Monday morning presentation

The resistomes of rural and urban pigs and poultry in Ghana

Importance

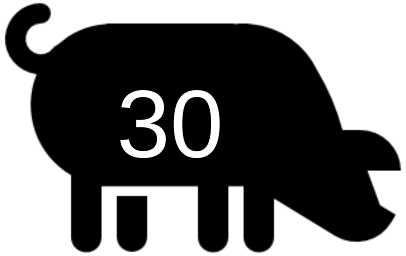
Limited data from Sub-Saharan Africa livestock:-

- No previous metagenomic study quantifying the AMR levels

Changes in the livestock production

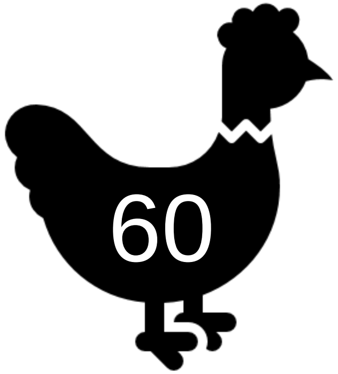
- From small to large scale

Samples



30

February to August 2020



60

January to May 2020

Samples

30

16

Rural setting

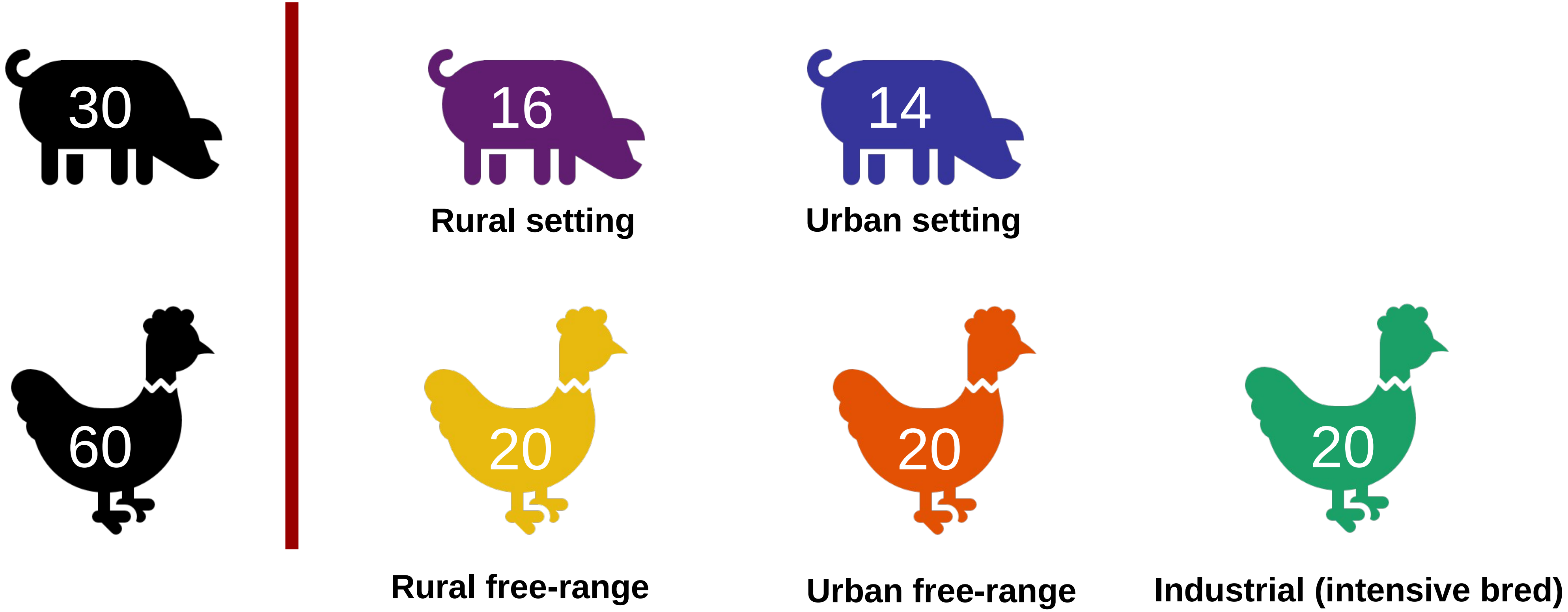
- Low-end biosecurity (n = 12)
- High-end biosecurity (n = 4)

14

Urban setting

- Low-end biosecurity (n = 4)
- High-end biosecurity (n = 10)

Samples



30

60

16

Rural setting

14

Urban setting

20

Rural free-range

20

Urban free-range

20

Industrial (intensive bred)

Data summary

5.48 billion PE reads were obtained from the 90 samples (average 60.9 million PE reads per sample, range 15.7-154.3 million PE reads per sample, SD: 189 million).

On average **0.119%** of the reads per sample aligned to ARGs from the **ResFinder** database.

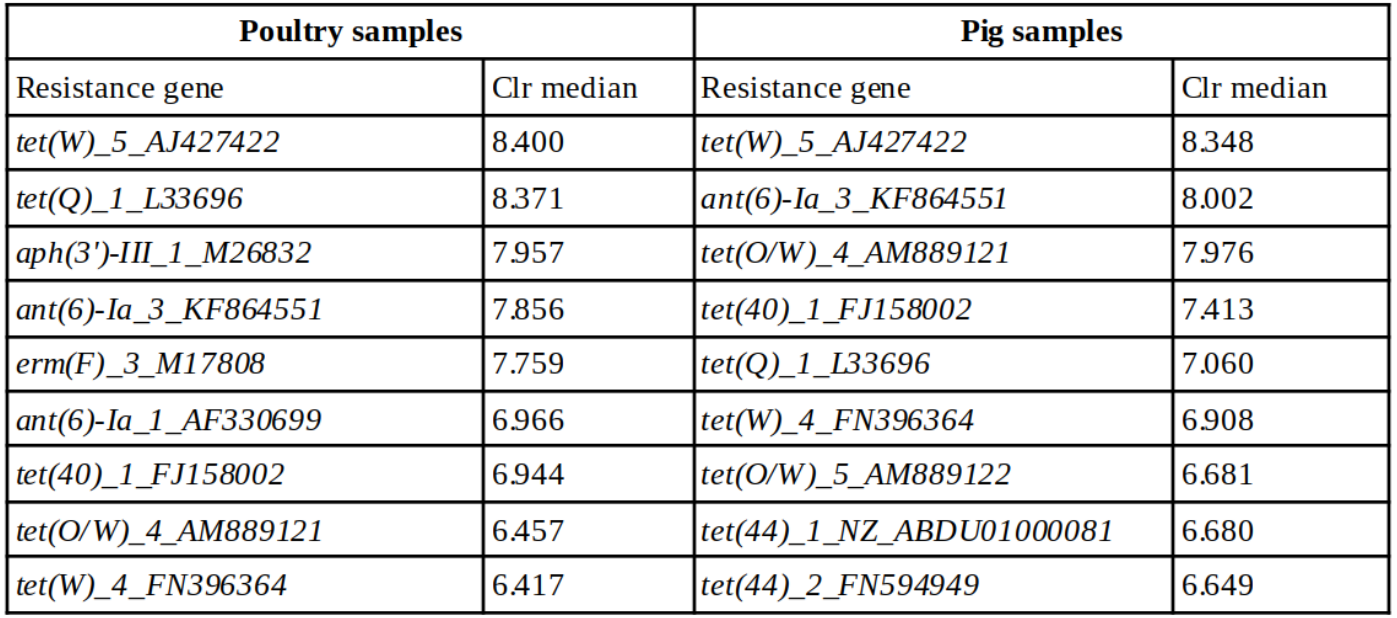
On average, **0.291%** of the reads per sample aligned to 16/18 S SSU rRNA from the **Silva** database.

- Of which, an average of **96.199%** per sample were assigned to **Bacteria**
- Of which, an average of **3.674%** per sample were assigned to **Eukaryotes**

The aquired resistome

544 different ARGs among poultry samples

688 different ARGs among pig samples



Poultry samples		Pig samples	
Resistance gene	Clr median	Resistance gene	Clr median
tet(W)_5_AJ427422	8.400	*tet(W)_5_AJ427422*	8.348
tet(Q)_1_L33696	8.371	*ant(6)-Ia_3_KF864551*	8.002
aph(3')-III_1_M26832	7.957	*tet(O/W)_4_AM889121*	7.976
ant(6)-Ia_3_KF864551	7.856	*tet(40)_1_FJ158002*	7.413
erm(F)_3_M17808	7.759	*tet(Q)_1_L33696*	7.060
ant(6)-Ia_1_AF330699	6.966	*tet(W)_4_FN396364*	6.908
tet(40)_1_FJ158002	6.944	*tet(O/W)_5_AM889122*	6.681
tet(O/W)_4_AM889121	6.457	*tet(44)_1_NZ_ABDU01000081*	6.680
tet(W)_4_FN396364	6.417	*tet(44)_2_FN594949*	6.649

Table S5: Top 10 most abundant resistance genes in poultry and pig samples from Ghana.

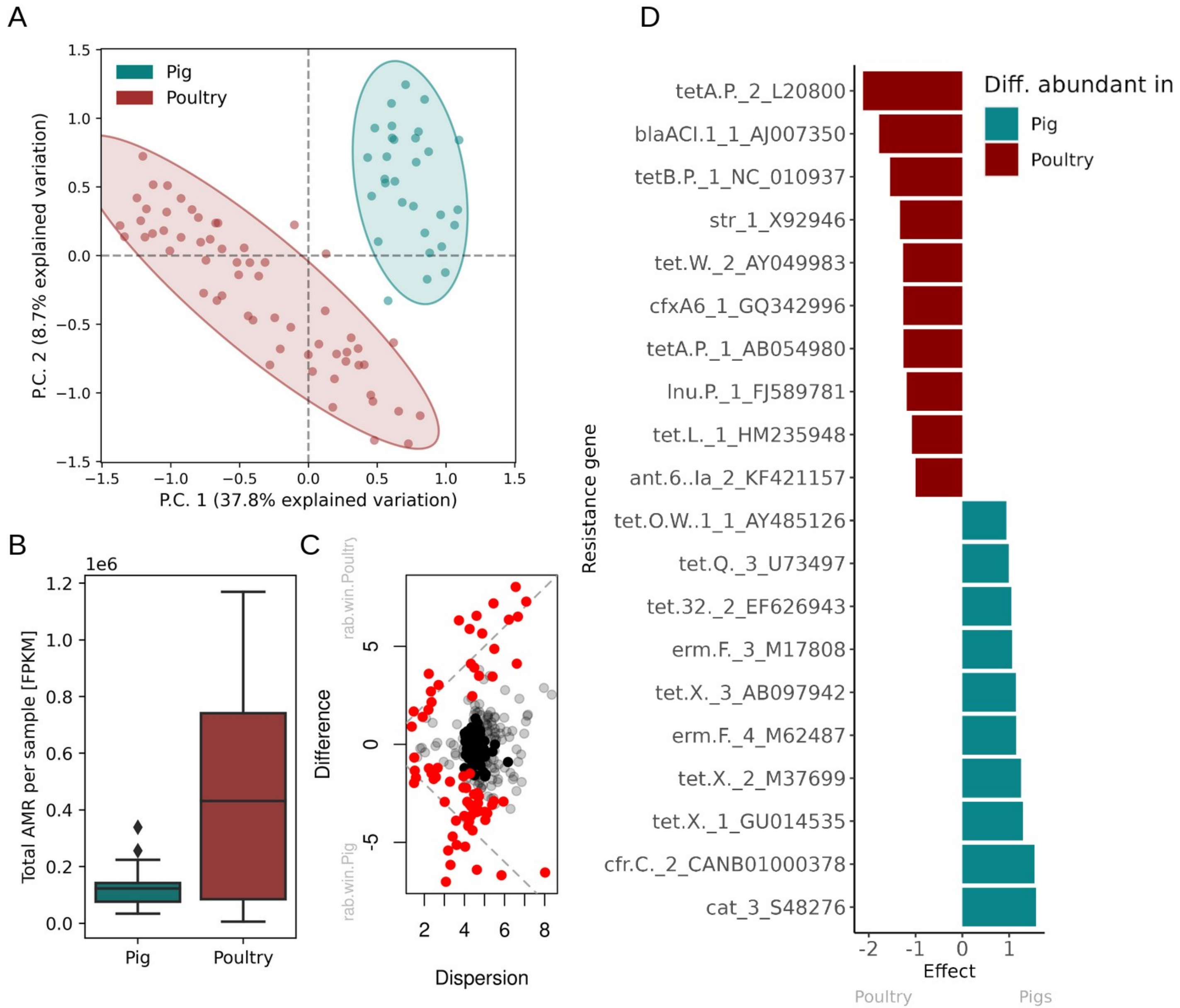


Figure 3: Differences in resistomes of pig and poultry from Ghana. **A)** Resistance genes PCA clustering. The ordination analysis was performed on the most abundant, most variant centered log-ratio (CLR) transformed size-adjusted counts (CLR variance > 2.2, CLR median > 0) **B)** Total AMR per sample calculated as the total AMR fragments per kilobase per million fragments per sample (FPKM), stratified by host. **C)** Differential abundance effect plot showing the within-group dispersion of CLR values of each resistance gene compared to the between-group differences. Statistical significant resistance genes with a Benjamini-Hochberg false-discovery rate (FDR) correction < 0.05 are colored red. The gray dotted line indicates an effect size of 1. **D)** Top 10 statistical significant resistance with FDR correction < 0.05 identified from the differential abundance analysis (see Table S8 for full list).

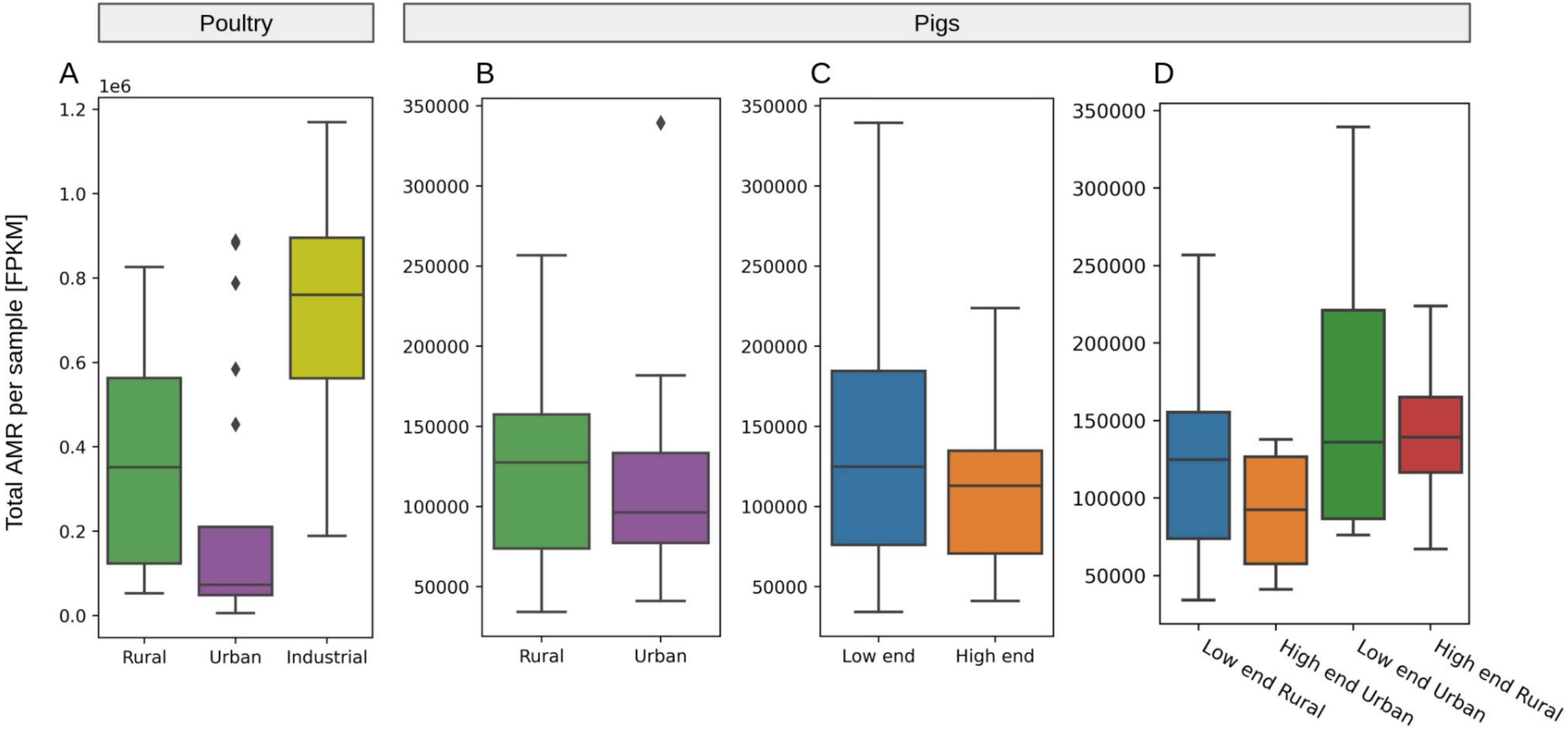


Figure S4: Total AMR per sample calculated as the total AMR fragments per kilobase per million fragments per sample (FPKM), stratified by host. **A)** Rural (n=20), urban (n=20) and industrial poultry (n=20) from Ghana. **B)** Urban (n=14) and rural (n=16) pigs from Ghana. **C)** Low-end (n=16) and high-end (n=10) biosecurity pigs from Ghana. **D)** Biosecurity combined with location pigs from Ghana: Rural low-end biosecurity pigs (n=12), urban high-end biosecurity pigs (n=10), urban low-end biosecurity pigs (n=4) and rural high-end biosecurity pigs (n=4).

Bacterial community

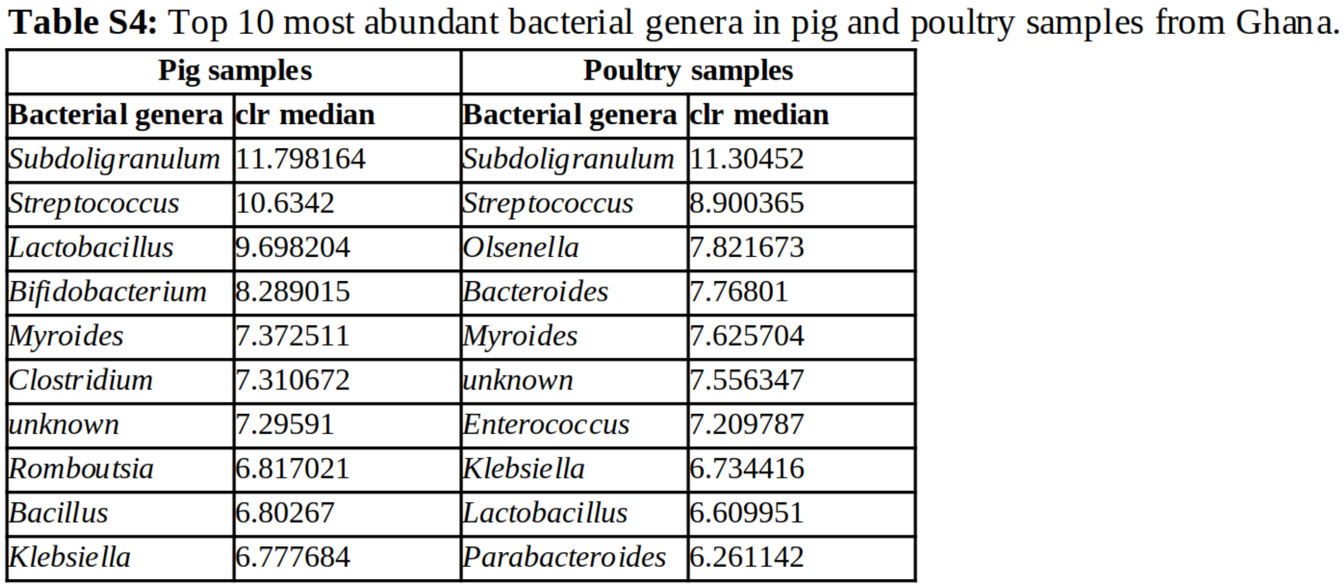


Table S4: Top 10 most abundant bacterial genera in pig and poultry samples from Ghana.

Pig samples		Poultry samples	
Bacterial genera	**clr median**	**Bacterial genera**	**clr median**
Subdoligranulum	11.798164	*Subdoligranulum*	11.30452
Streptococcus	10.6342	*Streptococcus*	8.900365
Lactobacillus	9.698204	*Olsenella*	7.821673
Bifidobacterium	8.289015	*Bacteroides*	7.76801
Myroides	7.372511	*Myroides*	7.625704
Clostridium	7.310672	*unknown*	7.556347
unknown	7.29591	*Enterococcus*	7.209787
Romboutsia	6.817021	*Klebsiella*	6.734416
Bacillus	6.80267	*Lactobacillus*	6.609951
Klebsiella	6.777684	*Parabacteroides*	6.261142

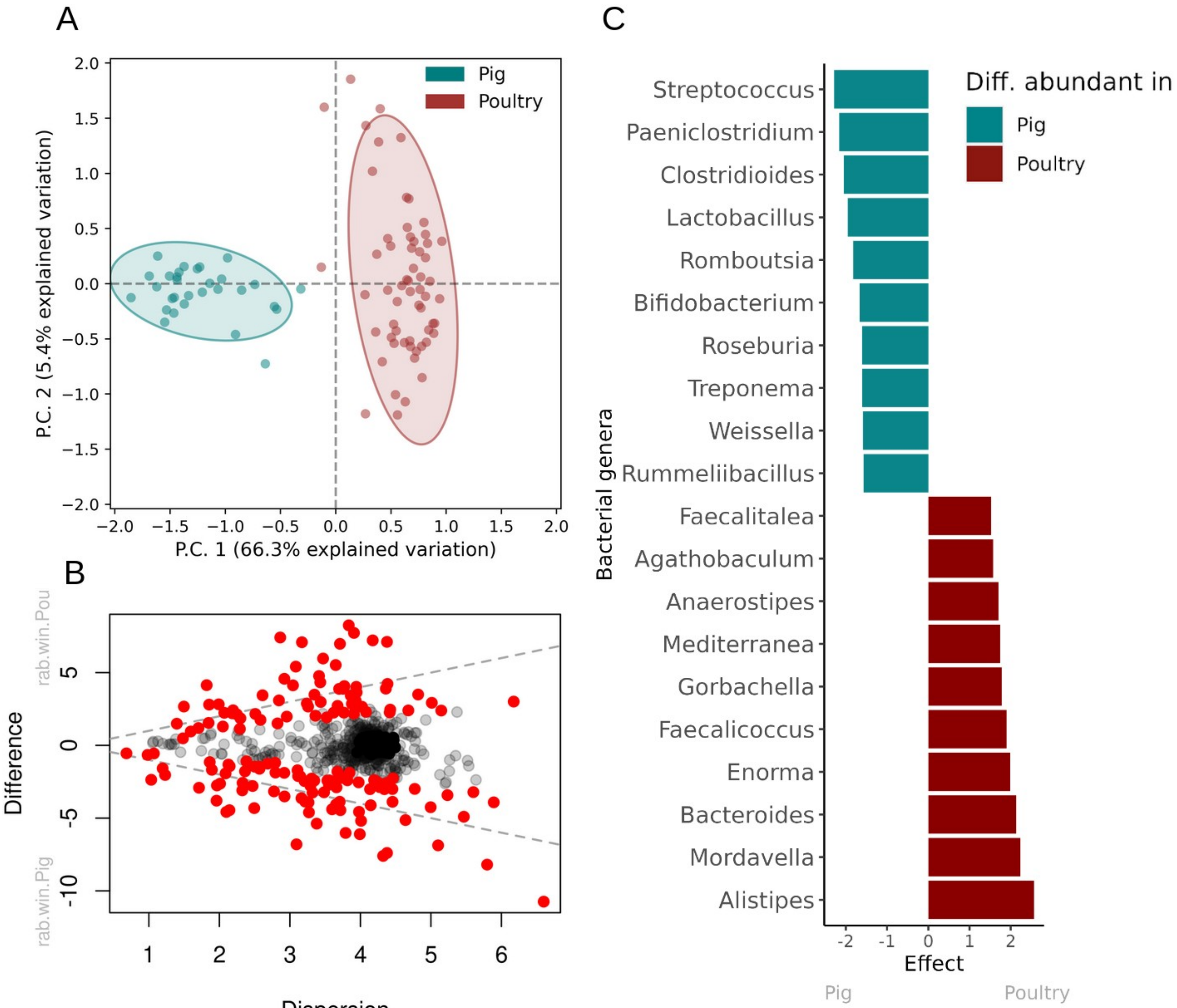
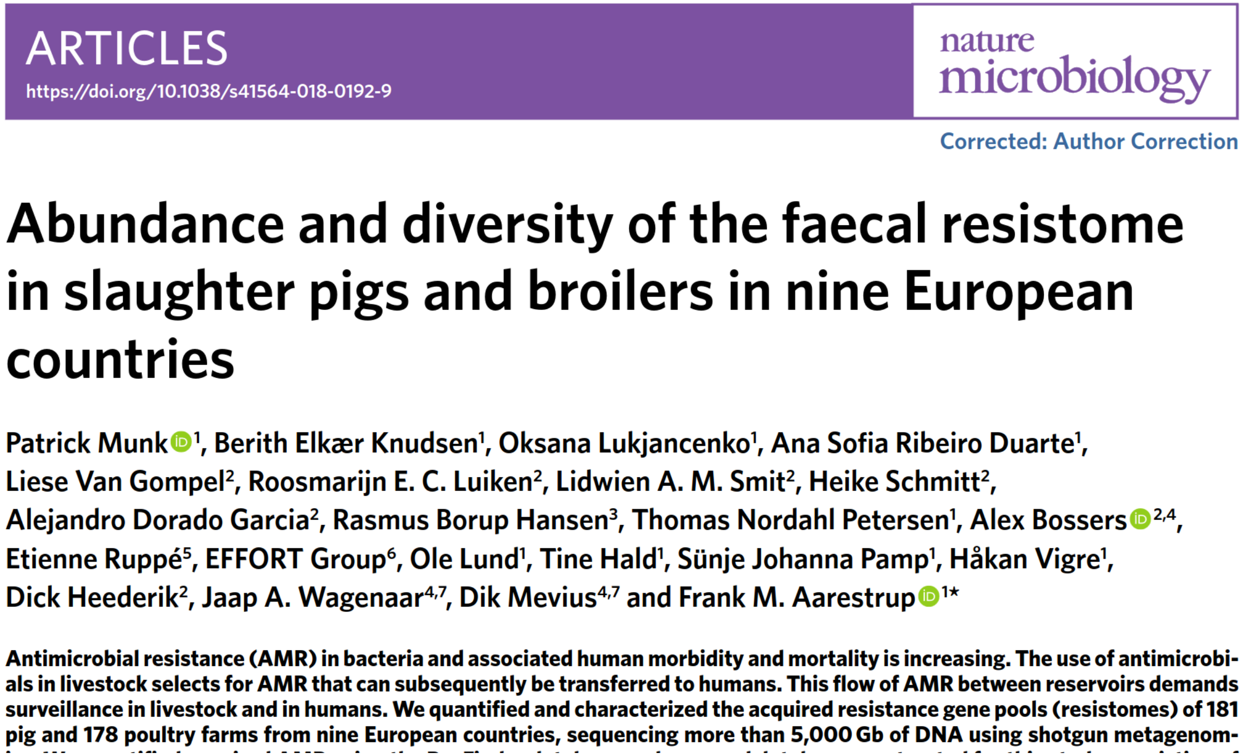


Figure 5: Bacterial genus differences between pig and poultry in Ghana. **A)** Clustering of pig and poultry samples from Ghana. The ordination analysis was performed on the most abundant, most variance centered log-ratio (CLR) transformed size-adjusted counts CLR variance > 2, CLR median > 0). **B)** Effect plot showing the within-group dispersion of CLR values of each bacterial genus compared to the between-group differences. Statistical significant bacterial genera with a Benjamini-Hochberg false-discovery rate (FDR) correction < 0.05 are colored red. The gray dotted line indicates an effect size of 1. **C)** Top 10 statistical significant bacterial genera with FDR correction < 0.05 (see Table S11 for full list).

Comparison with nine European countries

EFFORT samples

- BE, Belgium
- BG, Bulgaria
- DE, Germany
- DK, Denmark
- ES, Spain
- FR, France
- IT, Italy
- NL, the Netherlands
- PL, Poland



ARTICLES
https://doi.org/10.1038/s41564-018-0192-9
nature microbiology
Corrected: Author Correction

Abundance and diversity of the faecal resistome in slaughter pigs and broilers in nine European countries

Patrick Munk[1], Berith Elkær Knudsen[1], Oksana Lukjancenko[1], Ana Sofia Ribeiro Duarte[1], Liese Van Gompel[2], Roosmarijn E. C. Luiken[2], Lidwien A. M. Smit[2], Heike Schmitt[2], Alejandro Dorado Garcia[2], Rasmus Borup Hansen[3], Thomas Nordahl Petersen[1], Alex Bossers[2,4], Etienne Ruppé[5], EFFORT Group[6], Ole Lund[1], Tine Hald[1], Sünje Johanna Pamp[1], Håkan Vigre[1], Dick Heederik[2], Jaap A. Wagenaar[4,7], Dik Mevius[4,7] and Frank M. Aarestrup[1]*

Antimicrobial resistance (AMR) in bacteria and associated human morbidity and mortality is increasing. The use of antimicrobials in livestock selects for AMR that can subsequently be transferred to humans. This flow of AMR between reservoirs demands surveillance in livestock and in humans. We quantified and characterized the acquired resistance gene pools (resistomes) of 181 pig and 178 poultry farms from nine European countries, sequencing more than 5,000 Gb of DNA using shotgun metagenom-

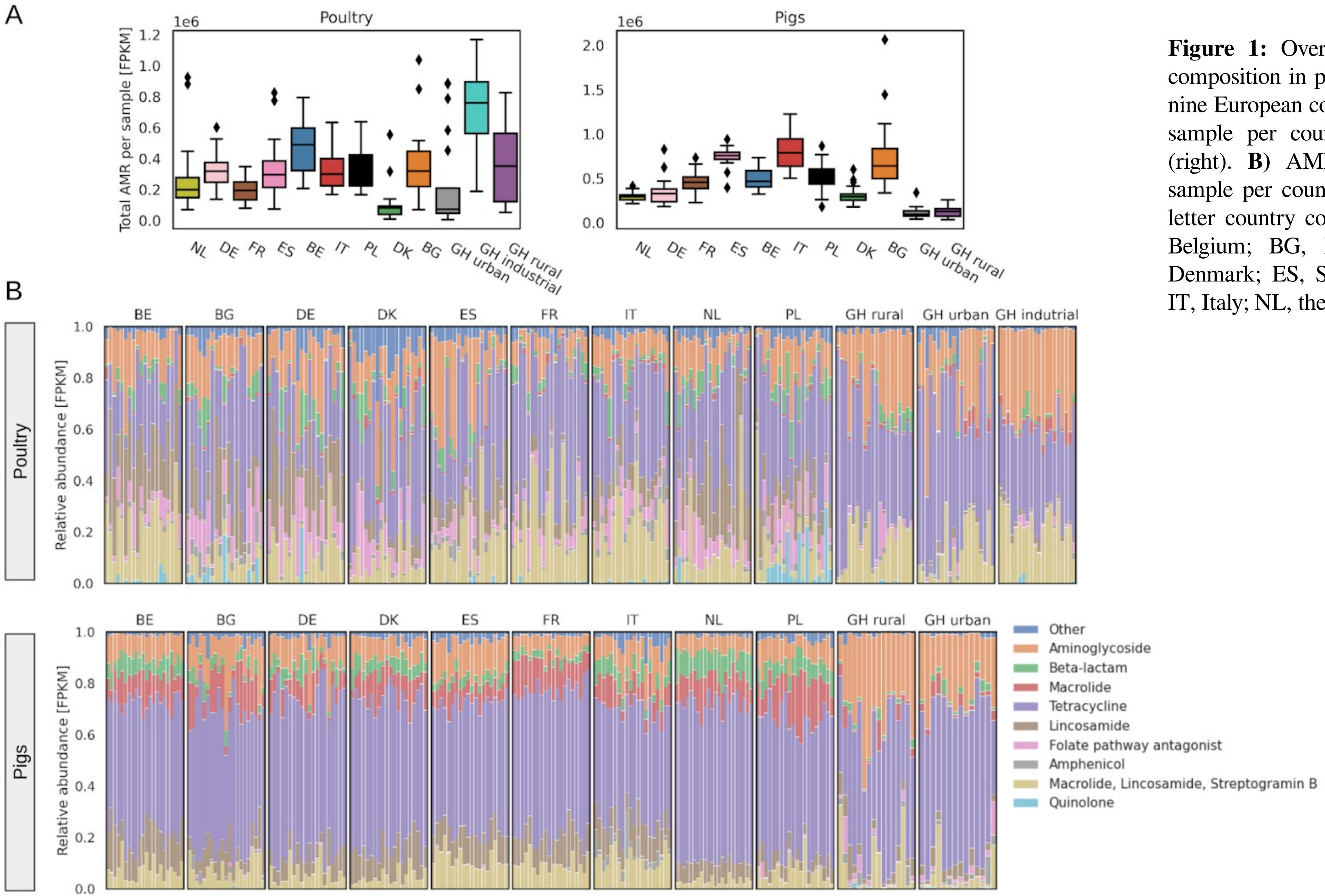


Figure 1: Overview of AMR abundance and composition in pigs and poultry from Ghana and nine European countries. **A)** Total AMR level per sample per country in poultry (left) and pigs (right). **B)** AMR gene class composition per sample per country, stratified by host. The two-letter country codes are used in all figures: BE, Belgium; BG, Bulgaria; DE, Germany; DK, Denmark; ES, Spain; FR, France; GH, Ghana; IT, Italy; NL, the Netherlands; PL, Poland.

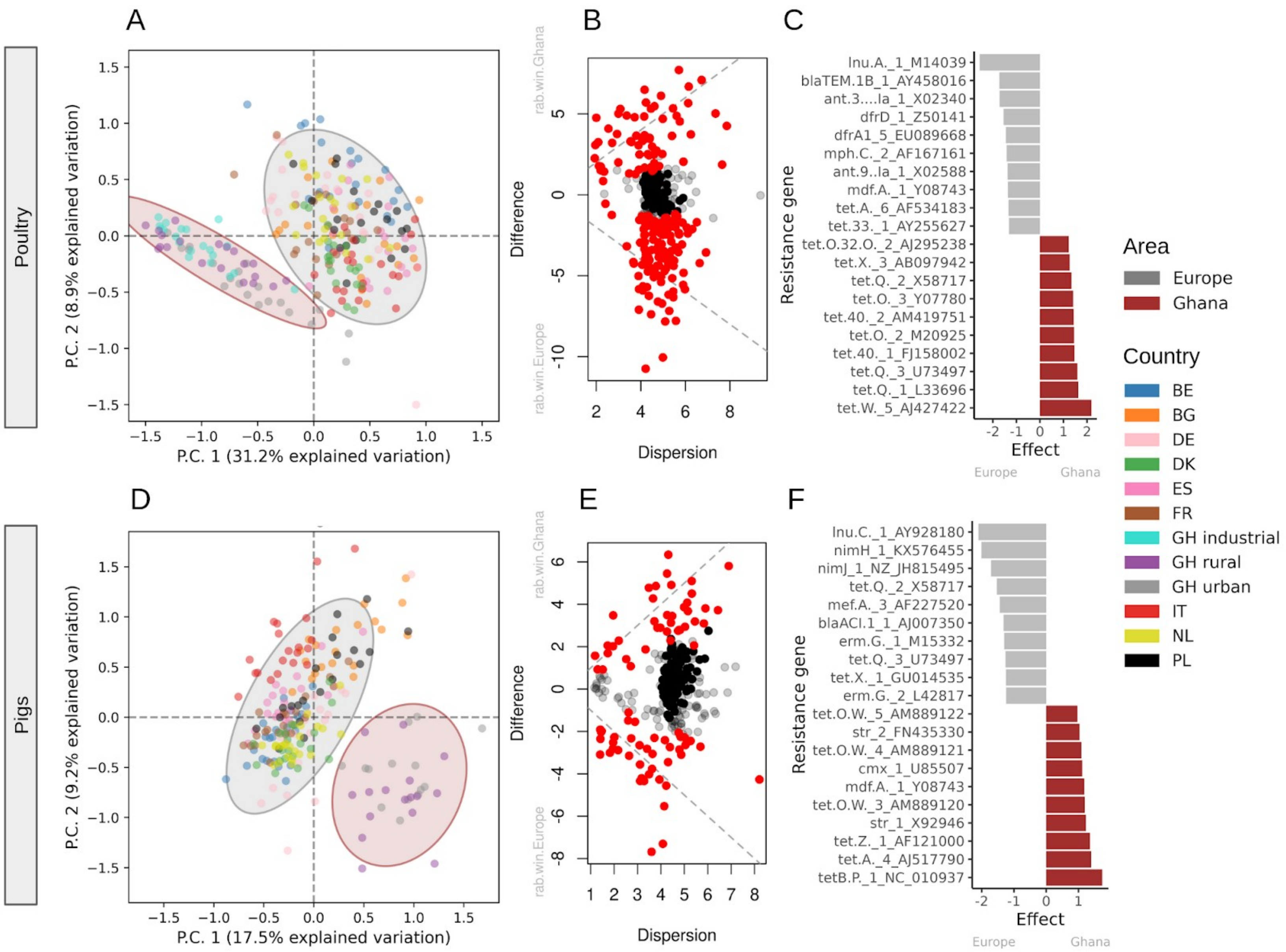


Figure 2: Differences in resistance genes between pig (D-F) and poultry (A-C) from Ghana and 9 European countries. **A)** Poultry resistance genes clustering. The ordination analysis was performed on the most abundant, most variant centered log-ratio (CLR) transformed size-adjusted counts (CLR variance > 1, CLR median > 0). **B)** Poultry effect plot from the differential abundance analysis further investigated in **C)** Top 10 Statistical significant poultry resistance genes between Europe and Ghana with FDR correction < 0.05 (see Table S6 for full list) **D)** Pig resistance genes clustering. The ordination analysis was performed on the most abundant, most variant centered log-ratio (CLR) transformed size-adjusted counts (CLR variance > 1, CLR median > 0). **E)** Pig effect plot from the differential abundance analysis further investigated in **F)** Top 10 statistical significant pig resistance genes with FDR correction < 0.05 (see Table S7 for full list).

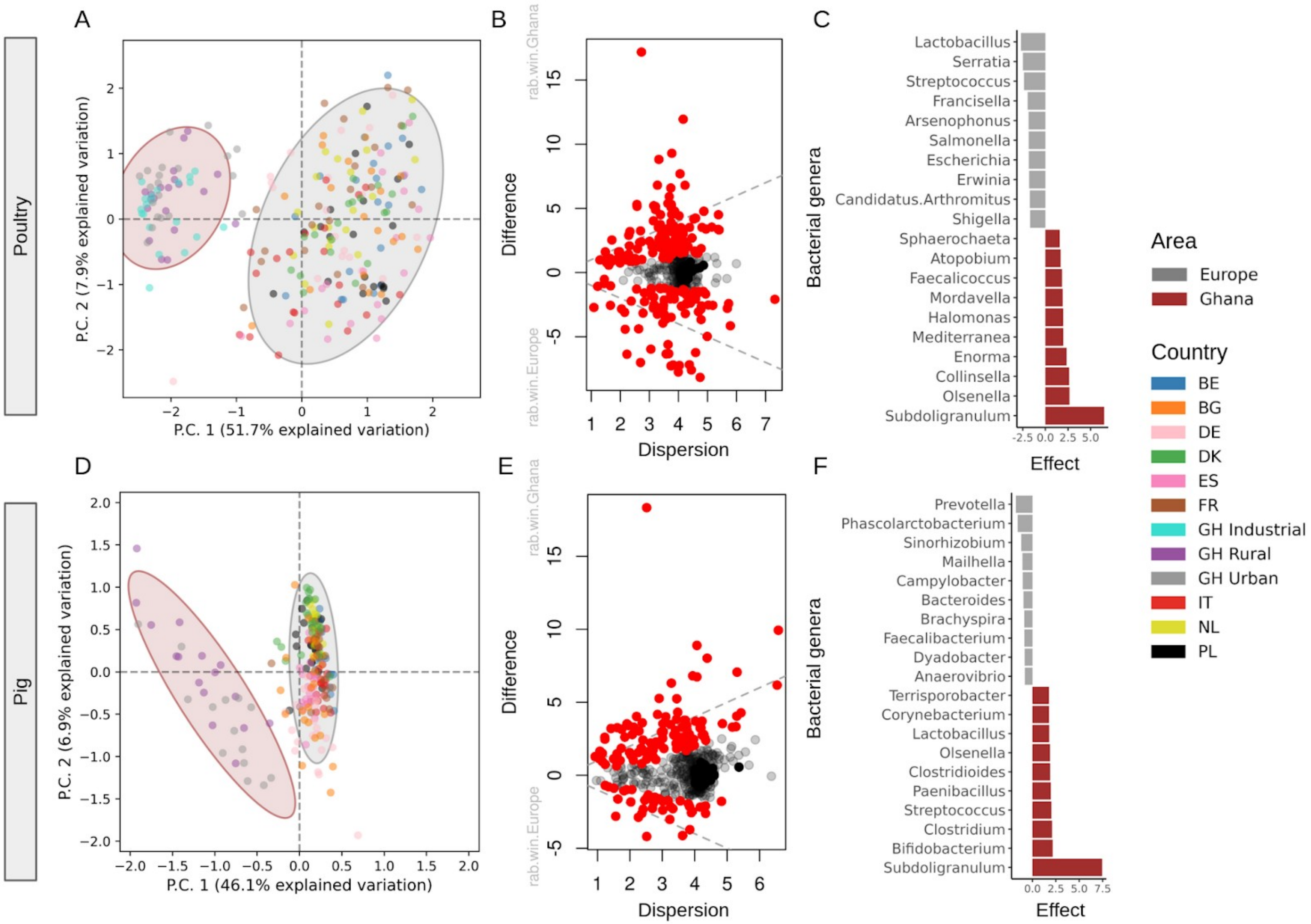


Figure 6: Differences in bacteria genera between poultry (A-C) and pigs (D-F) from Ghana and 9 European countries. **A+D)** Bacteria genera clustering. The ordination analysis was performed on the most abundant, most variant centered log-ratio (CLR) transformed size-adjusted counts (CLR variance > 1, CLR median > 0.5). **B+E)** Effect plot showing the within-group dispersion of CLR values of each resistance gene compared to the between-group differences. Statistical significant genera with a Benjamini-Hochberg false-discovery rate (FDR) correction < 0.05 are colored red. The gray dotted line indicates an effect size of 1. **C+F)** Top 10 statistical significant genera with FDR correction < 0.05 (see Table S13 for full list).

Summary

Increase in AMR levels with transition from small-scale and free-range production to large-scale industrialized production system.

Country-specific factors impacts the resistome and bacterial community.

Ghanaian samples driven by a high abundance of *Subdoligranulum.*

Increased biosecurity seemed to not affect the ARG levels.

Questions?