Using metagenomics for global surveillance of antimicrobial resistance

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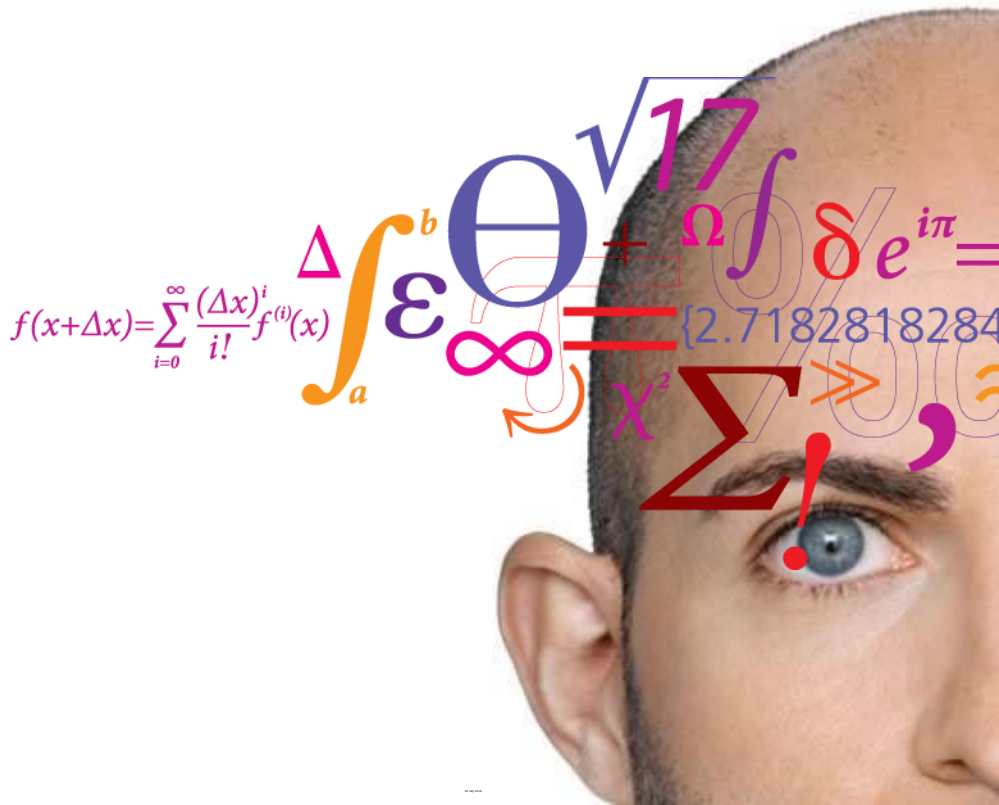


DTU Food
National Food Institute

Background – Shotgun metagenomics

Wastewater, fecal or pooled fecal sample

DNA extraction from sample

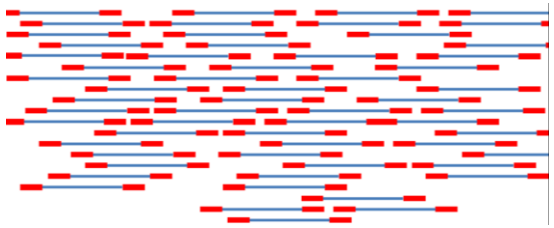
DNA sheared to 300 bp

(PCR-free) DNA library preparation

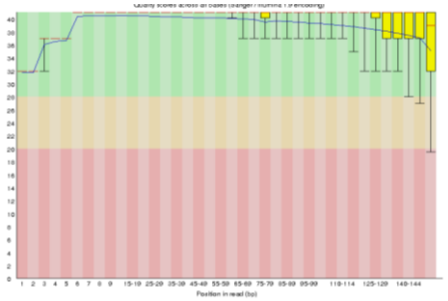
HiSeq PE Sequencing (2x100/150bp)

>35 million PE reads/sample

Background – Bioinformatics

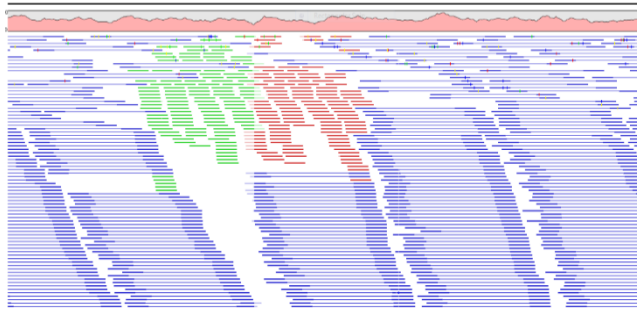


PE DNA reads
>35 mill / sample



QC, quality- and

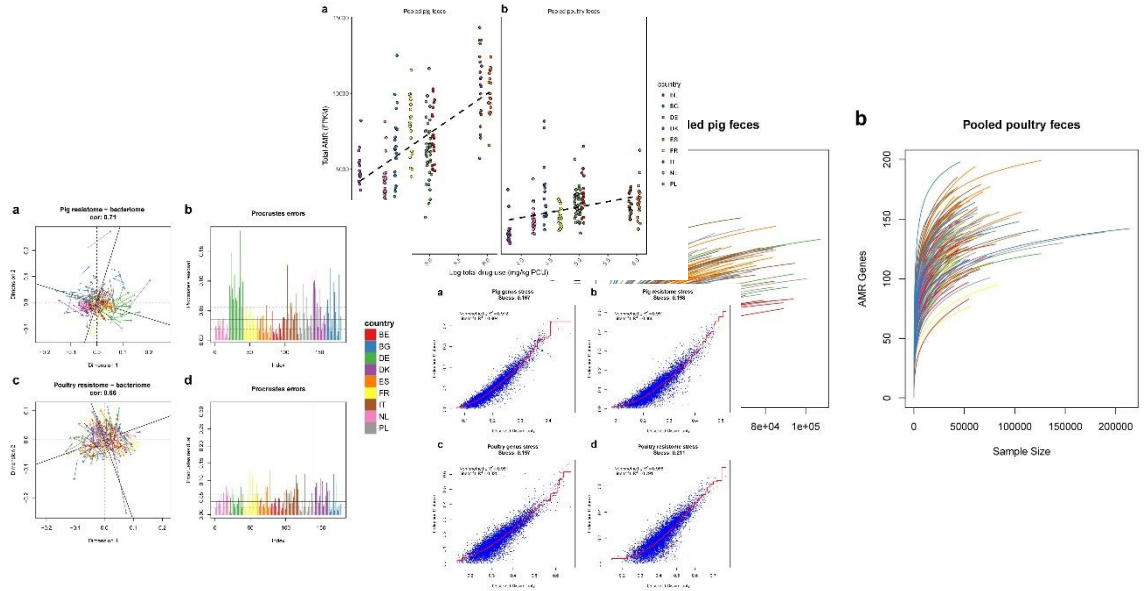
adapter-trimming



MGmapper (BWA-MEM)
Reads mapped to **ResFinder**,
Bacterial genomes etc.

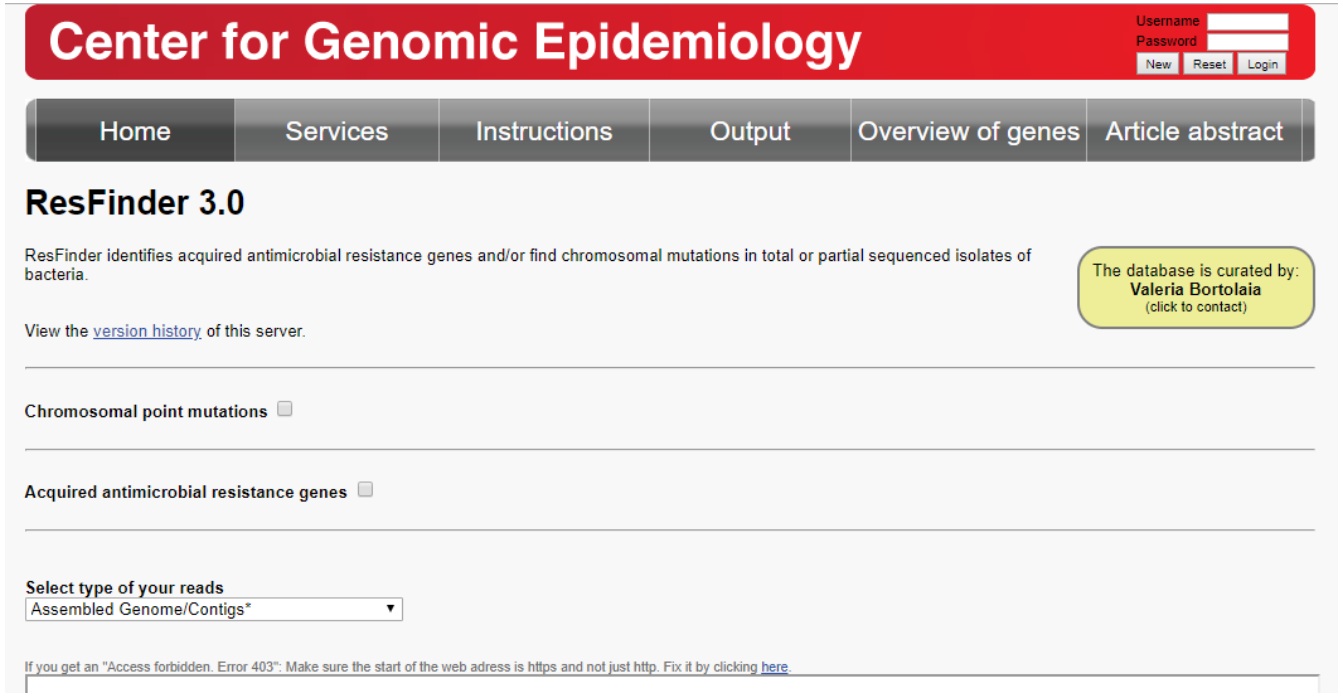
	Farm1	Farm2	Farm3
Ref1	17	19	14
Ref2	2	4	0
Ref3	31	0	2

feature x sample
Count matrices



ResFinder

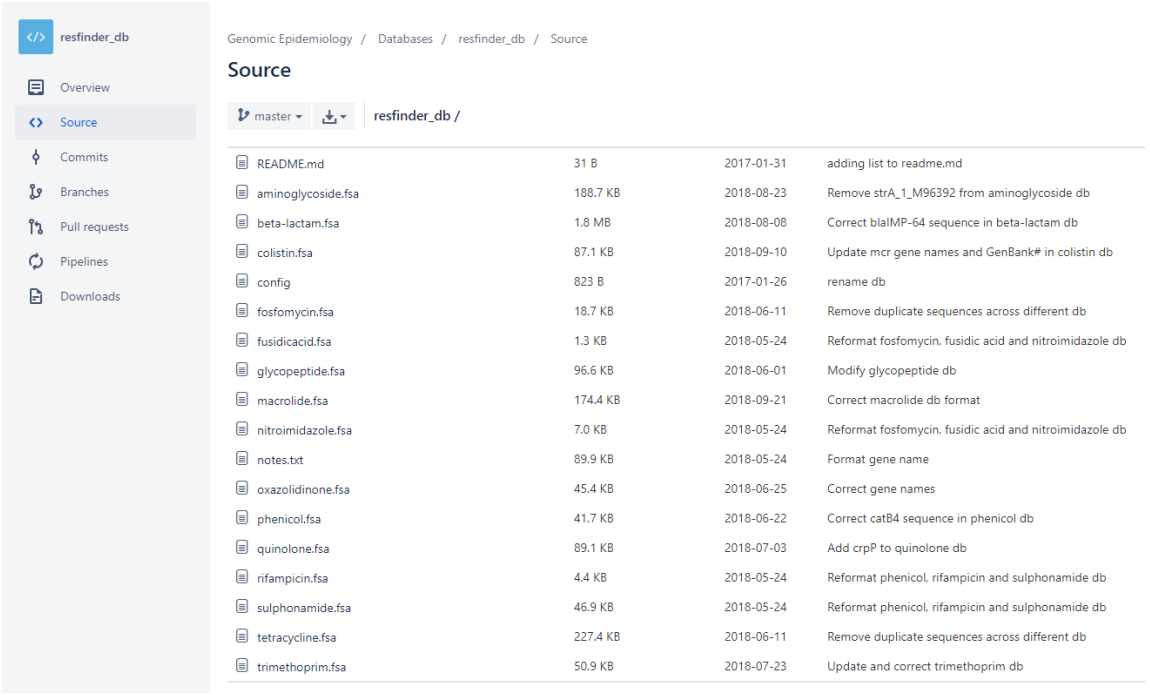
The Tool



cge.cbs.dtu.dk/services/ResFinder/

E Zankari et al (2012)

The Database



bitbucket.org/genomicepidemiology/resfinder_db

Pilot study

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Journal of Antimicrobial Chemotherapy

A sampling and metagenomic sequencing-based methodology for monitoring antimicrobial resistance in swine herds

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Objectives: Reliable methods for monitoring antimicrobial resistance (AMR) in livestock and other reservoirs are essential to understand the trends, transmission and importance of agricultural resistance. Quantification of AMR is mostly done using culture-based techniques, but metagenomic read mapping shows promise for quantitative resistance monitoring.

Methods: We evaluated the ability of: (i) MIC determination for *Escherichia coli*; (ii) cfu counting of *E. coli*; (iii) cfu counting of aerobic bacteria; and (iv) metagenomic shotgun sequencing to predict expected tetracycline resistance based on known antimicrobial consumption in 10 Danish integrated slaughter pig herds. In addition, we evaluated whether fresh or manure floor samples constitute suitable proxies for intestinal sampling, using cfu counting, qPCR and metagenomic shotgun sequencing.

Results: Metagenomic read-mapping outperformed cultivation-based techniques in terms of predicting expected tetracycline resistance based on antimicrobial consumption. Our metagenomic approach had sufficient resolution to detect antimicrobial-induced changes to individual resistance gene abundances. Pen floor manure samples were found to represent rectal samples well when analysed using metagenomics, as they contain the same DNA with the exception of a few contaminating taxa that proliferate in the extraintestinal environment.

Conclusions: We present a workflow, from sampling to interpretation, showing how resistance monitoring can be carried out in swine herds using a metagenomic approach. We propose metagenomic sequencing should be part of routine livestock resistance monitoring programmes and potentially of integrated One Health monitoring in all reservoirs.

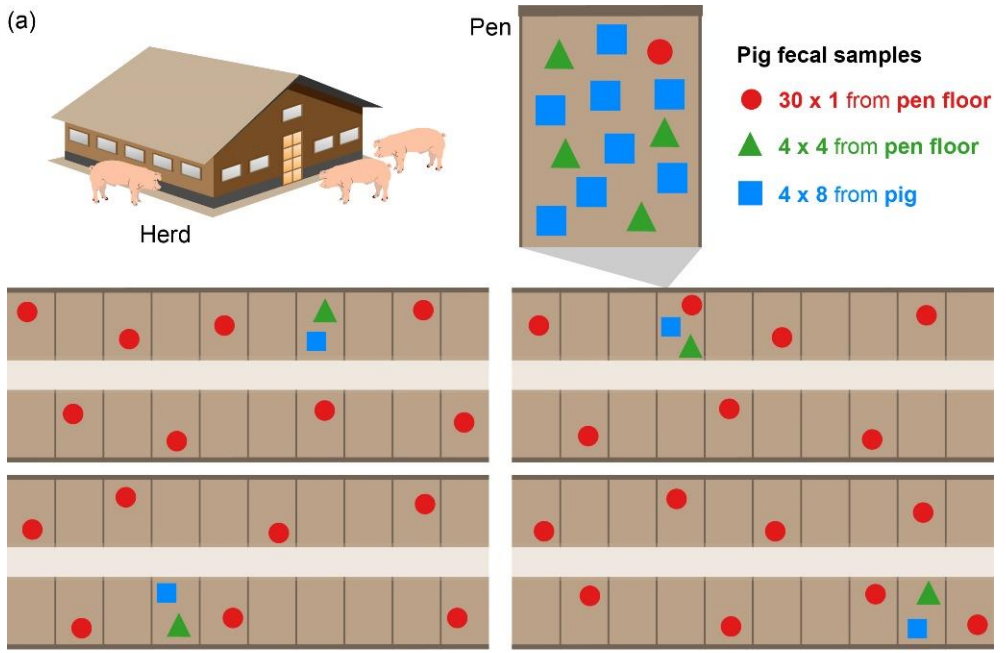
- Compare geno- and phenotypic AMR monitoring methods in Danish pig herds
- Compare different sampling strategies
- Assess the feasibility of using metagenomics to monitor AMR in pig herds
- Assess ability of metagenomics to detect differences in AMR abundance

P Munk et al (2017)

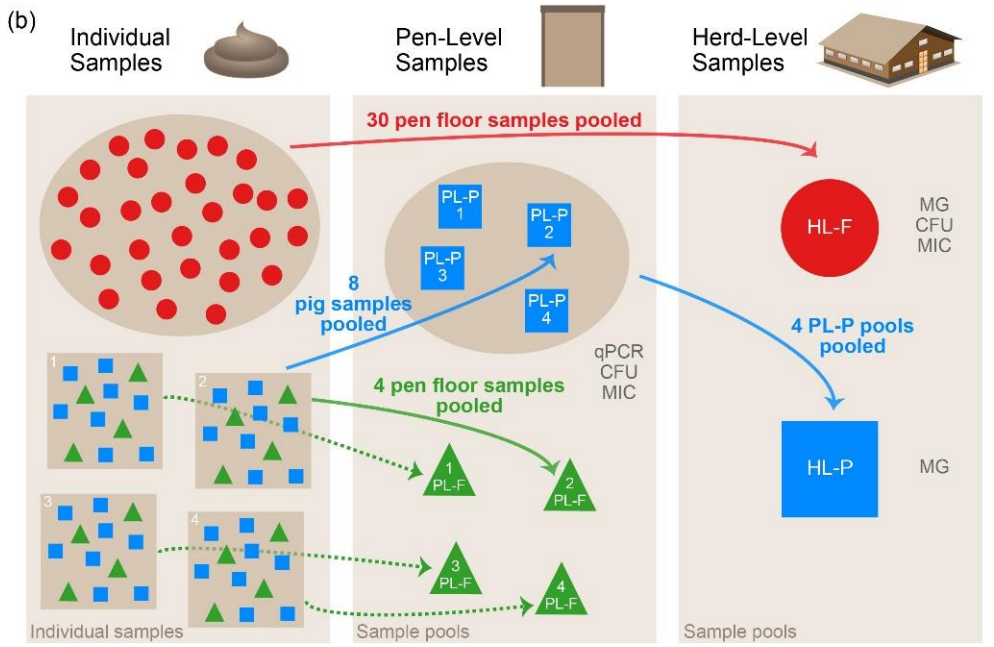
P Munk et al (2017)

Pilot study

- 10 Danish pig herds
- Both high and low AMU herds included
- Metagenomics, CFU counting, MIC, qPCR
- Stratified sampling of 30 pens
- Paired floor and rectal samples from 4 pens



Drug class	Pigs	Floors
Aminoglycosides	*apmA* *ant(6)-I* *strB* *tet(44)*	*apmA*
Macrolides	*erm(B)* *erm(G)* *erm(F)* *mef(A)* *msr(D)*	*erm(B)* *erm(G)*
Tetracyclines	-	*tet(44)*



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

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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 metagenomics. We quantified acquired AMR using the ResFinder database and a second database constructed for this study, consisting of AMR genes identified through screening environmental DNA. The pig and poultry resistomes were very different in abundance and composition. There was a significant country effect on the resistomes, more so in pigs than in poultry. We found higher AMR loads in pigs, whereas poultry resistomes were more diverse. We detected several recently described, critical AMR genes, including *mcr-1* and *optrA*, the abundance of which differed both between host species and between countries. We found that the total acquired AMR level was associated with the overall country-specific antimicrobial usage in livestock and that countries with comparable usage patterns had similar resistomes. However, functionally determined AMR genes were not associated with total drug use.

Antimicrobial resistance (AMR) is considered one of the largest threats to human health[1]. In addition to the use of antimicrobial agents in humans, livestock is considered an important source of AMR, potentially compromising human health[2]. Besides AMR in zoonotic pathogens, AMR in commensal bacteria is worrisome because of its ability to spread horizontally to pathogens.

Multiple studies have shown that the use of antimicrobials in livestock will lead to an increased occurrence of AMR and that the reduction of usage will eventually lead to reduced resistance[3–8]. Several national surveillance programmes have been implemented to monitor the occurrence of AMR in different reservoirs and follow trends over time[1,9–11]. There are major differences in antimicrobial consumption patterns between different countries globally and also within Europe[12]. Major differences in the occurrence of AMR have also been observed among indicator organisms (for example, *Escherichia coli*) isolated from different European countries[3,13]. Current monitoring efforts are mainly based on culturing indicator bacteria followed by phenotypic AMR determination[13,14]. This procedure only targets a limited number of species present in the gut microbiota and, therefore, probably represents only a fraction of its resistome (the collective pool of AMR genes). Metagenomic approaches have been used in several recent studies and have shown that metagenomic read mapping describes AMR abundance in bacterial communities more accurately than commonly used technologies on selected indicator organisms[15–17]. A recent study focused on sampling a diverse group of individual pigs from 11 farms in 3 countries and showed that genetics, age, diet and geography all probably influence the pig microbiota, but little information is available for the poultry microbiota[16].

As part of the European Union-funded EFFORT project (www.effort-against-amr.eu), we sampled >9,000 animals in 181 pig and 178 poultry herds in 9 European countries, generating herd-level composite samples as previously described[17]. Metagenomic sequencing of these samples gives us a unique insight into the abundance, diversity and structure of the acquired pig and broiler resistomes in Europe. An association between AMR gene abundance and national veterinary antimicrobial usage (AMU) was also analysed. The results and raw data presented here can be used as a baseline for future metagenomic AMR monitoring. To our knowledge, this study represents the single largest metagenomic AMR monitoring effort of livestock: both in terms of countries (9), herds included (359), individual animals sampled (>9,000) and sequencing effort (>5,000 Gb)[16].

Results

Acquired resistome characterization. The total AMR load varied significantly across samples, depending on both the host animal

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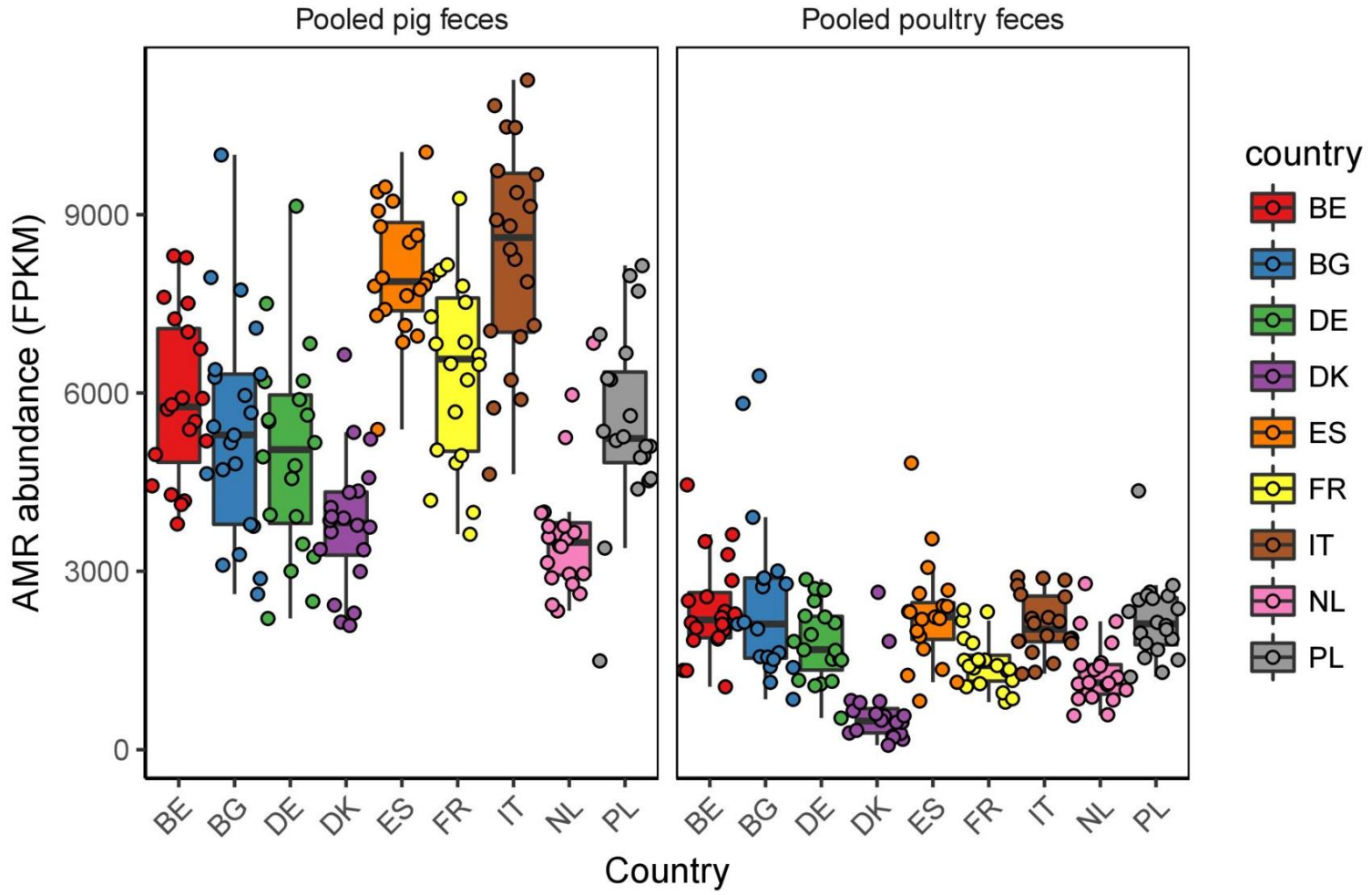
- Monitor AMR in integrated pig and poultry herds in nine European countries
- Over 9000 animals sampled
- 181 pig and 178 poultry herds
- >5,000,000,000,000 bp sequenced

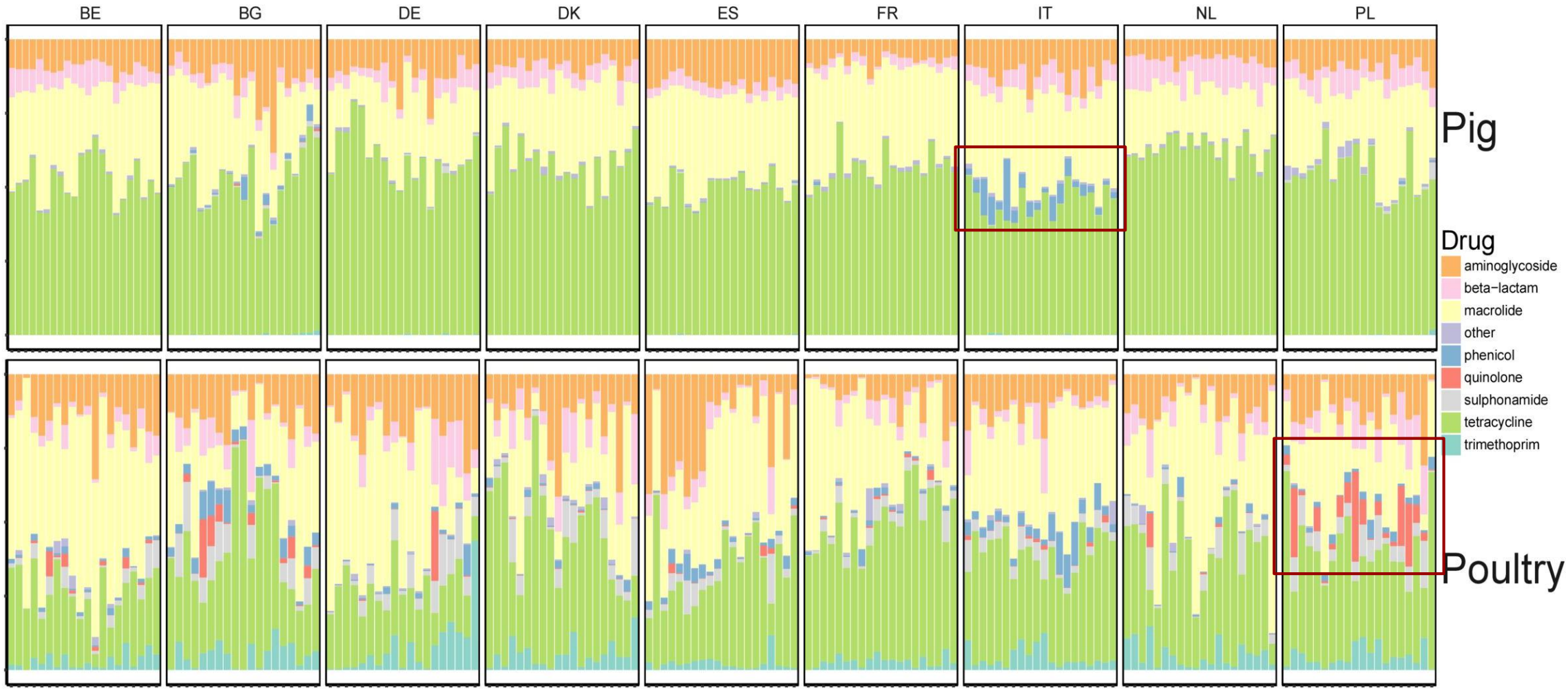
P Munk et al (2018)

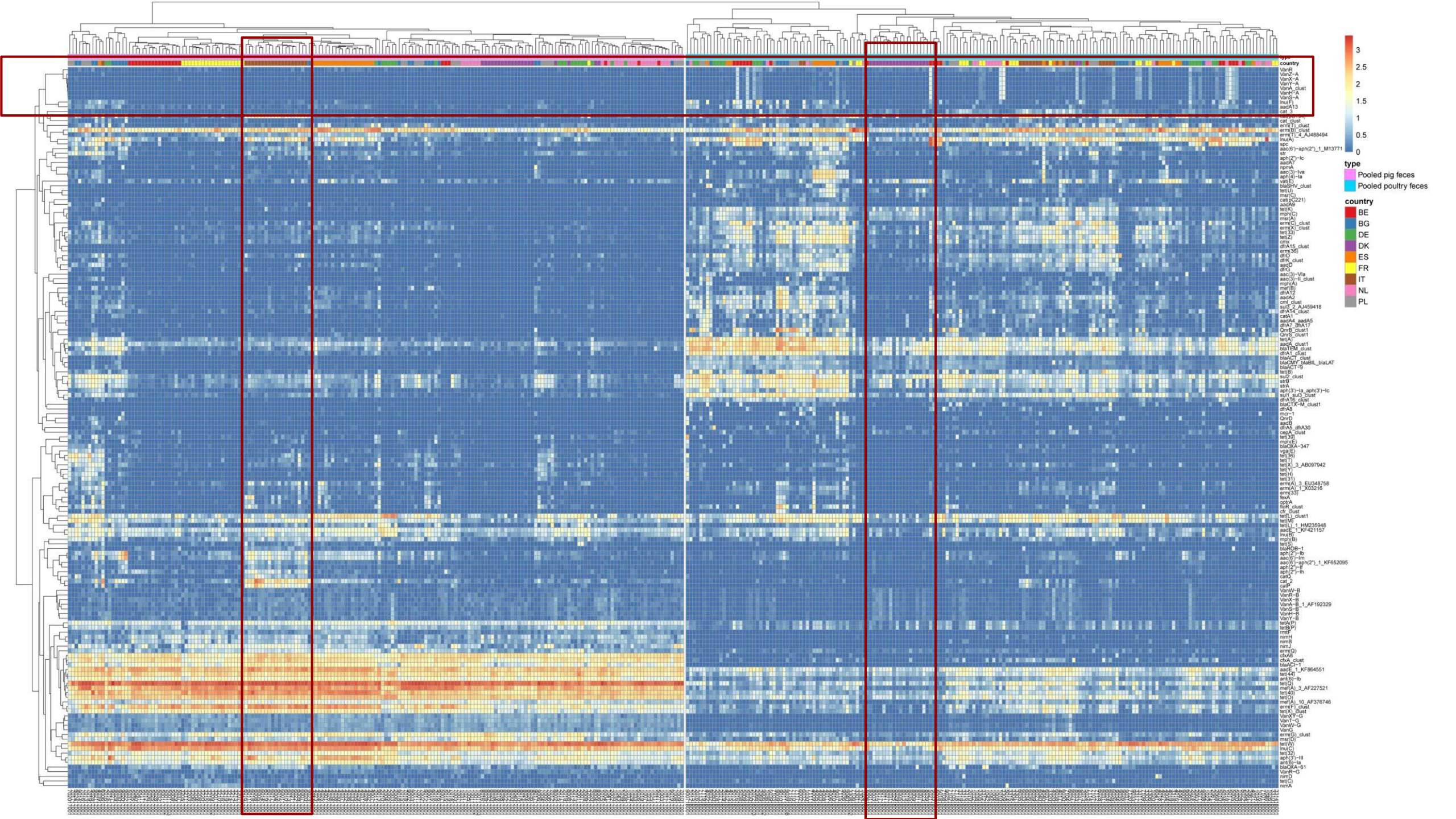
rdcu.be/3ora

Total AMR


Pooled pig feces
Pooled poultry feces
AMR abundance (FPKM)
9000
6000
3000
0
BE
BG
DE
DK
ES
FR
IT
NL
PL
Country
country
BE
BG
DE
DK
ES
FR
IT
NL
PL

AMR per drug class




type
country
VanR
VanZ−A
VanX−A
VanY−A
VanA_clust
VanRF−A
VanS−A
lnu(F)
aadA13
cat_3
cat_clust
erm(T)_clust
erm(B)_clust
erm(T)_4_AJ488494
lnu(A)
spc
aac(6')−aph(2")_1_M13771
str
aph(2")−Ic
aadA7
npmA
aac(3)−Iva
aph(4)−Ia
vat(E)
blaSHV_clust
tet(U)
msr(C)
cat(pC221)
aadA9
tet(K)
mph(C)
msr(A)
erm(C)_clust
erm(X)_clust
tet(33)
tet(Z)
cmx
dfrA15_clust
erm(36)
dfrD
dfrK_clust
aadD
dfrG
aac(3)−VIa
aac(3)−II_clust
mph(A)
mef(B)
dfrA12
aadA2
cml_clust
sul3_2_AJ459418
dfrA14_clust
catA1
aadA4_aadA5
dfrA7_dfrA17
QnrB_clust1
Qnr5_clust1
tet(A)
aadA_clust1
blaTEM_clust
dfrA1_clust
blaACT_clust
blaCMY_blaBIL_blaLAT
blaACT−9
tet(B)
sul2_clust
str6
strA
aph(3')−Ia_aph(3')−Ic
sul1_sul3_clust
dfrA16_clust
blaCTX−M_clust1
dfrA8
mcr−1
QnrD
aadB
dfrA5_dfrA30
cepA_clust
tet(39)
mph(E)
blaOXA−347
vga(E)
tet(36)
tet(T)
tet(X)_3_AB097942
tet(Y)
tet(H)
tet(31)
erm(A)_3_EU348758
erm(A)_1_X03216
erm(33)
fexA
optrA
floR_clust
cfr_clust
tet(L)_clust1
tet(M)
tet(L)_1_HM235948
aadE−1_KF421157
lnu(B)
mph(B)
tet(S)
blaROB−1
aph(2")−Ib
aac(6')−Im
aac(6')−aph(2")_1_KF652095
aph(2")−If
aph(2")−Ih
catQ
cat_2
catP
VanW−B
VanR−B
VanX−B
VanA−B_1_AF192329
VanS−B_1_AF192329
VanH−B
VanY−B
tet(A/P)
tet(B/P)
rmtF
nimH
nimB
nimJ
erm(Q)
cfxA6
cfxA_clust
blaACI−1
aadE_1_KF864551
tet(44)
ant(6)−Ib
tet(Q)
mef(A)_3_AF227521
tet(40)
tet(O)
mef(A)_10_AF376746
erm(F)_clust
tet(X)_clust
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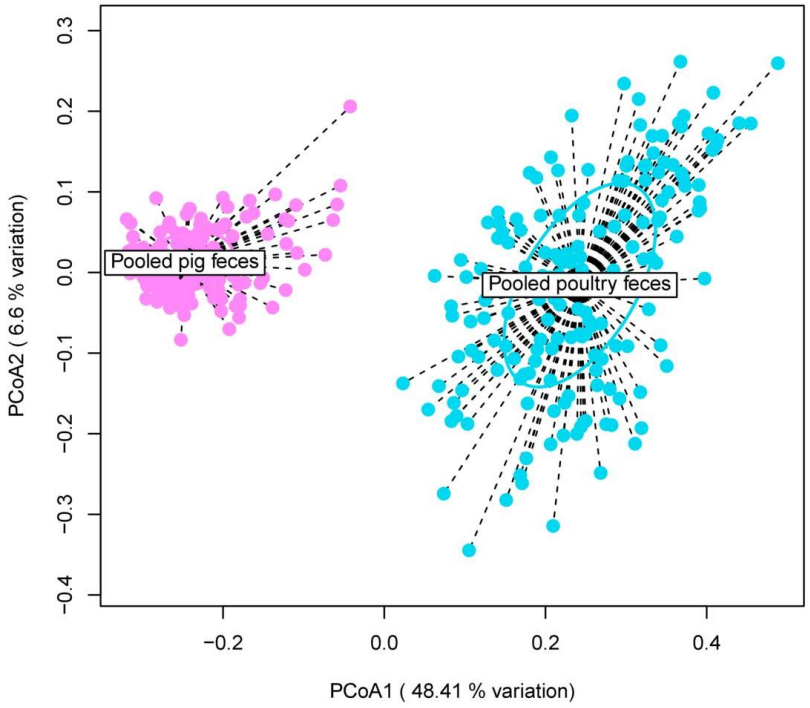
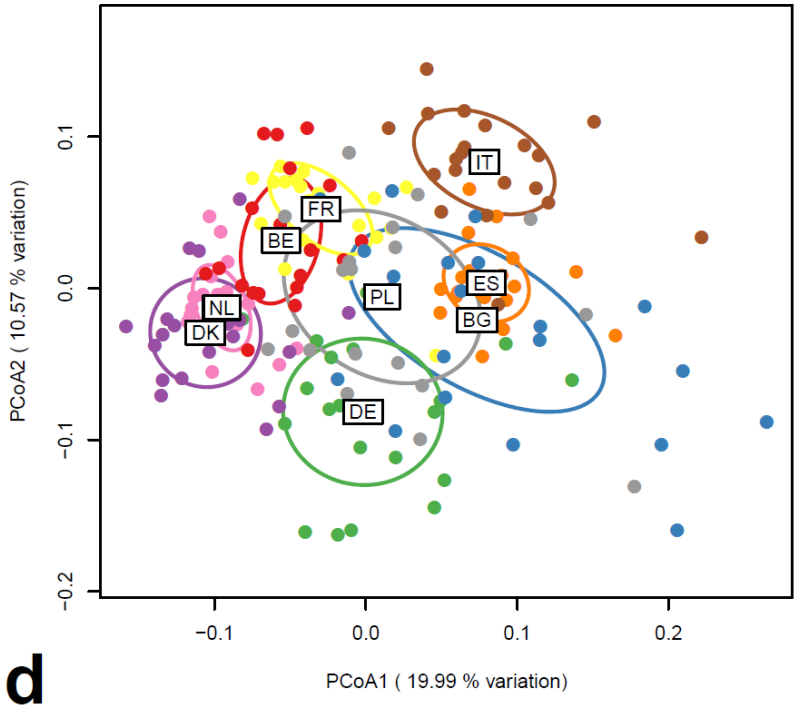
VanT−G
VanW−G
VanG
erm(G)_clust
msr(D)
tet(W)
lnu(C)
tet(32)
aph(3")−III
ant(6)−Ia
blaOXA−61
VanR−G
nimD
tet(C)
nimA
3
2.5
2
1.5
1
0.5
0
type
Pooled pig feces
Pooled poultry feces
country
BE
BG
DE
DK
ES
FR
IT
NL
PL

Resistome clustering

All samples

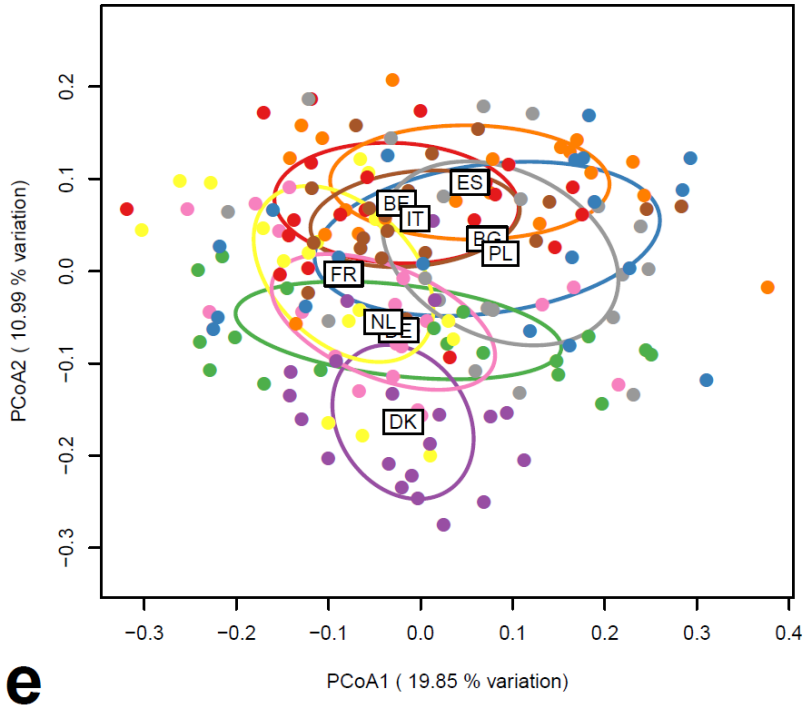


Pig



d

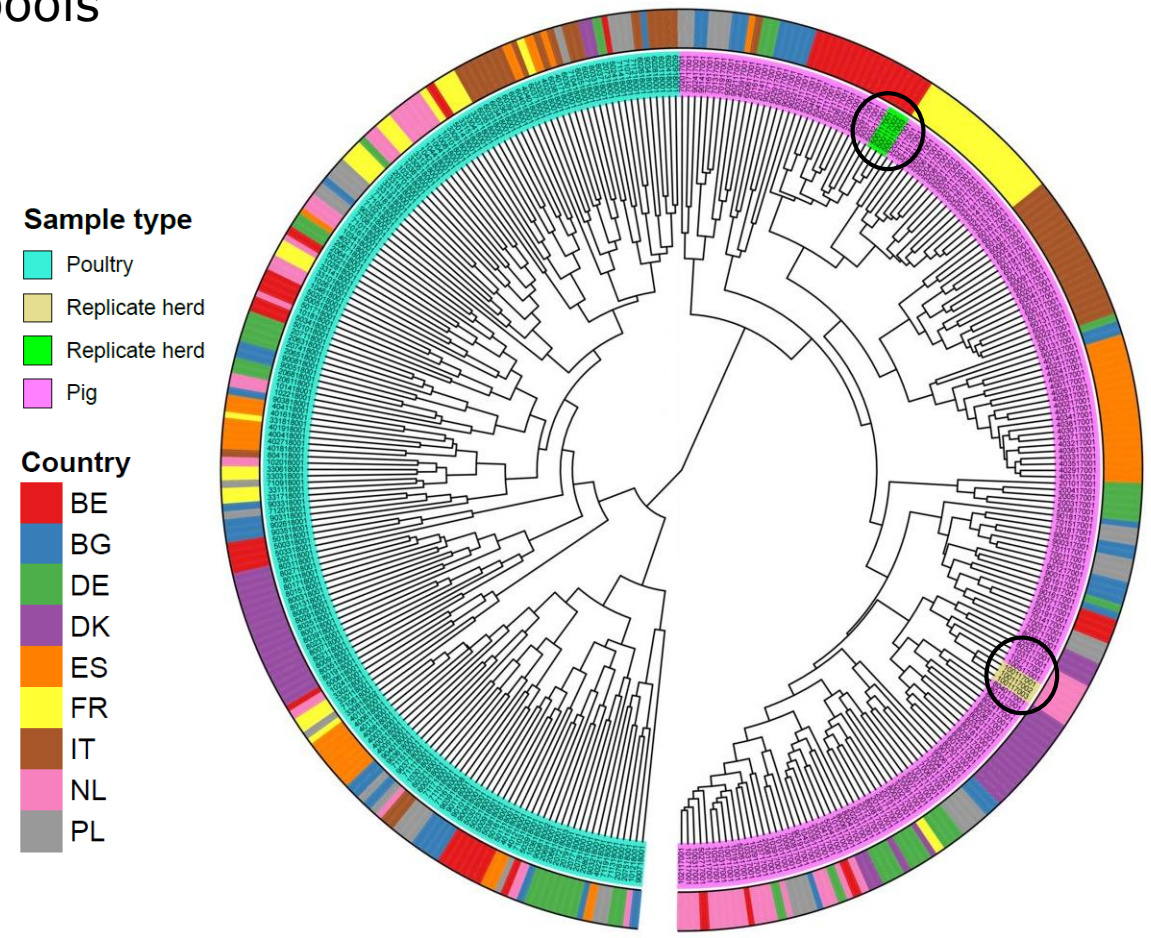
Poultry



e

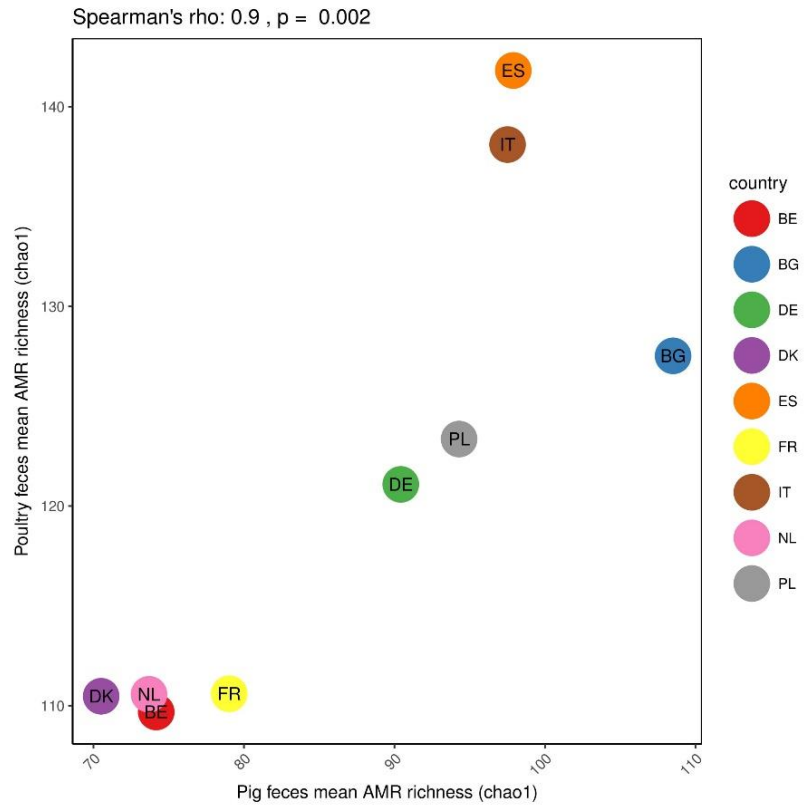
Sampling reproducibility

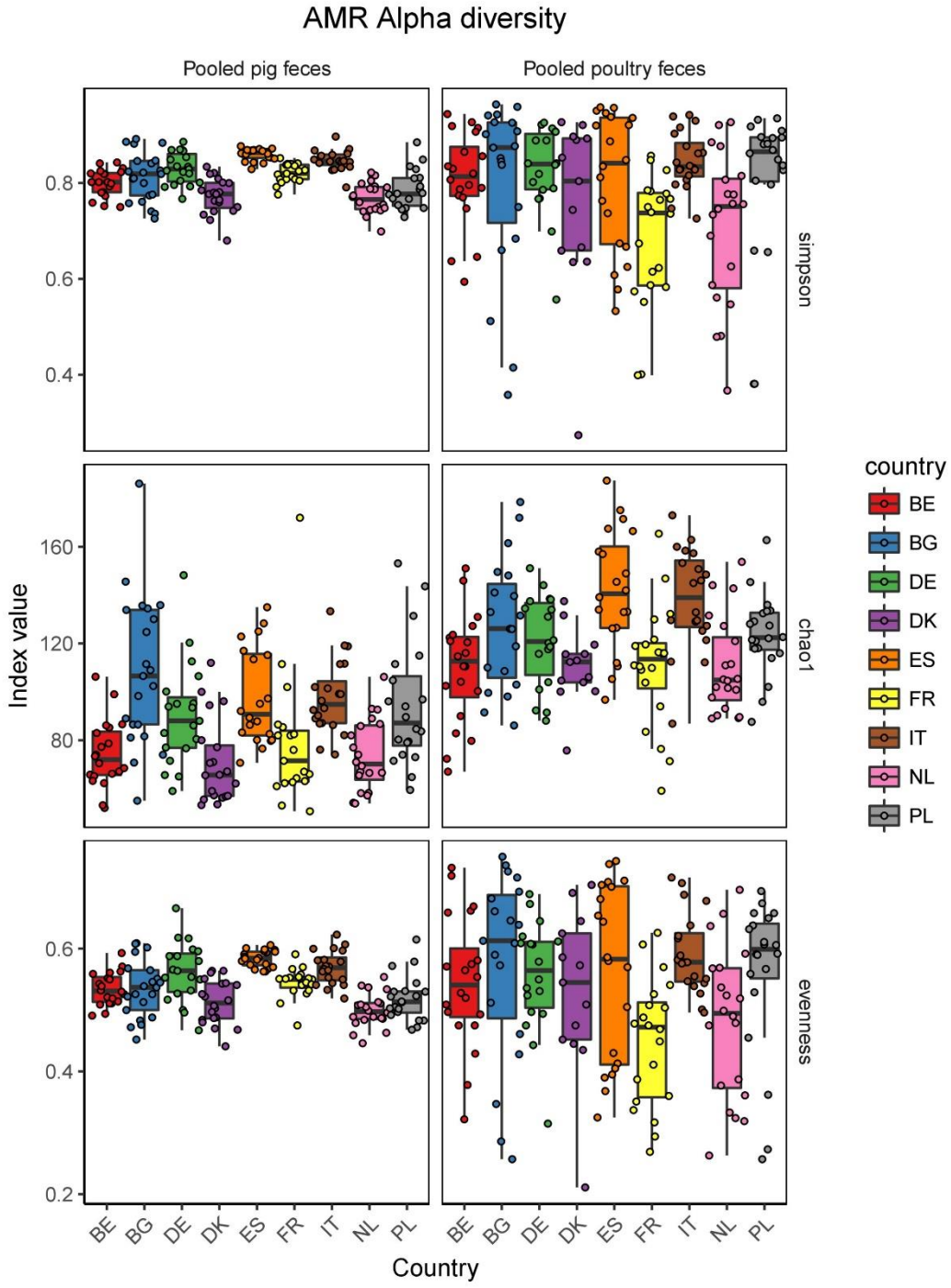
- Two pig farms were sampled 3 times on same day
- 3 sampling rounds x 25 individual samples = 3 pools
- 91.5 – 93.3% Bray Curtis similarity (BE)
- 93.6 – 93.7% Bray Curtis similarity (NE)



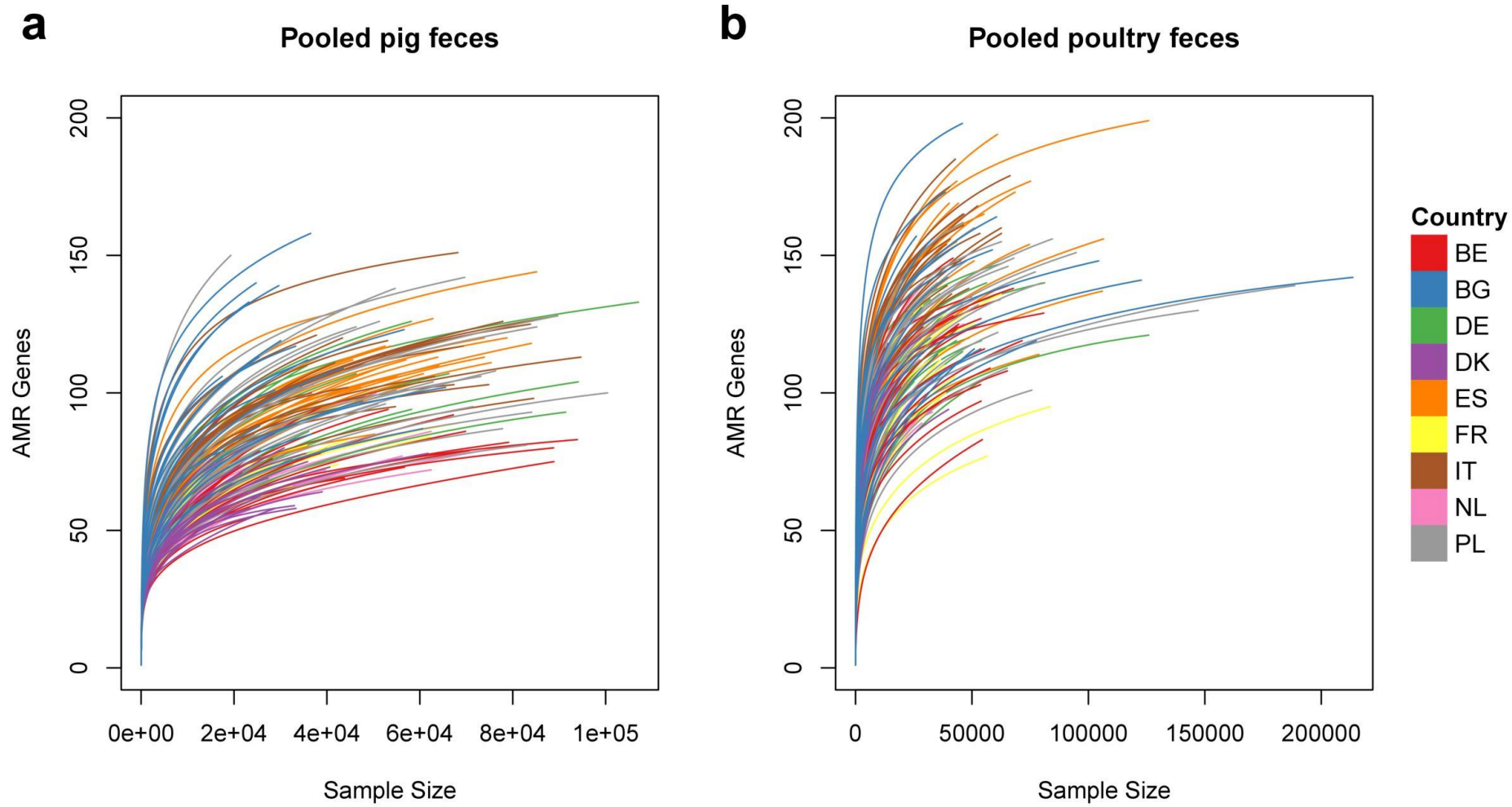
AMR gene alpha diversity

- Alpha diversity in sample resistomes:
  - Evenness (Pielou)
  - Richness (Chao1)
  - Diversity (Simpson)

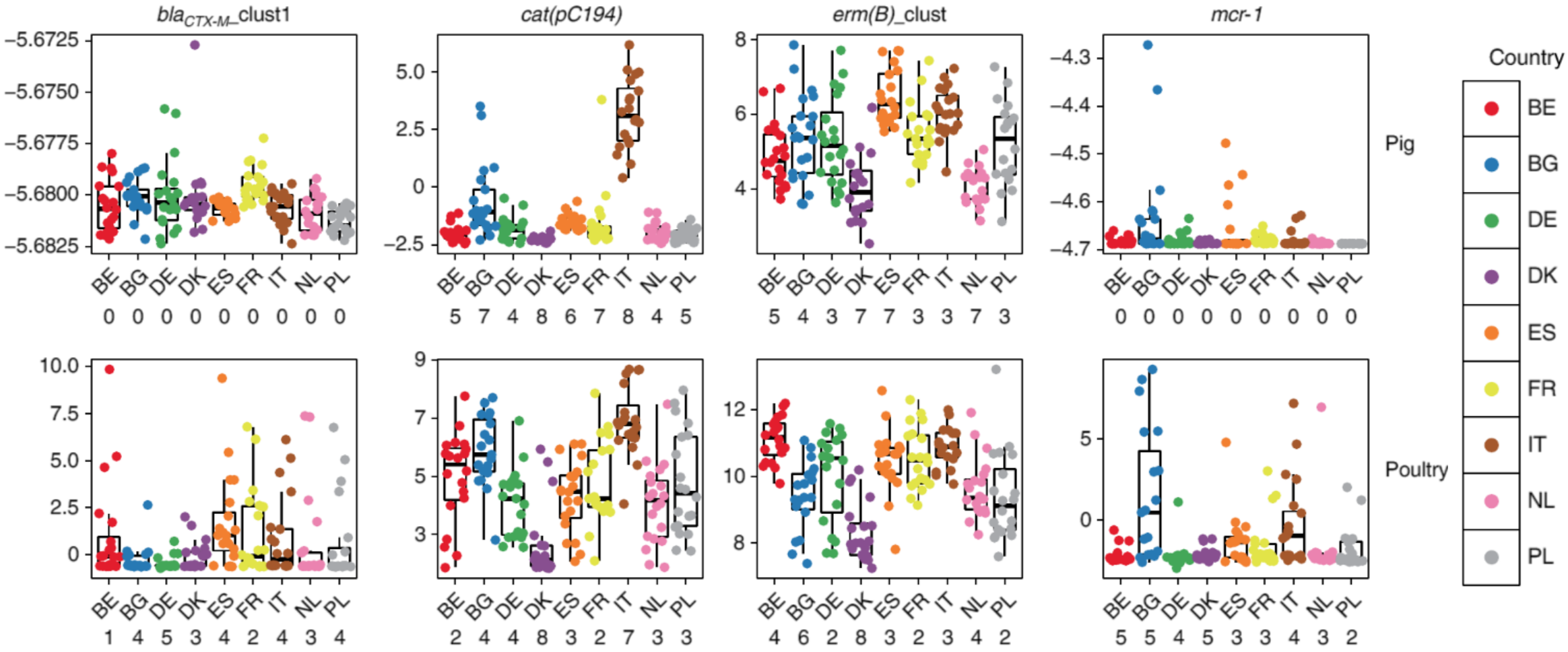




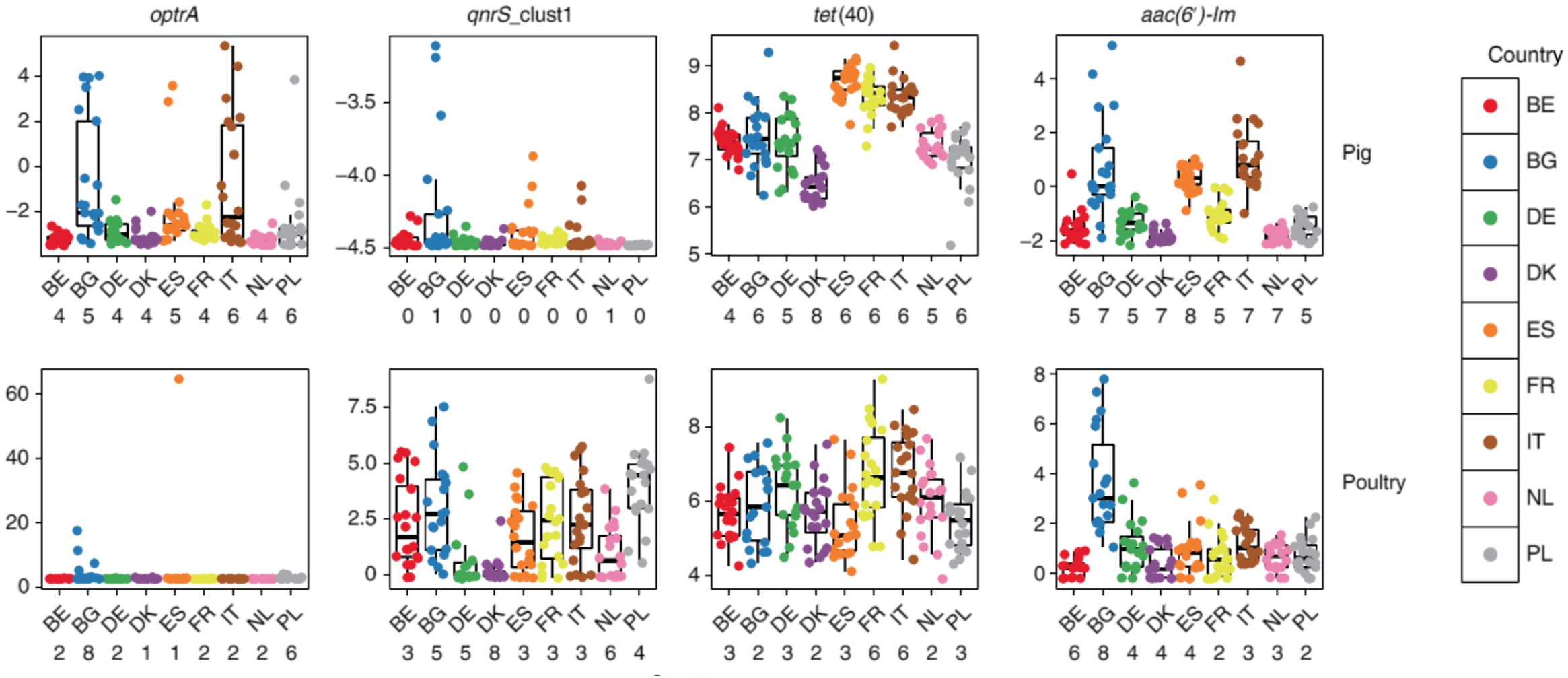
AMR gene alpha diversity



Differentially abundant genes



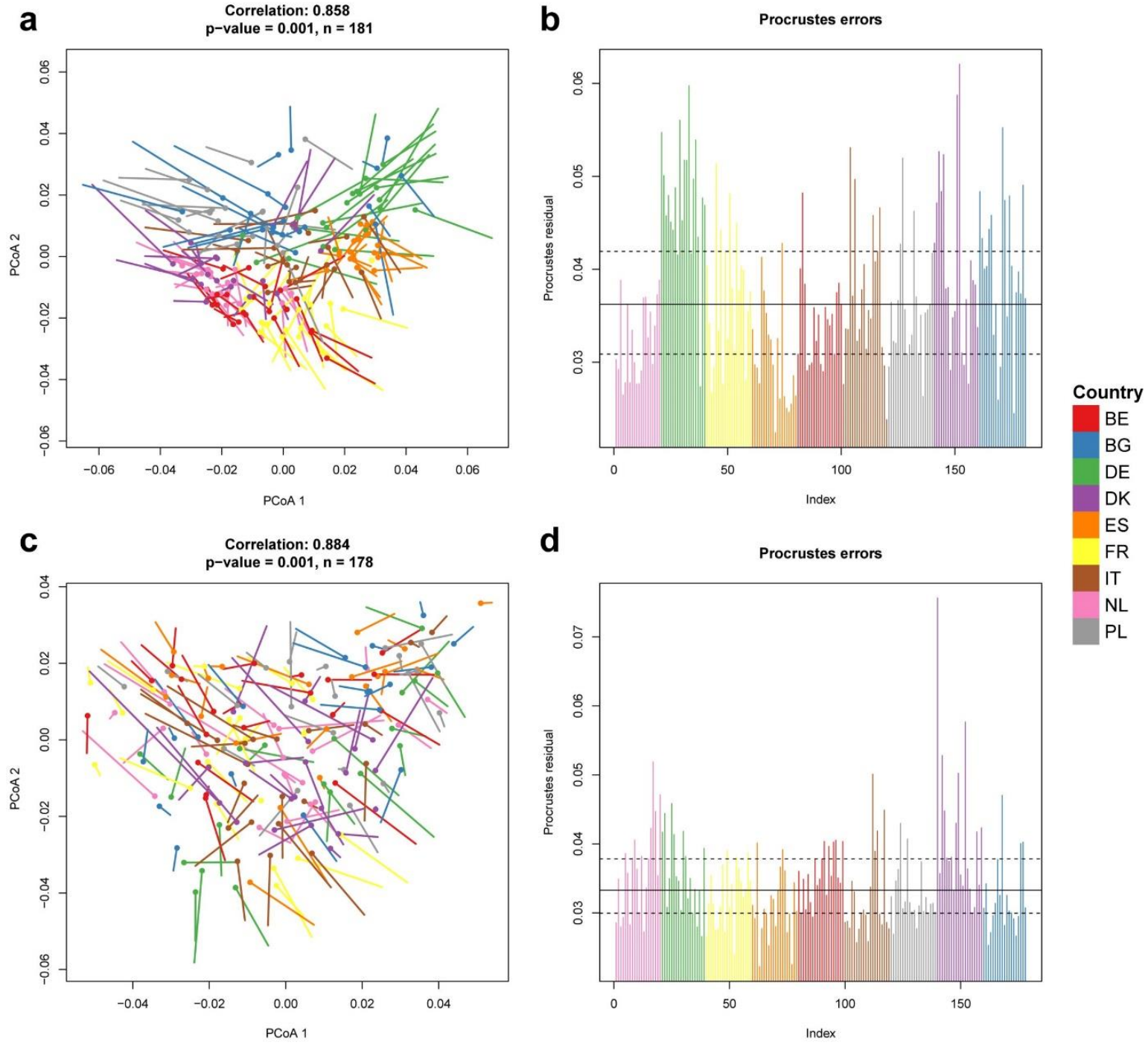
Differentially abundant genes



Effect of bacteriome

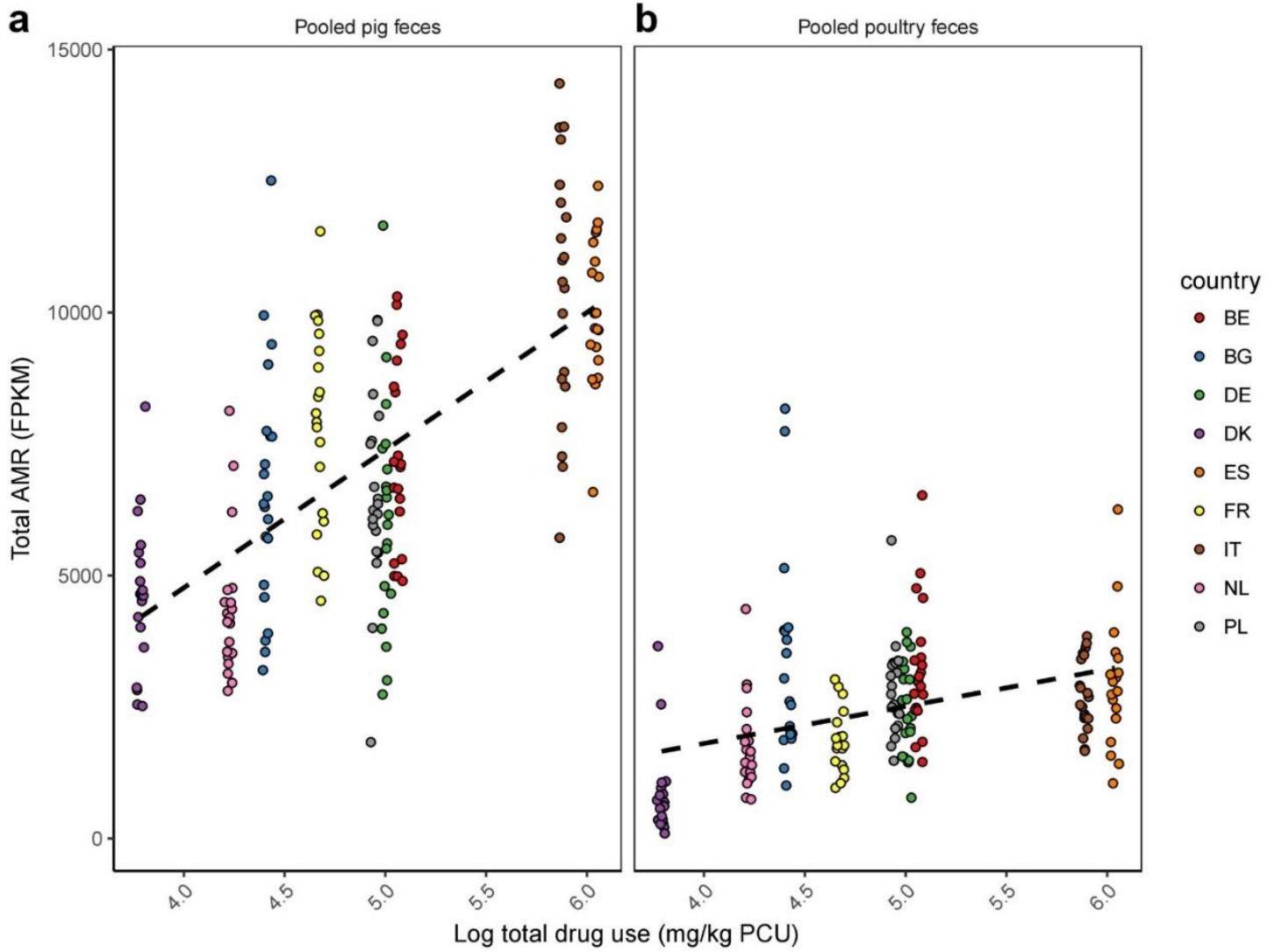
Pig farms

Poultry farms



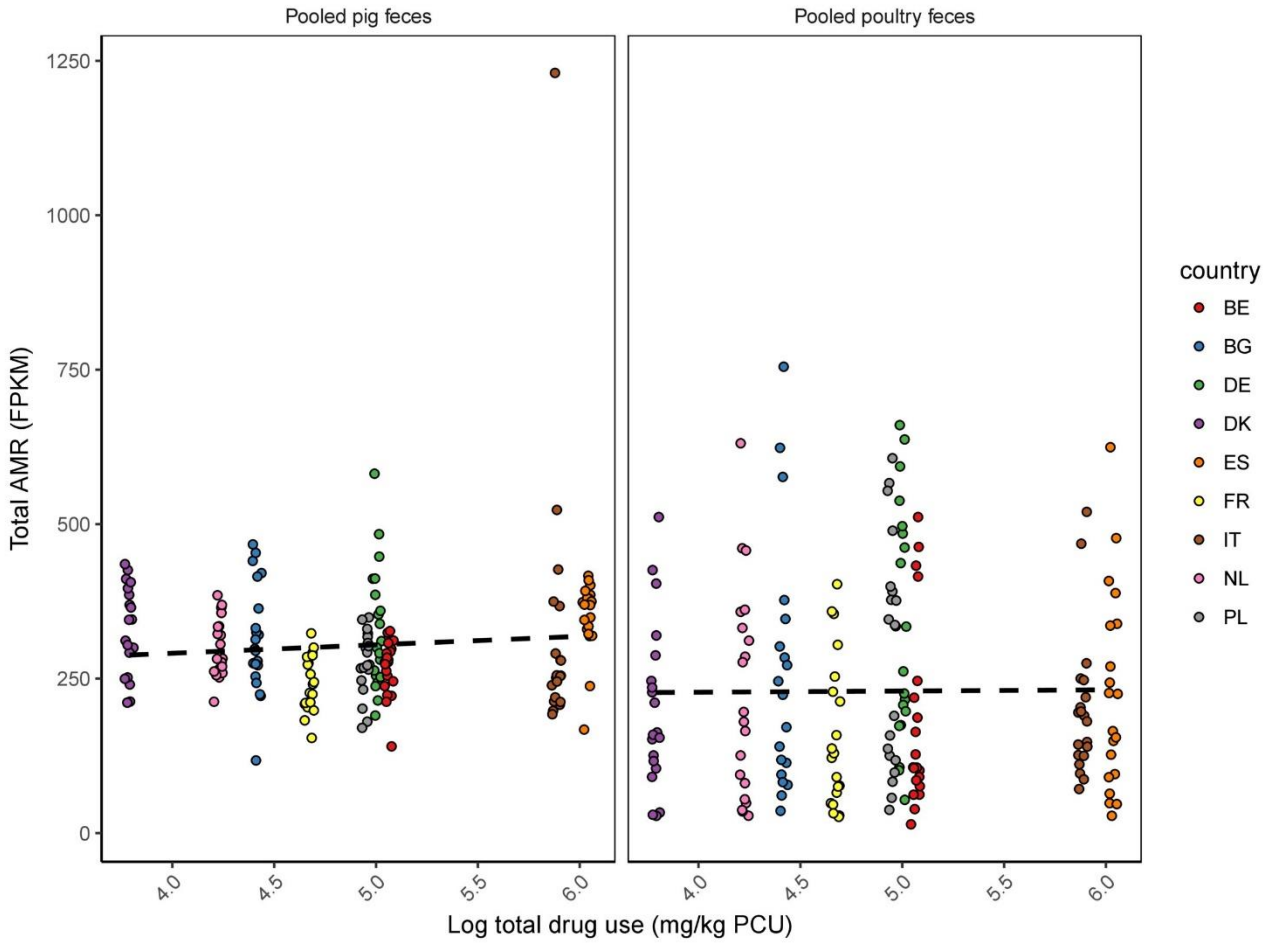
Total ResFinder AMR~AMU

- Total AMR measured follows veterinary drug use



Functional AMR~AMU association

- We introduce a database (FRD) of resistance genes determined through functional metagenomics
- Regression models repeated with FRD total abundance
- No association to drug use!



EFFORT project final conference

- www.effort-against-amr.eu
- Data from more animal species
- Specific risk factors analyzed
- Farmer survey data analyzed

What about human monitoring?

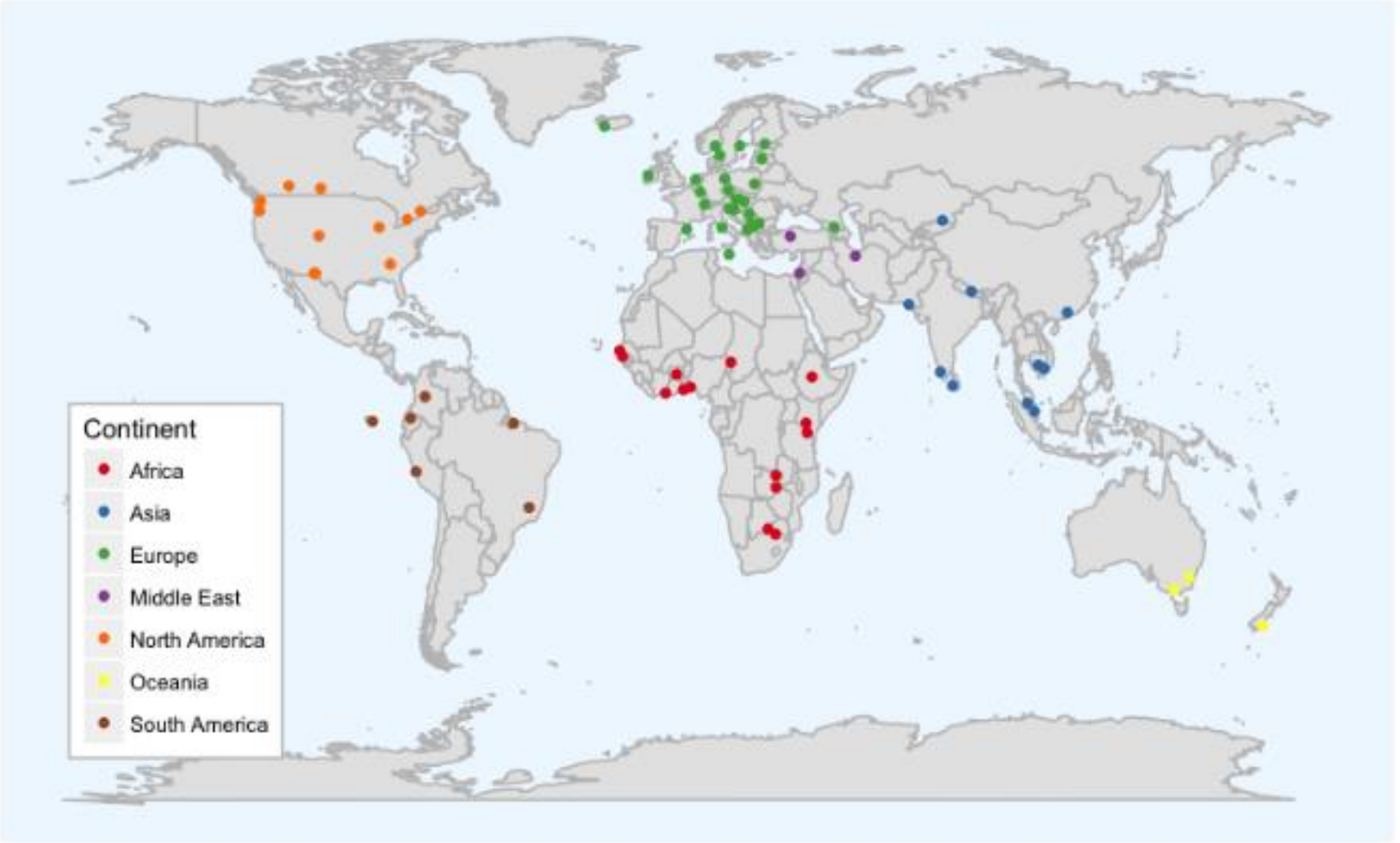
Sewage

Why sewage?

- Easy to get permission
- Less sensitive data
- Able to monitor large, healthy populations
- Metagenomics lends itself to fewer / pooled samples

Sewage sampling 2016 – Round 1

- 80 samples
- 63 countries




HARD
ROCK
HALLELUJAH
PAKISTAN
KARACHI
S.SHAKOOR
001
CPH
FRAGILE
DHL

7 slides removed

Conclusions

- Our metagenomic approach is able to quantify hundreds of known AMR genes in pooled samples
- Our method is precise and sensitive enough to detect effects of e.g. differential drug use
- The digital sequence data lends itself sharing and re-use, giving it further value
- The little hands-on time and ability to automate computational workflows is valuable

Future perspectives

- At currently feasible sequencing depths, there are still AMR genes below our detection level
- Further work should be done to ensure compatibility with many sample types
- A metagenomics approach should supplement existing monitoring. It is unable to replace it and relies on updated AMR gene databases and phenotypically-derived annotation

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Rene S. Hendriksen
Sofia Duarte
Lasse Bergmark
Berith E. Knudsen
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Heike Schmitt
Lidwien Smit
Roosmarijn Luiken
Liese Van Gompel
Alex Bossers
And many more

Miljø- og Fødevareministeriet
Fødevarestyrelsen

novo nordisk fonden

Thank you for listening!