

Metagenomic Profiles of Antibiotic Resistance Genes Associated with Bar-headed Goose (*Anser indicus*)

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Abstract

Bar-headed goose (*Anser indicus*), a species endemic to Asia, has become one of the most popular economic species in recent years for wild birds artificial rearing industries in several provinces of China. The use of antibiotics may accelerate the development of antibiotic resistance genes (ARGs) and bacteria which shade health risks to humans and animals. The emerging of ARGs related to Bar-headed goose is becoming an increasing concern. In this study, the profiles of ARGs in both wild and artificially reared Bar-headed geese were investigated using a high-throughput functional metagenomic approach. Analysis of the data showed the existence of a broad-spectrum of different ARGs in both two groups, and the types and subtypes of ARGs were much more abundant and diverse in the artificially reared Bar-headed geese than wild ones. The ARGs related to resistance to streptomycin and trimethoprim have been detected at higher abundance in wild group. ARGs contributed to the resistance to acriflavin, aminoglycoside, beta_lactam, glycylcycline, bacitracin, staphylococcus, fluoroquinolone, thiostrepton and teicoplanin were found to be higher in artificially reared group. These findings provided a broad spectrum profile of ARGs in Bar-headed geese, and may help in the design of future studies to elucidate how artificial rearing environmental factors contributed to the spread of ARGs.

Keywords

Bar-headed Goose, Gut Metagenome, Antibiotic Resistance Genes, High-throughput Sequencing, Artificial Rearing

1. Introduction

Since the introduction in medicine to treat millions of people, antibiotics have considerably reduced infectious mortality and can account for the biggest medical revolution of the 20th century. Unfortunately, due to unmonitored use of antibiotics and the release of residuals into the environment, widespread of antibiotic resistance genes (ARGs) and antibiotic resistant bacteria (ARB) have become a great public concern [1]. The high concentrations of antibiotics used in clinical, agricultural, livestock and poultry settings provide strong selective pressure favoring these ARGs exchange between pathogens and microbes from virtually any ecological niches (e.g. soil, wastewater and gut) [2, 3, 4,

5]. Bacterial communities in these ecological niches can thus be considered as natural reservoirs from where they can spread into other environments and other non-resistant microbial populations [6]. The human gastrointestinal tract contains the largest collection of microorganisms, including harmless symbionts, commensals and opportunistic pathogens, which are collectively named "gut microbiota". The human gut microbiota are also a reservoir of ARGs [7], and studies to examine the human gut resistome (gut microbiota and the ARGs) [8] have identified thousands of ARGs in gut bacteria [9]. Additionally, a growing number of studies have shown that gut microbiota play an important role in several fundamental and crucial processes such as host development [10], immune homeostasis [11], nutrient assimilation [12], vitamins synthesis and sterols metabolism

in the host [13], and diseases (e.g., obesity, diabetes, and cancer) in humans and other animals [14, 15]. Therefore, for the important roles in health and disease, gut microbiota and resistome have become one of the hotspots in microbiological research.

In poultry, the public concern is that the use of antibiotics in birds promotes the growth of ARB that can then be transferred to humans via food processing and distribution systems. Many studies on gut resistome have been conducted on some popular domestic bird species (e.g., chicken, turkey, duck, geese and quail) [16, 17]. However, as a new species for the modern poultry breeding companies, there is therefore no literature available to date on the gut resistome of wild or artificially reared Bar-headed goose (*Anser indicus*). The Bar-headed goose is endemic to Asia, breeding in selected wetlands on the high plateaus of central Asia [18], wintering in the south-central Tibet [19] and India [20]. As one of the dominant waterfowl species in wetland areas in Qinghai-Tibetan Plateau, Bar-headed geese are increasingly being reared in several provinces of China since year 2003 for the purpose of both conservation and economic development [21].

Various methods have been applied in most of the previous studies to detect ARGs in environments, including isolation and culture, PCR, quantitative PCR, multiplex PCR and DNA microarray [22]. However, these methods are limited by the low throughput in ARGs detection. In the last decade, metagenomic technologies combined with next generation sequencing can simultaneously explore a broad-spectrum profile of ARGs, and it has been successfully used in detecting various ARGs in various environments [23, 24]. In our previous studies [25], we have found that the core gut microbiome of Bar-headed geese were dominated by *Firmicutes*, *Proteobacteria*, *Actinobacteria*, and *Bacteroidetes*. Furthermore, *Bacteroidetes* were found to be significant higher in artificially reared Bar-headed geese compared to wild ones [26]. In the present study, we draw attention to the ARGs present in the gut microbiota through metagenomic analysis based on Illumina high throughput sequencing, and the aim of this study was to provide a description of the gut resistome of Bar-headed geese, and to compare resistome differences between wild and artificially reared Bar-headed geese.

2. Materials and Methods

2.1. Ethics Statement

This study was carried out in strict accordance with the Animal Management Rule of the National Health and Family Planning Commission, People's Republic of China (documentation 55, 2001). The research protocol was reviewed and approved by the Animal Care and Use Committee of the Chinese Academy of Sciences. The manuscript does not contain experiments using animals and human studies.

2.2. Faecal Samples Collection

Two groups of faecal samples were obtained in Qinghai province, China, on 2nd July, 2014. The wild Bar-headed

geese faecal samples ($n = 2$) were collected from Ha Da-tan wetland (37°07'41.3"N, 99°43'39.9"E, elevation 3,100 m). The artificially reared (abbreviation: AR) Bar-headed geese faecal samples ($n = 2$) were derived from Bu Ha River Estuary (36°58'25.5"N, 99°50'19.2"E, elevation 3,197 m) in Qinghai Lake. The AR populations lived freely in both wild and captivity environments, fed on both natural and artificial diets (commercial blended feed for chicken). These populations were not treated with antibiotics. About 1 g of faecal samples were collected from faecal balls, avoiding collection of faecal material that was touching the ground. All samples were placed in sterile containers and transported to the laboratory in car-carried refrigerator. In laboratory, faecal samples were kept frozen at -80°C until processing.

2.3. DNA Extraction and Shotgun Metagenomic Sequencing

Genomic DNA was isolated from approximately 1 g of faecal sample using the E.Z.N.A.® stool DNA Kit (Omega Bio-tek, Norcross, GA, USA) following the manufacturer's instruction. DNA concentration and quality were determined using QuantiFluor™ - ST (Promega, MadisonCity, WI, USA) and gel electrophoresis respectively. With the extracted DNA, library construction was performed on an Illumina Hiseq2500 platform according to the standard protocols. Metagenome sequences data are now available at NCBI under the Sequence Read Archive (SRA) database with accession no.SRP072790 and no.SRP072793.

2.4. Bioinformatic Analysis of Sequencing Data

Raw sequences obtained from 4 metagenomic samples were subjected to a quality check using the FastQC software (version v0.11.3) [27]. All samples showed satisfactory values for each parameter tested. Next, the sequences were run through Trimmomatic (version 0.33) [28] to remove low quality base pairs using these parameters (SLIDINGWINDOW: 4:15 MINLEN: 36). Further, the host specific and other eukaryotic sequences were removed by parsing the NCBI non-redundant protein database (NCBI-nr) taxonomy assignment using the lowest common ancestor (LCA) algorithm in MEGAN [29]. All the resulting cleaned sequences obtained from four samples were mixed together and assembled *de novo* using MEGAHIT (version 1.0.2) [30]. From the resulting contigs, microbial genes were predicted using Prodigal (version 2.6.2) [31]. The function assignment of all ORFs was conducted using DIAMOND (version 0.7.9) and BLASTX (version 2.2.31+) against antibiotic resistance genes database. Student's t-test was applied to compare differences of ARGs types and subtypes between the AR and Wild group. A p value of <0.05 was considered statistically significant. All figures were generated with customized R scripts.

3. Results

3.1. Types of ARGs

Through the application of metagenomic approaches, a

more comprehensive profile of the types of ARGs can be created as they are listed in the antibiotic resistance genes database. A total of 80 types of ARGs were detected in wild group (Table A1). Among all of the detected ARGs, the top 5 most abundant types were macab (the average scale number is 282.49), bcr (135.63), mls_abc (53.01), pbp (43.45) and vand (34.44). In the AR group, 82 types of ARGs were found (Table A1). The top 5 most abundant types were macab (196.70), bcr (88.99), vand (59.72), tet_rpp (51.07) and vana (39.76).

Furthermore, types of ARGs in different gut metagenomes can be compared for the analysis of difference. A total of 12 types of ARGs were found to be statistically different between two groups (Fig. 1). Of them, 7 types of ARGs were increased in AR group compared to wild group, including mecr1 (antibiotic resistance: methicillin), mexcd (antibiotic resistance: erythromycin, fluoroquinolone, glycylcycline, roxithromycin), mexef (antibiotic resistance: chloramphenicol, fluoroquinolone), mexhi, mexvw, smedef (antibiotic resistance: fluoroquinolone) and tsnr (antibiotic resistance: thiostrepton). Another 5 types of ARGs were found to be higher in wild group compared to AR group, including mdtl (antibiotic resistance: chloramphenicol), qac (antibiotic resistance: qa_compound), qnr (antibiotic resistance: fluoroquinolone), sul (antibiotic resistance: sulfonamide) and ykk (antibiotic resistance: na_antimicrobials).

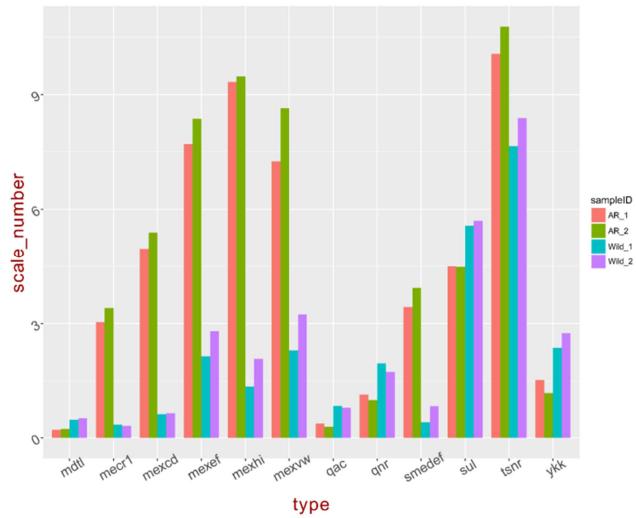


Figure 1. The significantly different ARGs types between AR and wild group ($p < 0.05$).

3.2. Subtypes of ARGs

A more comprehensive profile of ARGs subtypes showed that a total of 247 and 296 subtypes were identified in wild and AR group, respectively (Table A2). Figure 2 showed the 23 significantly different subtypes between both groups. Among them, 13 subtypes were found to be higher in AR group, while the remaining 10 subtypes higher in wild group. All the corresponding antibiotic resistance for these 23 subtypes were listed in Table 1.

Table 1. The significantly different subtypes and the corresponding antibiotic resistance in both the AR and wild group.

Group	Subtypes	Antibiotic resistance
Higher in AR group	Acrb	acriflavin, aminoglycoside, beta_lactam, glycylcycline, macrolide
	Bcr	bacitracin
	Berc	bacitracin
	cata11	chloramphenicol
	ermr	lincosamide, macrolide, streptogramin_b
	mecrl	Staphylococcus
	mexw	-
	Opra	acrilavine, aminoglycoside, macrolide
	Qnrs	fluoroquinolone
	tet40	tetracycline
	Tsnr	thiostrepton
	vanya	teicoplanin, vancomycin
	vanyd	teicoplanin, vancomycin
Higher in wild group	aph33ib	streptomycin
	dfra24	trimethoprim
	dfra26	trimethoprim
	mdtl	chloramphenicol
	mefa	macrolide
	msra	lincosamide, macrolide, streptogramin_b
	Qac	qa_compound
	tet31	tetracycline
	vansg	vancomycin
	ykkc	na_antimicrobials

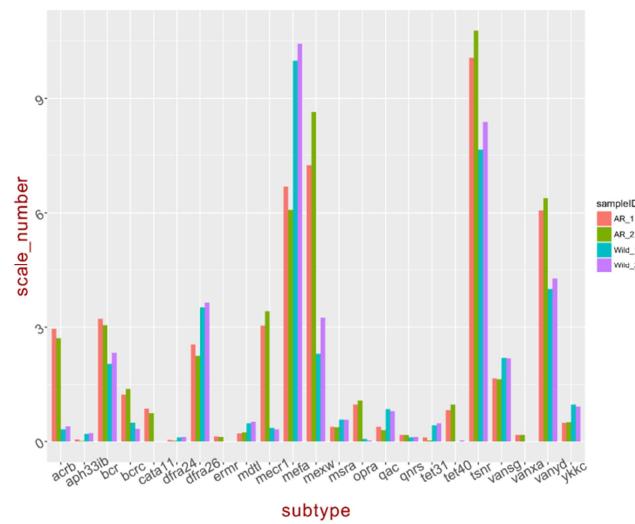


Figure 2. The significantly different ARGs subtypes between AR and wild group ($p < 0.05$).

4. Discussion

To the best of our knowledge, this is the first documented broad-spectrum scan investigation of different ARGs related to Bar-headed geese using the metagenomic approach combined with next generation sequencing. This approach may overcome the drawbacks of PCR / qPCR largely caused by the limitation of primers or the bias in the amplification process. Using this promising technology to study ARGs in both wild and artificially reared samples, as demonstrated in the present study, will help to better understand the changes of ARGs during the early domestication of economically important wildfowls.

The existence of ARGs in wild Bar-headed geese supports the view that antibiotic resistance is naturally originated. Soil, one of the largest and most diverse microbial habitats on

earth, is increasingly recognized as a vast repository of antibiotic resistance genes [32]. Consequently, it is very difficult to find a pristine modern environment that has never been exposed to anthropogenic antibiotics or resistance genes evolving in response to the use of anthropogenic antibiotics. In the present study, one feature of the profiles of ARGs in the wild group which is distinct from those in the AR group was the high abundance of ARGs related to 2 commonly used antibiotics (streptomycin and trimethoprim) (Table 1). Streptomycin was found to be a water-soluble, thermostable, acid- and alkali-labile, levorotatory, basic compound [33]. It is widely used in the treatment of serious bacterial infections, and the primary mechanism of action of streptomycin is by inhibition of binding aminoacyl tRNA to position "A" in elongation phase, which finally causes to stop bacterial protein synthesis [34]. Streptomycin resistance is mainly related to mutation in gene *rpsL* and in lesser amount to gene *rrs* [35]. In the wild Bar-headed geese, the streptomycin resistance mechanism is related to aminoglycoside O-phosphotransferase, which was considered as slightly effective factor. Trimethoprim antagonize the actions of folate by inhibition of dihydrofolate reductase [36]. The mechanisms for resistance against this antibiotics in wild Bar-headed geese are related to *dfra24* and *dfra26*. This may serve to diminish the folate deficiency caused by trimethoprim in this bird.

China is the largest producer and consumer of antibiotics in the world, and more than 46% of antibiotics are used for growth augmentation and disease control in livestock and poultry industries [37]. Therefore, compared to wild Bar-headed geese, the types and subtypes of ARGs were much more abundant and diverse in the artificially reared Bar-headed geese. ARGs encoding resistances to 9 groups of commonly used antibiotics were found to be higher in AR group, including acriflavin, aminoglycoside, beta_lactam, glycyclcycline, bacitracin, staphylococcus, fluoroquinolone, thiostrepton and teicoplanin (Table 1). There are four different mechanisms of antibiotic resistance, including efflux pumps, target modification, target bypass, and the

inactivation of antibiotics [38]. The major mechanism of these 9 resistance in the AR group is efflux pumps (*acrb* and *brcr*) capable of reducing intracellular concentrations of antibiotics.

Another notable finding of this study is that the same antibiotics present in both groups related to the distinctive mechanisms of antibiotic resistance. For instance, the resistance genes regarding macrolide in AR group were *acrb* and *opra* (both of them associated with multidrug resistance efflux pump) (Table 1). However, *mefa* and *msra* were two major macrolide resistance genes in wild group (both of them also associated with multidrug resistance efflux pump). It is highly possible that gut microbes in artificially reared Bar-headed geese develop new resistance genes, mechanisms, or gene mutations to deal with the higher selective pressure exerted by various contaminants in human impacted environments.

5. Conclusion

In summary, metagenomic profiling of ARGs is a good approach to characterize the unique molecular trait of antibiotic resistance in both wild and artificially reared Bar-headed geese since it can provide broader profiles of ARGs. In the present study, various ARGs were found in both two groups. In comparison, the data suggest that he ARGs types and subtypes were much more abundant and diverse in the artificially reared Bar-headed geese than in the wild Bar-headed geese. This study can form a basis for further investigations into gut resistome and the gut microbes harboring them, on much larger flocks of Bar-headed geese.

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Appendix

Table A1. All the detected types of ARGs in each sample. P value was given in the last column.

Types of ARGs	AR_1	AR_2	Wild_1	Wild_2	p-value
vana	34.0546	45.4594	29.6279	30.5085	0.33731
bla_d	0.35618	0.65451	0.30578	0.41418	0.50103
str	0.09981	0.01028	0.09537	0.12546	0.42067
tet_efflux	19.7882	15.6883	17.4106	18.1809	0.98224
sta	4.1789	1.95784	6.43912	6.62371	0.19546
meca	2.01873	1.22635	1.14378	1.0716	0.4162
ere	0.12501	0.00343	0.36507	0.31014	0.09706
mdtef	6.66009	10.3791	0.32797	0.7396	0.14195
catb	1.8765	5.5863	1.42487	1.39232	0.42898
emea	3.27355	0.55197	6.81161	6.8584	0.17162
tet_rpp	46.8772	55.268	30.8981	31.509	0.13088
cata	4.72822	8.23513	0.16893	0.46959	0.17422
mfpa	1.06947	0.22267	1.48157	2.00747	0.1828
emrd	0.06098	0.04777	0.06089	0.12265	0.43352
vane	22.2463	25.318	18.7976	19.3757	0.1911
mexcd	4.97647	5.39641	0.61501	0.64694	0.02844
bcr	100.796	77.1761	134.818	136.44	0.15642
mepa	27.435	41.0395	3.03583	3.87308	0.1373
erm	0.76644	2.15987	0.42503	0.63475	0.40455

Types of ARGs	AR_1	AR_2	Wild_1	Wild_2	p-value
tet_xprt	0.02298	0.05204	0	0	0.23525
tet_flavo	3.79184	6.60643	0.51858	0.52667	0.18609
aac	4.11138	2.45972	6.38241	5.73511	0.14884
cml	4.88094	2.76073	4.61055	4.8827	0.54181
lsa	4.15775	2.44254	7.13141	6.79355	0.13533
blt	0.39634	0.05843	0.89397	1.10148	0.08056
tet_mod	0.32129	0.24365	0.54953	0.74165	0.12752
mls_abc	41.841	36.8362	53.6066	52.4143	0.10029
bla_c	3.36883	2.52557	4.46947	4.61498	0.15577
vanb	34.7479	39.7192	22.5861	26.5793	0.06249
mls_mfs	12.8809	6.69625	22.8726	23.4405	0.14216
pbp	37.0705	32.3377	43.5686	43.3287	0.16745
fos	1.77277	0.78724	3.51755	3.20837	0.12276
ble	0.07298	0.1011	0.12907	0.18383	0.19427
lnu	8.60945	12.5035	0.88037	1.08006	0.1269
mdtl	0.20986	0.23072	0.46915	0.50826	0.01687
mexef	7.68276	8.36995	2.15359	2.8067	0.00729
norm	4.5317	7.24198	0.71194	1.05782	0.16351
qacab	0.57134	0.01419	1.31051	1.37035	0.16194
mdtk	4.0387	6.26302	1.3426	1.36914	0.18144
tcma	2.82789	1.02513	3.92678	4.57433	0.20625
qac	0.37383	0.29265	0.83298	0.78212	0.01735
vang	32.3743	35.3587	30.7197	31.4561	0.30065
rosab	6.88228	5.34305	10.0355	10.6468	0.08064
pmra	2.26661	0.41583	4.66592	5.33387	0.12625
mph	0.24331	0.03605	0.24614	0.36922	0.32336
mdth	0.18854	0.01145	0.473	0.43559	0.14319
vanc	18.3211	20.5537	17.6753	18.4587	0.42261
dfra	3.98212	4.32385	4.50593	4.46074	0.2996
smeabc	1.70353	5.28434	0.15422	0.28494	0.31823
ceo	14.4165	23.4288	1.27939	2.34322	0.1597
bmr	0.27318	1.13415	0.44259	0.41834	0.63998
ksga	6.09889	6.28743	6.40261	6.51917	0.16155
mexvw	7.23472	8.64213	2.303	3.24528	0.03537
baca	8.61133	8.69402	7.61436	7.20758	0.09248
emre	0.31669	0.07912	0.33537	0.46886	0.30346
mexab	14.7442	27.3026	2.03304	2.53691	0.20535
mdtg	1.29508	0.98415	1.77326	1.86984	0.11809
amr	2.64819	4.25399	0.25048	0.3054	0.15741
bla_b	3.16478	3.90536	1.44373	1.4789	0.11183
mexhi	9.32974	9.47402	1.3606	2.08978	0.02472
vat	9.36656	15.9152	5.97983	5.7058	0.28529
mecrl	3.04135	3.41267	0.3464	0.31002	0.03896
smedef	3.43505	3.9321	0.40907	0.82623	0.01211
bla_a	2.11904	4.36121	0.91616	1.05871	0.29293
macab	225.925	167.469	278.17	286.802	0.20267
acr	7.2671	13.3757	1.1417	1.42923	0.20684
mdtm	0.11704	0.05372	0	0	0.22606
mdtnop	6.97281	9.01665	1.55162	2.33662	0.07487
fusb	2.01545	0.04481	5.24673	5.15527	0.14702
tmr	0.34524	0.03062	0.96814	1.0147	0.11679
ant	2.02671	3.70779	2.70293	2.90629	0.95273
ykk	1.52999	1.16814	2.37841	2.75216	0.04291
vand	50.768	68.6663	33.5458	35.3344	0.21366
aph	3.11217	0.25327	4.81559	5.34812	0.24419
qnr	1.12369	0.98228	1.96921	1.74927	0.03657
sul	4.49797	4.4893	5.57217	5.69869	0.03443
adeabc	5.36546	9.94844	0.83069	1.35019	0.20995
arna	11.5994	10.6183	13.9966	15.3133	0.057
tsnr	10.0599	10.767	7.63581	8.38608	0.04337
fush	0.01149	0	0	0.04135	0.6
vanz	0.8338	0.55141	0.85485	0.77069	0.54718
mexxy	1.54617	4.04503	0.13033	0.17929	0.28123

Table A2. All the detected subtypes of ARGs in each sample. P value was given in the last column.

Subtypes of ARGs	AR_1	AR_2	Wild_1	Wild_2	p-value
bcrA	99.585	75.7906	134.329	136.111	0.15441
opcm	0.38905	0.21609	0.24617	0.1836	0.4875
mexy	1.54617	3.90949	0.13033	0.17929	0.27396
tetL	0.76988	0.08227	1.11539	1.38383	0.21992

Subtypes of ARGs	AR_1	AR_2	Wild_1	Wild_2	p-value
dfra22	0.49769	0.12116	0.45655	0.38319	0.66184
bl2b_rob	0.06359	0.0999	0	0	0.13913
vanhd	4.5847	4.33791	4.25374	4.43467	0.53022
vansg	1.66095	1.64111	2.19354	2.18559	0.00406
vata	0.18539	0.3899	0.03969	0.08883	0.25929
lnub	0.09373	0.01933	0.27197	0.24724	0.08921
tetz	0.08015	0.09681	0.08209	0.06856	0.34942
smea	0.49803	1.13169	0.07274	0.12713	0.26355
opra	0.95236	1.05848	0.06263	0.0241	0.0192
arna	11.5994	10.6183	13.9966	15.3133	0.057
vanwb	0.45064	1.12586	0.00604	0.03381	0.2631
aph33ib	0.0485	0.01412	0.19193	0.21479	0.02099
bl3_vim	0.50914	0.37871	0.45417	0.29351	0.57058
nora	2.47336	3.18983	2.87653	2.51778	0.77878
pmra	2.26661	0.41583	4.66592	5.33387	0.12625
cata15	0.25913	0.47371	0.09657	0.14123	0.24923
vang	2.06759	2.74882	2.2947	2.26738	0.77253
mdtk	4.0387	6.26302	1.3426	1.36914	0.18144
aac6ig	0.0204	0.03503	0.05468	0.15908	0.36734
catb3	0.99103	2.73299	0.89501	0.91039	0.46929
ant6ia	1.38338	1.84495	2.51935	2.67087	0.1201
amrb	1.38382	2.1403	0.18786	0.25267	0.15091
bl2_veb	0.02491	0.04762	0	0	0.19325
aac3vi	0	0.07125	0	0	0.5
bl3_cit	0.11048	0.02534	0.06095	0.05115	0.82715
bl2a_iii2	0.06421	0	0.02724	0.0792	0.66189
emre	0.31669	0.07912	0.33537	0.46886	0.30346
tetpb	13.2373	15.5658	9.04682	8.67162	0.12431
cml_e6	3.18938	1.66461	2.40403	2.58346	0.94449
tet36	2.2119	1.88438	0.8568	0.84773	0.08643
cml_e5	0.02962	0.02145	0.07144	0.0533	0.11113
ermr	0.13229	0.11879	0	0	0.0342
smed	1.20068	2.43988	0.29249	0.54296	0.25434
dfra26	2.54597	2.25301	3.51494	3.64238	0.04587
vanra	18.5745	24.5817	17.904	18.3357	0.45458
bl1_asba	0.01262	0.01962	0	0	0.13604
mexf	6.44187	8.06717	1.4835	1.82372	0.08072
ermf	0.00806	0.65906	0	0	0.49221
bl2a_pc	0	0.05948	0	0	0.5
otrb	0.1771	0.13962	0.19533	0.13204	0.9007
qaca	0.29467	0.0023	0.6254	0.7204	0.14626
vanyg	0.36337	0.38122	0.61836	0.78007	0.15066
vanyd	6.06495	6.38243	3.98983	4.26896	0.01059
vantg	6.25134	7.34532	6.23687	6.42097	0.54668
mexb	3.29407	7.5015	1.09282	1.33543	0.29576
opmd	0.15819	0	0.26324	0.41341	0.14095
catal	0.06011	0.07183	0.04971	0.05564	0.22322
lmra	0.81441	0.29719	1.58825	1.62593	0.15192
ble	0.07298	0.1011	0.12907	0.18383	0.19427
vansa	8.65866	13.0214	5.13532	5.47805	0.23727
aac6iia	0.00581	0.04947	0.05607	0.02129	0.7323
fosc	0	0.01432	0	0	0.5
vante	0.29901	0.49251	0.19306	0.32279	0.37284
emrd	0.06098	0.04777	0.06089	0.12265	0.43352
tet41	0.12614	0.05458	0.18992	0.29121	0.15022
mexi	4.94742	3.26434	0.88603	1.38723	0.15257
lmrp	1.22165	0.03454	3.32729	3.21436	0.13792
bl1_och	0.01574	0	0.05063	0.05666	0.07673
aac3iii	0	0.00604	0	0	0.5
qnra	0.68291	0.31188	1.47052	1.27921	0.08404
bcr	3.21467	3.05006	2.04006	2.3261	0.04706
adeb	1.28553	1.62526	0.58404	0.84315	0.08126
bcrc	1.21115	1.38554	0.48823	0.32836	0.01757
bl2be_ctxm	0	0.01261	0	0	0.5
ant3ia	0.09326	0.06223	0.04069	0.04554	0.26175
mexd	3.43729	4.35811	0.53758	0.52455	0.08647
vanha	3.86797	5.82398	3.12861	3.42076	0.34986
mdte	4.30233	5.78057	0.09913	0.28804	0.09189
aac2ia	0.19962	0.03251	0.27207	0.18234	0.39173
tetj	0.04152	0.00127	0.06163	0.06897	0.26517

Subtypes of ARGs	AR_1	AR_2	Wild_1	Wild_2	p-value
cata3	1.10631	2.52671	0	0	0.23727
mdtm	0.11704	0.05372	0	0	0.22606
mphb	0.092	0.00304	0.11586	0.18583	0.21636
vatd	0.10493	0.11358	0.09143	0.08569	0.07196
vatc	0.15307	0.20052	0.29226	0.25821	0.08891
dfra24	0.03867	0.01748	0.10657	0.11926	0.03379
aph3ic	0.01579	0	0.06039	0.11458	0.18699
teta	0.08884	0.01881	0.19072	0.11109	0.21066
bl1_fox	0.1163	0.33382	0.07606	0.24659	0.69222
cata14	0.37752	0.63482	0	0	0.15845
srmb	1.59144	3.83305	0.71893	0.84134	0.33396
dfra12	0.0073	0	0.08213	0.02626	0.31746
vcaa	7.91734	7.13505	8.995	9.07146	0.15875
mpha	0.04067	0.03242	0	0	0.07159
pac	0.10669	0.02341	0	0	0.36246
vand	1.14235	1.8163	0.47181	0.74213	0.2004
lmrb	5.3881	0.32362	11.3048	11.3851	0.18447
cml_e7	0.6091	0.30874	0.86929	0.78456	0.2271
vanc	0.50691	0.56014	0.21186	0.30343	0.0542
ceob	8.65976	12.503	0.48189	0.79116	0.1196
vanxyc	0.16429	0.39591	0.03314	0	0.2581
aph3iiia	0.21776	0.12236	0.18804	0.14255	0.93908
mpfc	0.11065	0.00059	0.13028	0.18339	0.28452
tetq	1.85338	1.66099	1.03363	1.33883	0.10781
bl2d_lcr1	0.03895	0.04148	0.04175	0	0.52399
ant2ia	0	0	0.05358	0.06996	0.08389
tetc	16.2674	13.318	14.0425	14.3948	0.76379
ykkc	0.47709	0.50039	0.94983	0.90177	0.01291
cata5	0	0.02317	0	0	0.5
bl1_sm	1.66865	1.51782	1.86552	1.88495	0.16136
vanxd	0.40808	1.13905	0	0.01705	0.28358
bl2c_bro	0.09998	0.00343	0.2716	0.24812	0.1288
tett	7.82493	11.264	4.2008	4.02147	0.19429
sull	0.60539	0.46439	0.35519	0.31812	0.19931
fosx	0	0.0129	0	0	0.5
tmrb	0.34524	0.03062	0.96814	1.0147	0.11679
vanya	1.67725	0.96248	3.16168	3.00709	0.11434
vanyb	1.10721	2.28187	0.67995	0.6852	0.33478
aac6ic	0.35929	0.22589	0.39087	0.27093	0.71132
pbp2x	5.29296	2.12498	9.79554	9.34367	0.16196
bl2e_cfxa	0.05028	0.76811	0	0.00559	0.46054
pbp1a	13.2899	9.39916	17.7721	17.3445	0.18951
fosa	0.15832	0.01005	0.40528	0.44279	0.11945
aac3ix	0.15746	0.07132	0.18763	0.19028	0.33323
oprm	9.4237	17.1619	0.54619	0.67188	0.18841
vanxa	0.16796	0.16535	0	0	0.00497
vatb	8.61682	14.6898	5.30014	5.11076	0.27993
mxh	4.38232	6.20968	0.47457	0.70255	0.11752
ermt	0.03418	0.1277	0	0	0.33351
mefa	6.67834	6.07544	9.97953	10.4294	0.01229
ermh	0.01227	0.00856	0	0	0.1123
aph6id	0.09157	0.00928	0.2462	0.17294	0.10342
qnrb	0.27216	0.49824	0.3905	0.35763	0.93764
bl1_cmy2	0.15253	0.10576	0.18613	0.31784	0.29
amra	0.31201	1.05521	0	0.02863	0.3223
tetv	0.30274	0.00434	0.66684	0.64717	0.18207
bl1_mox	0.2547	0.16961	0.6044	0.44771	0.10341
tet40	0.81197	0.95514	0	0.02685	0.04533
bl2f_nmca	0	0.03208	0	0	0.5
cml_e1	0.04166	0.02451	0	0	0.16146
mfpa	1.06947	0.22267	1.48157	2.00747	0.1828
emea	3.27355	0.55197	6.81161	6.8584	0.17162
aac6ie	0.50552	0.19391	0.6064	0.43412	0.46262
pbp1b	4.63671	3.3639	5.51829	5.92514	0.20052
bl3_imp	0.18257	0.0802	0.2656	0.34794	0.12186
meca	2.01873	1.22635	1.14378	1.0716	0.4162
mepa	27.435	41.0395	3.03583	3.87308	0.1373
oprj	1.04786	0.7017	0.07743	0.12239	0.13489
sul2	0.46597	0.15473	0.27196	0.2878	0.87695
bl1_acc	0.2771	0.08007	0.23574	0.26245	0.60384

Subtypes of ARGs	AR_1	AR_2	Wild_1	Wild_2	p-value
catb1	0.38459	2.31181	0.2719	0.3205	0.47202
ereb	0.12501	0.00343	0.36507	0.31014	0.09706
cata4	0.03652	0.06732	0	0	0.18354
cata16	1.17827	0.94678	0	0	0.06908
vangr	13.674	12.8244	10.3458	10.2275	0.08555
bl1_pse	0.27108	0.03829	0.3823	0.51107	0.1941
bl2_ges	0	0.02736	0	0	0.5
aph6ia	0.08532	0.00118	0.15557	0.1627	0.21958
otra	3.07361	3.71598	1.87612	1.80717	0.1264
ermv	0.01304	0.00087	0.05591	0.02346	0.26602
bl2a_nps	0.04961	0.04373	0.15952	0.20598	0.10315
teto	0.94696	2.37548	0.56116	0.64604	0.37745
vanxyg	0.46911	0.36778	0.51776	0.4514	0.40397
tsnr	10.0599	10.767	7.63581	8.38608	0.04337
tetm	3.61543	4.35043	2.4469	2.52946	0.14966
acra	1.55999	2.46943	0.26159	0.26158	0.16156
vansb	5.83735	3.93755	3.88824	4.06826	0.5131
vanug	6.80647	8.96466	7.44102	8.14827	0.94732
smef	0.84493	0.52617	0.07614	0.08431	0.1637
macb	225.925	167.469	278.17	286.802	0.20267
fusb	2.01545	0.04481	5.24673	5.15527	0.14702
cata6	0.03757	0.04297	0	0.09994	0.878
ermv	0.00847	0.05008	0	0	0.3933
aad9ib	0.55007	1.80061	0.0893	0.11992	0.33635
tcma	2.82789	1.02513	3.92678	4.57433	0.20625
tet31	0.10184	0.02599	0.41695	0.47308	0.01893
tolc	2.74494	8.19867	0.56681	0.7757	0.32852
bl2d_oxa10	0.26927	0.40829	0.18497	0.36994	0.65261
aac6ib	1.87509	0.14868	3.52363	3.34237	0.21478
baca	8.61133	8.69402	7.61436	7.20758	0.09248
smeb	0.43381	0.16615	0	0	0.26714
lnua	8.51572	12.4842	0.6084	0.83282	0.12648
vanb	0.87505	0.58349	0	0.03628	0.12417
adec	1.82658	3.94473	0.16106	0.29447	0.24025
ermg	0.00905	0.03508	0	0	0.33932
vanxb	0.0334	0.17783	0	0.01384	0.40054
mexc	0.49132	0.33661	0	0	0.11761
blt	0.39634	0.05843	0.89397	1.10148	0.08056
catb2	0.50088	0.48245	0.25795	0.16144	0.09808
tet37	3.79184	6.60643	0.51858	0.52667	0.18609
tetx	0.32129	0.24365	0.54953	0.74165	0.12752
tet38	0.00543	0.02672	0	0	0.37228
oleb	4.16753	2.70224	6.04876	5.14205	0.15324
acrb	2.96217	2.70764	0.31329	0.39195	0.02057
aac6i	0.05912	0.25974	0	0	0.35753
vanhb	4.91679	5.93497	2.65704	3.1226	0.08416
cata11	0.84895	0.72773	0	0	0.04885
cata13	0.61465	2.12677	0	0.07144	0.32753
tcr3	0.28012	0.10399	0.24078	0.25672	0.63564
vansd	35.8433	51.3742	22.9392	23.7407	0.23214
tet34	0.02298	0.05204	0	0	0.23525
aph3iva	0.31685	0.07451	0.78809	0.61501	0.0881
pbp2	8.07791	8.80698	8.33086	8.26798	0.76203
ermd	0	0.00803	0	0.01451	0.74291
bl2be_shv2	0	0.03492	0	0	0.5
sme	1.38944	0.96604	0.04044	0.19896	0.09368
catb4	0	0.02999	0	0	0.5
vanse	3.5221	3.71902	3.25357	3.32978	0.15042
dfra20	0.89249	1.9322	0.34573	0.28965	0.28148
oprn	0.58081	0.02921	0.52411	0.70644	0.45287
aph3va	0	0.01532	0	0	0.5
mexw	7.23472	8.64213	2.303	3.24528	0.03537
tlrc	5.11941	4.43619	7.1032	7.34219	0.06271
rosa	0.82866	1.08025	0.9987	1.25883	0.43713
vant	0.6017	0.66877	0.74528	0.70943	0.17389
tetpa	0.5488	0.89536	0.07707	0.08009	0.16743
aph3ia	2.2098	0.01575	3.01963	3.83852	0.25391
tets	3.65703	2.42102	4.73957	5.38243	0.13917
ermx	0.11907	0.00672	0.20362	0.41781	0.21652
aac3iia	0	0.00879	0	0	0.5

Subtypes of ARGs	AR_1	AR_2	Wild_1	Wild_2	p-value
ermB	0	0.01928	0	0	0.5
vanRE	14.4421	16.8206	10.8396	10.9188	0.15577
vanXYE	0.22966	0.08908	0.43349	0.35683	0.13236
tet32	4.45313	3.84762	2.07161	2.12223	0.09128
mecR1	3.04135	3.41267	0.3464	0.31002	0.03896
bl3_cpha	0	0.01851	0	0	0.5
bl1_ec	0.27778	0.1792	0.38896	0.26078	0.36224
vanz	0.8338	0.55141	0.85485	0.77069	0.54718
fush	0.01149	0	0	0.04135	0.6
cml_e4	0.52085	0.52264	0.53405	0.49262	0.75444
ykkD	1.0529	0.66776	1.42857	1.85039	0.11318
vgab	6.49506	4.4718	10.8591	10.8439	0.11857
mdfa	0.08637	0.22887	0	0	0.27028
bl2e_cbla	0	0.16569	0	0	0.5
aac3viii	0.37742	0.22103	0.62837	0.61325	0.14901
mdth	0.18854	0.01145	0.473	0.43559	0.14319
aac3ia	0.08397	0.14002	0.2904	0.18186	0.2201
aac6iib	0.06736	0.16039	0.1124	0.15887	0.72856
pbp2b	5.77308	8.64264	2.15179	2.44734	0.17782
tet	0	0.0523	0	0	0.5
mdtf	2.35776	4.59848	0.22884	0.45156	0.21539
qac	0.37383	0.29265	0.83298	0.78212	0.01735
vansc	7.28721	9.94062	8.28222	8.16205	0.81722
smeC	0.77169	3.98649	0.08148	0.1578	0.39355
aac3x	0.11893	0.02917	0	0	0.34687
vate	0.30635	0.52142	0.25629	0.16231	0.27735
cara	16.1723	13.8927	19.3147	18.6116	0.15508
tet39	0.0643	0.01535	0.06853	0.04881	0.58029
mdtg	1.29508	0.98415	1.77326	1.86984	0.11809
vanwg	1.08152	1.08542	1.0717	0.975	0.43096
bl1_ampC	0.19282	0.05303	0.33771	0.29021	0.19578
bl2d_oxa2	0.00819	0	0.07905	0.04423	0.17161
aac3iv	0	0.02406	0	0	0.5
mdtp	0.17139	0.00368	0.19467	0.33174	0.25109
mdtn	6.80141	9.01297	1.35695	2.00488	0.09074
adeA	2.25334	4.37845	0.0856	0.21258	0.20501
pur8	0.36698	0.0163	0.43957	0.53827	0.32434
qaCB	0.27667	0.01189	0.68511	0.64995	0.15247
mdr	4.33312	0.29994	8.48858	8.99943	0.18874
mexA	2.02647	2.63924	0.39402	0.5296	0.09064
ksgA	6.09889	6.28743	6.40261	6.51917	0.16155
bmr	0.27318	1.13415	0.44259	0.41834	0.63998
vanRD	2.7247	3.61641	1.89114	2.1309	0.21522
bl3_1	2.36259	3.4026	0.66302	0.7863	0.1463
rosB	6.05362	4.2628	9.03678	9.38798	0.12745
aph6ic	0.12659	0.00074	0.16574	0.08703	0.50129
lsa	4.15775	2.44254	7.13141	6.79355	0.13533
catB5	0	0.02905	0	0	0.5
vanRB	21.5275	25.6776	15.3548	18.6194	0.13597
tetW	6.00361	8.12997	4.06466	4.142	0.21889
ermA	0.04787	0.00114	0.12661	0.08307	0.12891
cml_e3	0.1753	0.05877	0.11654	0.11255	0.97276
mexE	0.66008	0.27357	0.14599	0.27655	0.39836
qrns	0.16862	0.17216	0.10819	0.11243	0.00244
bl1_pao	0.1295	0.02835	0.34202	0.33672	0.12128
norm	4.5317	7.24198	0.71194	1.05782	0.16351
mdlT	0.20986	0.23072	0.46915	0.50826	0.01687
fosB	1.61445	0.74997	3.11228	2.76558	0.11701
erme	0.12034	0.21668	0.03889	0.07254	0.23008
cata2	0.13794	0.47158	0	0	0.31884
ermQ	0.2618	0.90788	0	0.02336	0.32643
sul3	3.42661	3.87018	4.94502	5.09277	0.07692
teth	0.0136	0.00136	0.02734	0.03059	0.15757
msra	0.37788	0.36518	0.56699	0.56172	0.00821
bl2a_1	0.08908	0.14169	0.20416	0.23208	0.10834
tet33	0.01149	0	0.03695	0.09304	0.27111
bl2b_tle	0	0.02645	0	0	0.5
cata8	0.07125	0.12174	0.02264	0.10134	0.54869
sta	4.1789	1.95784	6.43912	6.62371	0.19546
aac6ia	0.1614	0.59384	0.2599	0.18071	0.59836

Subtypes of ARGs	AR_1	AR_2	Wild_1	Wild_2	p-value
bl2a_iii	0.05904	0.0306	0.11252	0.13192	0.05771
str	0.09981	0.01028	0.09537	0.12546	0.42067
aac6if	0.11997	0.18859	0	0	0.13931
vana	1.10824	0.90455	0.29825	0.26694	0.08286
tetb	0.22306	0.00328	0.18848	0.36524	0.37021
bl2d_oxa5	0.03976	0.20474	0	0	0.37788
mexx	0	0.13554	0	0	0.5
vanrc	9.761	8.98824	8.40283	9.28383	0.46165
cml_e8	0.31502	0.16001	0.61521	0.85621	0.09195
ceoa	5.36769	10.7097	0.55133	1.36845	0.22306
bl2e_cepa	1.53581	2.85401	0	0.01164	0.18616
bl2b_tem	0.08255	0.01353	0.14111	0.14418	0.22207
vane	3.75337	4.19675	4.07786	4.44757	0.42686

References

- [1] Wright GD. The antibiotic resistome: the nexus of chemical and genetic diversity. *Nat. Rev. Microbiol.* 2007; 5(3): 175-186.
- [2] Dantas G and Sommer MO. Context matters - the complex interplay between resistome genotypes and resistance phenotypes. *Curr. Opin. Microbiol.* 2012; 15(5): 577-582.
- [3] Sommer MO, Dantas G and Church GM. Functional characterization of the antibiotic resistance reservoir in the human microflora. *Science*. 2009; 325(5944): 1128-1131.
- [4] Forsberg KJ, Patel S, Gibson MK, Lauber CL, Knight R, Fierer N and Dantas G. Bacterial phylogeny structures soil resistomes across habitats. *Nature*. 2014; 509(7502): 612-616.
- [5] Durso LM, Miller DN and Wienhold BJ. Distribution and quantification of antibiotic resistant genes and bacteria across agricultural and non-agricultural metagenomes. *PLoS one*. 2012; 7(11): e48325.
- [6] Allen HK, Donato J, Wang HH, Cloud-Hansen KA, Davies J and Handelsman J. Call of the wild: antibiotic resistance genes in natural environments. *Nat. Rev. Microbiol.* 2010; 8(4): 251-259.
- [7] van Schaik W. The human gut resistome. *Phil. Trans. R. Soc. B*. 2015; 370(1670): 20140087.
- [8] Sommer MO and Dantas G. Antibiotics and the resistant microbiome. *Curr. Opin. Microbiol.* 2011; 14(5): 556-563.
- [9] Hu Y, Yang X, Qin J, Lu N, Cheng G, Wu N, Pan Y, Li J, Zhu L, Wang X, Meng Z, Zhao F, Liu D, Ma J, Qin N, Xiang C, Xiao Y, Li L, Yang H, Wang J, Yang R, Gao GF, Wang J and Zhu B. Metagenome-wide analysis of antibiotic resistance genes in a large cohort of human gut microbiota. *Nat. Commun.* 2013; 4: 2151.
- [10] Malmuthuge N, Griebel PJ and Guan L. The Gut Microbiome and Its Potential Role in the Development and Function of Newborn Calf Gastrointestinal Tract. *Front. Vet. Sci.* 2015; 2: 36.
- [11] Ahern PP, Faith JJ and Gordon JI. Mining the human gut microbiota for effector strains that shape the immune system. *Immunity*. 2014; 40(6): 815-823.
- [12] Kau AL, Ahern PP, Griffin NW, Goodman AL and Gordon JI. Human nutrition, the gut microbiome and the immune system. *Nature*. 2011; 474(7351): 327-336.
- [13] O'Mahony SM, Clarke G, Borre YE, Dinan TG and Cryan JF. Serotonin, tryptophan metabolism and the brain-gut-microbiome axis. *Behav. Brain. Res.* 2015; 277: 32-48.
- [14] Lee WJ and Hase K. Gut microbiota-generated metabolites in animal health and disease. *Nat. Chem. Biol.* 2014; 10(6): 416-424.
- [15] Kinross JM, Darzi AW and Nicholson JK. Gut microbiome-host interactions in health and disease. *Genome Med.* 2011; 3(3): 14.
- [16] Singer RS and Hofacre CL. Potential impacts of antibiotic use in poultry production. *Avian. Dis.* 2006; 50(2): 161-172.
- [17] Faldynova M, Videnska P, Havlickova H, et al. Prevalence of antibiotic resistance genes in faecal samples from cattle, pigs and poultry. *Vet. Med.* 2013; 58(6): 298-304.
- [18] Takekawa J Y, Heath S R, Douglas D C, et al. Geographic variation in Bar-headed Geese Anser indicus: Connectivity of wintering areas and breeding grounds across a broad front. *Wildfowl*. 2012; 59(2009): 100-123.
- [19] Bishop MA, Song YL, Canjue ZM and Gu BY. Bar-headed Geese Anser indicus wintering in South-central Tibet. *Wildfowl*. 1997; 48: 118-126.
- [20] Javed S, Takekawa J Y, Rahmani A R, et al. Tracking the spring migration of a Bar-headed Goose (Anser indicus) across the Himalaya with satellite telemetry. *Global. Environ. Res.* 2000; 4: 195-205.
- [21] Feare CJ, Kato T and Thomas R. Captive rearing and release of Bar-headed Geese (Anser indicus) in China: a possible HPAI H5N1 virus infection route to wild birds. *J. Wildl. Dis.* 2010; 46: 1340-1342.
- [22] Card R, Zhang J, Das P, Cook C, Woodford N and Anjum MF. Evaluation of an expanded microarray for detecting antibiotic resistance genes in a broad range of gram-negative bacterial pathogens. *Antimicrob. Agents Ch.* 2013; 57(1): 458-465.
- [23] Elbeherly AH, Aziz RK and Siam R. Antibiotic Resistome: Improving Detection and Quantification Accuracy for Comparative Metagenomics. *Omics*. 2016; 20(4): 229-238.
- [24] Xiao KQ, Li B, Ma L, Bao P, Zhou X, Zhang T and Zhu YG. Metagenomic profiles of antibiotic resistance genes in paddy soils from South China. *FEMS. Microbiol. Ecol.* 2016; 92(3).
- [25] Wang W, Cao J, Yang F, Wang X, Zheng S, Sharshov K and Li L. High-throughput sequencing reveals the core gut microbiome of Bar-headed goose (Anser indicus) in different wintering areas in Tibet. *MicrobiologyOpen*. 2016; 5(2): 287-295.
- [26] Wang W, Cao J, Li JR, Yang F, Li Z and Li LX. Comparative analysis of the gastrointestinal microbial communities of bar-headed goose (Anser indicus) in different breeding patterns by high-throughput sequencing. *Microbiol. Res.* 2016; 182: 59-67.

- [27] Andrews S, et al. FastQC: A Quality Control tool for High Throughput Sequence Data. 2012; <http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>.
- [28] Bolger AM, Marc L and Bjoern U. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*. 2014; 30: 2114-2120.
- [29] Huson DH, Auch AF, Qi J and Schuster SC. MEGAN analysis of metagenomic data. *Genome. Res.* 2007; 17: 377-386.
- [30] Li D, Liu CM, Luo R, Sadakane K and Lam TW. Megahit: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct de bruijn graph. *Bioinformatics*. 2015; 31: 1674-1676.
- [31] Hyatt D, Locascio PF, Hauser LJ and Uberbacher EC. Gene and translation initiation site prediction in metagenomic sequences. *Bioinformatics*. 2012; 28: 2223-2230.
- [32] Forsberg KJ, Reyes A, Wang B, Selleck EM, Sommer MO and Dantas G. The shared antibiotic resistome of soil bacteria and human pathogens. *Science*. 2012; 337(6098): 1107-1111.
- [33] Dyer JR and Todd AW. The Absolute Configuration of Streptidine in Streptomycin. *J. Vasc. Intervent. Radiol.* 2015; 26(2): S115.
- [34] Iscla I, Wray R, Wei S, Posner B and Blount P. Streptomycin potency is dependent on MscL channel expression. *Nat. Commun.* 2014; 5: 4891.
- [35] Arjomandzadegan M and Gravand S. Analysis of rpsL and rrs genes mutations related to streptomycin resistance in Mdr and Xdr clinical isolates of *Mycobacterium tuberculosis*. *Tuberkulozve. Toraks.* 2015; 63(4): 235-242.
- [36] Falagas ME, Vardakas KZ and Roussos NS. Trimethoprim/sulfamethoxazole for *Acinetobacter* spp.: A review of current microbiological and clinical evidence. *Int. J. Antimicrob. Ag.* 2015; 46(3): 231-241.
- [37] Zhu YG, Johnson TA, Su JQ, Qiao M, Guo GX, Stedtfeld RD, et al. Diverse and abundant antibiotic resistance genes in Chinese swine farms. *Proc. Natl. Acad. Sci. USA*. 2013; 110: 3435-3440.
- [38] Zhang XX, Zhang T, and Fang HH. Antibiotic resistance genes in water environment. *Appl. Microbiol. Biotechnol.* 2009; 82(3): 397-414.