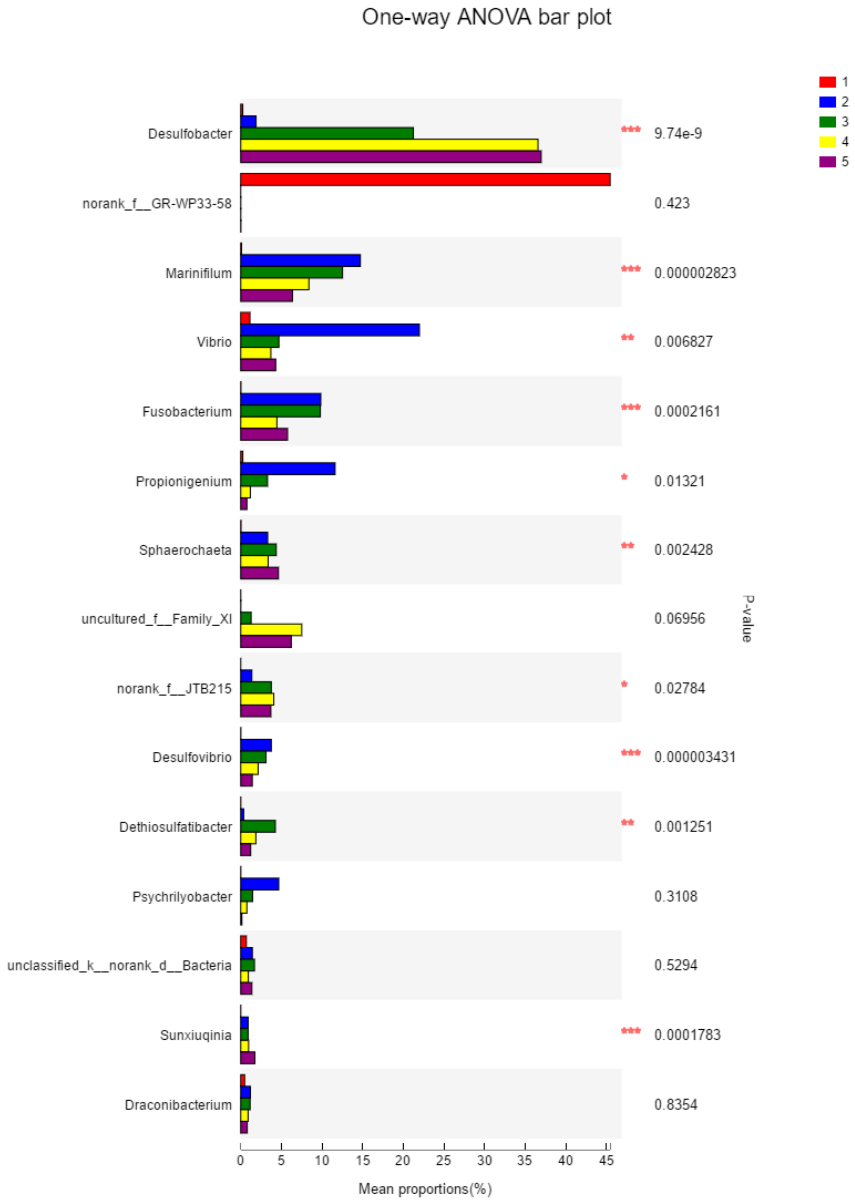

Figure S8 Comparison of samples of different enrichment time at genus level (one-way ANOVA). * $0.01 < P \leq 0.05$, ** $0.001 < P \leq 0.01$, *** $P \leq 0.001$. 1, the samples without enrichment; 2, the samples incubating for 5 days; 3, the samples incubating for 12 days; 4, the samples incubating for 21 days; 5, the samples incubating for 30 days.