

Figure S1. Bray-Curtis dissimilarity plots showing the genera detected on carcasses from the conventional and antibiotic free farm.

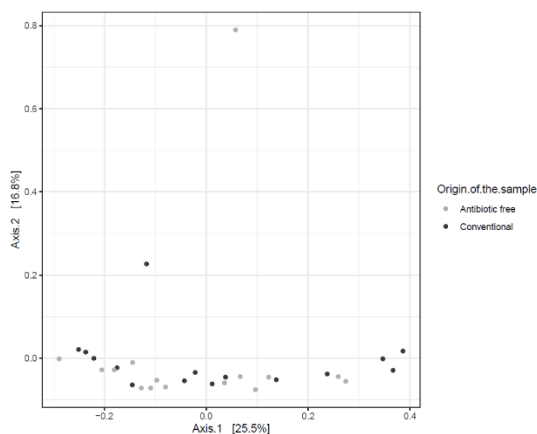


Figure S2. Bray-Curtis plot beta diversity of functional genes in the carcasses of birds reared in the antibiotic free and conventional farm.

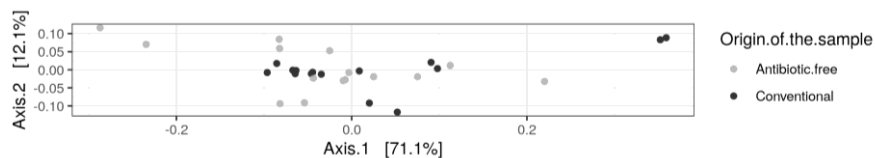


Figure S3. Beta diversity of carcasses samples shown as PCoA of Bray-Curtis diversity computed based on the AMR gene family abundances normalized with DESeq2.

Table S1. Numbers identifying the tested broilers during slaughtering and corresponding metagenome numbers for the caeca and the carcass samples.

Broiler number	Type of farm	Metagenome number for the caeca sample	Metagenome number for the carcass sample
31	Conventional	1343	1280
32	Conventional	1344	1281
33	Conventional	1345	1282
34	Conventional	1346	1283
35	Conventional	1347	1284
36	Conventional	1348	1285
37	Conventional	1349	1286
38	Conventional	1350	1287
39	Conventional	1351	1288
40	Conventional	1352	1289
41	Conventional	1353	1290
42	Conventional	-	1291
43	Conventional	1355	1292
44	Conventional	1356	1293
45	Conventional	1357	1294
46	Antibiotic free	1358	1295
47	Antibiotic free	1359	1296
48	Antibiotic free	1360	1297
49	Antibiotic free	1361	1298
50	Antibiotic free	1362	1299
51	Antibiotic free	1363	1300
52	Antibiotic free	1364	1301
53	Antibiotic free	1365	1302
54	Antibiotic free	1366	1303
55	Antibiotic free	1367	1304
56	Antibiotic free	1368	1305
57	Antibiotic free	1369	1306
58	Antibiotic free	1370	1307
59	Antibiotic free	1371	1308
60	Antibiotic free	1372	1309

Table S2. Normalized mean (DESeq2 normalized counts) of the top 20 genera significantly different ($p < 0.05$) in the caeca of the birds reared in the conventional and antibiotic-free farm.

Genus	Norm_mean_ conventional	Norm_mean_ antibiotic free	p-value
<i>Alistipes</i>	121394.483	201764.198	0.00
<i>Alkaliphilus</i>	65854.658	37406.434	0.00
<i>Anaerotruncus</i>	81830.348	141769.053	0.00
<i>Bacillus</i>	121574.408	80982.300	0.00
<i>Bacteroides</i>	903112.032	5541950.681	0.00
<i>Coprococcus</i>	56576.507	94133.578	0.00
<i>Desulfitobacterium</i>	83821.616	49267.150	0.00
<i>Dorea</i>	69937.780	121675.154	0.00

<i>Escherichia</i>	40613.940	215143.602	0.00
<i>Ethanoligenens</i>	126272.735	80004.036	0.00
<i>Holdemania</i>	87562.749	113518.336	0.00
<i>Lactobacillus</i>	162869.829	297992.157	0.02
<i>Parabacteroides</i>	54047.672	557109.972	0.00
<i>Prevotella</i>	41748.154	697359.162	0.00
<i>Roseburia</i>	95006.083	151647.561	0.00
<i>Ruminococcus</i>	522286.960	806960.484	0.00
<i>Streptococcus</i>	122827.116	90076.971	0.01
<i>Subdoligranulum</i>	342496.625	505311.498	0.00

Table S3. Mean values of the InvSimpson, Shannon, Chao1 indexes quantified for the genera identified in the caeca of birds from the conventional and the antibiotic free farm.

	InvSimpson	Shannon	Chao1
Mean_Conventional	13.733	3.747	559.631
Mean_Antibiotic free	7.08	3.067	554.05

Table S4: Norm_Mean (DESeq2 normalized counts) values of the top 20 functional genes significantly different ($p < 0.05$) in the caeca of the birds reared in the conventional and antibiotic-free farm.

Level function	Norm_Mean_ conventional	Norm_Mean_ antibiotic free	p-value
Beta-galactosidase (EC 3.2.1.23)	7282.341	0	0.00
Chaperone proteinDnaK	9293.178	12738.485	0.00
DNA topoisomerase III (EC 5.99.1.2) in PFGI-1-like cluster	2.005	0.214	0.00
Excinuclease ABC subunit A paralog in greater Bacteroides group	410.247	2013.301	0.00
Integrase	9163.519	15684.380	0.00
Ribonucleotide reductase of class III (anaerobic), large subunit (EC 1.17.4.2)	9822.277	14658.281	0.00
Translation elongation factor G	9751.141	18514.220	0.00

Table S5. Norm_Mean values (DESeq2 normalized counts) of the top 20 genera significantly different ($p < 0.05$) in the carcasses of the birds reared in the conventional and antibiotic-free farms.

Genus	Norm_Mean_ Conventional	Norm_Mean_ Antibiotic-free	p-value
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<i>Aeromonas</i>	118.652	325.106	0.00
<i>Anoxybacillus</i>	702.123	14.224	0.00
<i>Bacillus</i>	1032.502	183.119	0.00
<i>Bacteroides</i>	520.731	2011.121	0.00
<i>Geobacillus</i>	616.229	40.338	0.00
<i>Pedobacter</i>	429.521	127.025	0.00
<i>Prevotella</i>	54.755	339.248	0.00
<i>Ruminococcus</i>	142.057	361.581	0.00
<i>Sphingobacterium</i>	684.452	188.174	0.00

Table S6. Mean values of the InvSimpson, Shannon, Chao1 indexes quantified for the genera identified on carcasses of birds from the conventional and the antibiotic free farm.

Index	Mean conventional	Mean antibiotic-free	p-value
InvSimpson	13.312	10.738	0.44
Shannon	3.233	3.185	0.88
Chao1	491.491	503.355	0.75

Table S7. Genes classified as antibiotic resistant genes in the Level 2 category and showing normalized mean values significantly different ($p < 0.05$) in the caeca of birds from the conventional and antibiotic free farm. Genes in bold have been identified also on carcasses but with any statistical significant difference in the two tested groups.

Level function	Norm_Mean Conventional	Norm_Mean Antibiotic free	pval
Level 3-Aminoglycoside_adenylyltransferases			
AminoglycosideN6'-acetyltransferase (EC 2.3.1.82)	8.085	0.743	0.000
Spectinomycin9-O-adenylyltransferase	95.174	19.101	0.000
Streptomycin3"-O-adenylyltransferase (EC 2.7.7.47)	115.467	324.204	0.000
Level 3- Beta-lactamase			
Beta-lactamase class A	6.453	0.181	0.023
Beta-lactamase class C and other penicillin binding proteins	162.999	29.850	0.000
Beta-lactamaserepressorBlaI	395.078	36.980	0.001
Metal-dependent hydrolases of the beta-lactamase superfamily II	16.522	2.465	0.000
Negative regulator of beta-lactamase expression	5.177	0.897	0.003
Regulatory protein BlaR1	23.199	0.507	0.000
Level 3- BlaR1_Family_Regulatory_Sensor-transducer_Disambiguation			
Beta-lactamase repressor BlaI	0	51.574	0.000
Regulatory sensor-transducer. BlaR1/MecR1 family	1031.434	412.738	0.000

Transcriptional regulator. MecI family	33.457	83.803	0.001
Transcriptional repressor. BlaI/MecI family	261.786	462.161	0.000
Level 3-Erythromycin_resistance			
rRNA adenine N-6-methyltransferase (EC 2.1.1.48)	190.674	712.307	0.000
rRNA adenine N-6-methyltransferase (EC 2.1.1.48) (Macrolide-lincosamide-streptogramin B resistance protein) (Erythromycin resistance protein)	23.962	1.709	0.000
rRNA adenine N-6-methyltransferase (erm/ksgA)	3.797	16.206	0.000
Level 3- Methicillin_resistance_in_Staphylococci			
FmtA protein involved in methicillin resistance	3.757	0.481	0.017
Transposase for insertion sequence-like element IS431mec	0.618	4.946	0.005
UDP-N-acetylmuramoylalanyl-D-glutamate--2.6-diaminopimelate ligase (EC 6.3.2.13)	2541.379	0	0.000
Level 3- MexE-MexF-OprN_Multidrug_Efflux_System			
Multidrug efflux transporter MexF	67.028	32.709	0.000
Transcriptional regulator MexT	1.374	0.113	0.089
Level 3- Multidrug_Resistance_Efflux_Pumps			
Acriflavin resistance protein	2292.452	7129.559	0.000
Macrolide export ATP-binding/permease protein MacB (EC 3.6.3-)	1036.871	426.512	0.000
Macrolide-specific efflux protein MacA	210.917	668.432	0.000
Multi antimicrobial extrusion protein (Na+//drug antiporter-). MATE family of MDR efflux pumps	8815.673	3125.98	0.000
Multidrug efflux pump component MtrF	19.153	2.949	0.000
Multidrug efflux RND membrane fusion protein MexC	4.113	1.517	0.022
Multidrug efflux RND transporter MexD	19.084	6.185	0.002
Multidrug resistance efflux pump PmrA	20.989	3.974	0.000
Multidrug-efflux transporter. major facilitator superfamily (MFS) (TC 2.A.1)	14.734	47.616	0.000
Transcription regulator of multidrug efflux pump operon. TetR (AcrR) family	22.021	4.136	0.000
Transcription repressor of multidrug efflux pump acrAB operon. TetR(AcrR) family	4.972	24.051	0.001
Multiple antibiotic resistance protein MarA	0.307	3.607	0.003
Level3- Polymyxin_Synthetase_Gene_Cluster_in_Bacillus			
Polymyxin synthetase PmxA	1.953	0.255	0.029
Level 3- Resistance_to_fluoroquinolones			
Efflux pump Lde	25.941	69.768	0.024

Topoisomerase IV subunit B (EC 5.99.1-)	1796.567	0	0.000
Level 3- Resistance_to_Vancomycin			
Protein VanZ	1.472	0.085	0.027
Sensor histidine kinase VanS (EC 2.7.3)	847.058	136.855	0.000
Vancomycin B-type resistance protein VanW	882.44	147.837	0.000
Vancomycin B-type resistance protein VanX	65.047	29.341	0.000
Vancomycin resistance protein VanH	11.766	4.74	0.015
Vancomycin response regulator VanR	1340.459	856.222	0.005
Vancomycin Teicoplanin A-type resistance protein VanA	44.3	3.047	0.000
Level 3- Streptococcus_pneumoniae_Vancomycin_Tolerance_Locus			
ABC transporter. ATP-binding protein Vex2	84.104	8.606	0.000
Level 3- Streptothricin_resistance			
Streptothricin acetyltransferase. Streptomyces lavendulae type	149.21	248.089	0.001
Level 3- The_mdtABCD_multidrug_resistance_cluster			
Multidrug transporter MdtB	16.731	79.835	0.005
Multidrug transporter MdtC	15.583	69.334	0.002
Multidrug transporter MdtD	7.577	32.849	0.003
Probable RND efflux membrane fusion protein	709.051	1590.562	0.000
Response regulator BaeR	53.501	23.835	0.002
Sensory histidine kinase BaeS	16.449	35.314	0.019