

Novel methods to measure the occurrence & transmission of AMR

(From standards to chaos?)

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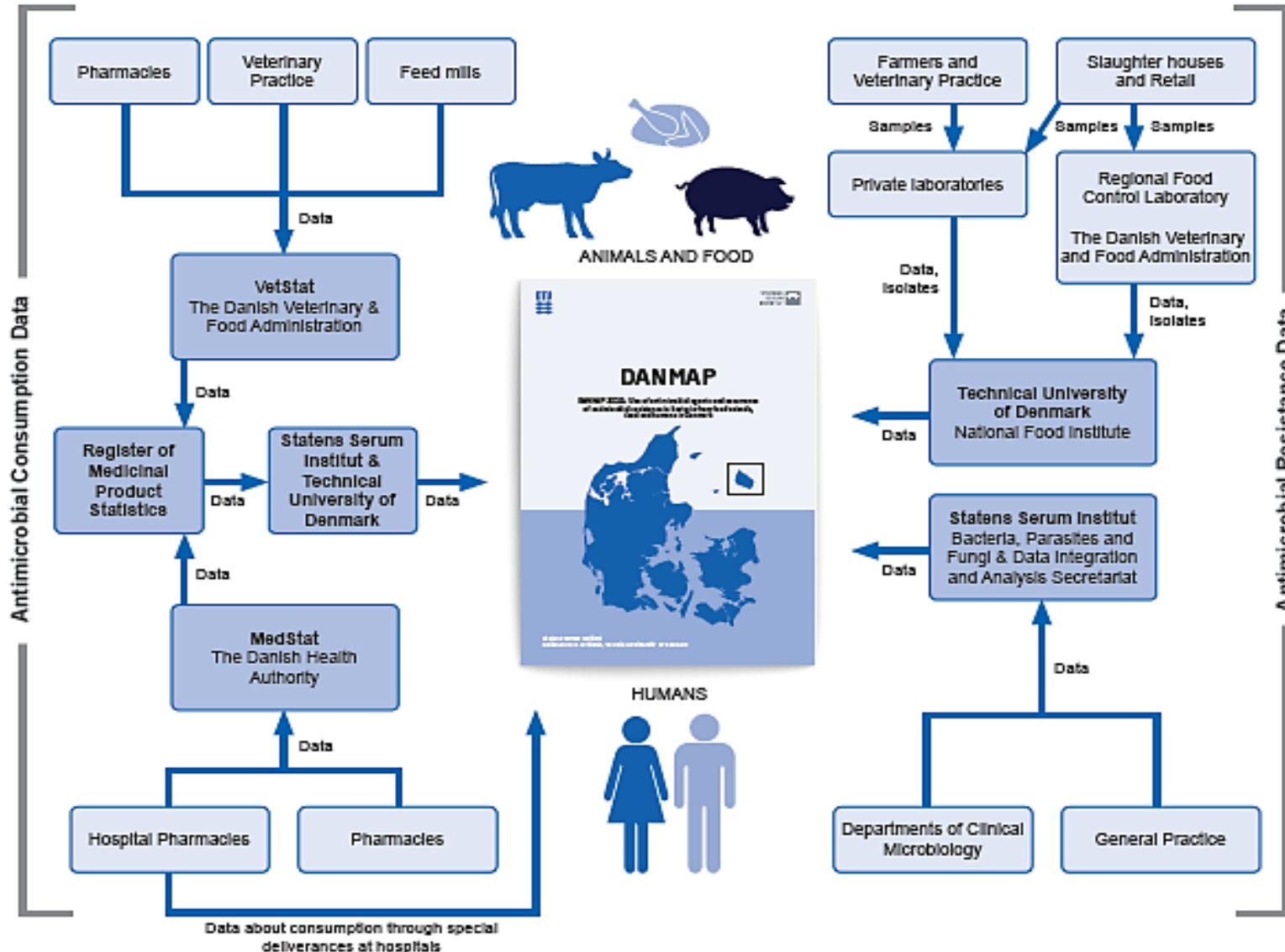
No matter how we look at it

- Surveillance is the basis of everything and what the world needs is:
- Real-time data on occurrences of all infectious agents and AMR everywhere
 - Geography, reservoir and pathogen independent
 - Observe trends and rapidly compare between data
 - Transfer of information to those who need to:
 - Take public health response
 - Develop tests and treatments
 - Take clinical decisions

DANMAP – AMR surveillance since 1995

Figure 3.1 Organisation of the DANMAP collaboration regarding data and data flow

DANMAP 2019



Clinical isolates from humans and animals and indicator isolates from animals

Structured number of samples and reservoirs

Phenotypic testing

Changing patterns

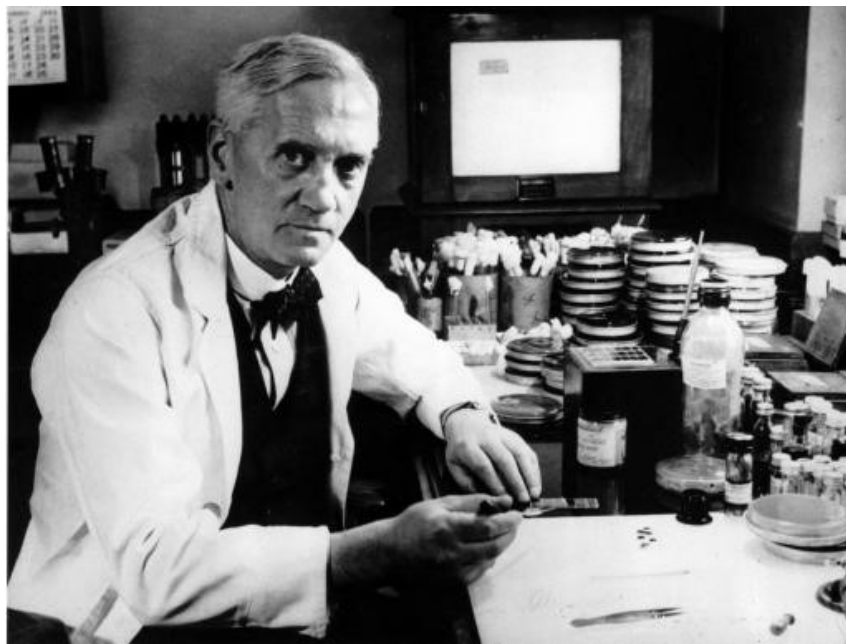
- Phenotype -> genotypes
- Single isolates -> resistomes
- Clinical -> community and hotspots
- Static -> dynamic (even standard -> chaos)

Advantages of Next Generation Sequencing (NGS)

- DNA/RNA are common across pathogens, therefore, methods to analyse genomes are potentially universal
- NGS provides a universal language
- Raw data are shared allowing for QC and re-analyses
- NGS capacity is developing fast
 - Less equipped labs may leapfrog

Sequence data:

```
>gi|218693476|ref|NC_011748.1| Escherichia coli 55989 chromosome, complete genome  
GTAAGTATTTTCAGCTTTTCATTCTGACTGCAACGGGCAATATGTCTCTGTGTGGATTAAAAAAGAGT  
GTCGTGATAGCAGCTTCTGAACTGGTTACCTGCCGTGAGTAAATTTAAATTTTATTGACTTAGGTCACTAA  
ATACTTTAACCAATATAGGCATAGCGCACAGACAGATAAAAAATTACAGAGTACACAACATCCATGAAACG  
CATTAGCACCCACCATTACCACCACCATCACCATTACCACAGGTAACGGTGCGGGCTGACGCGTACAGGAA  
ACACAGAAAAAAGCCCGCACCTGACAGTGCGGGCTTTTTTTTCGACCAAAGGTAACGAGGTAACAACCAT  
GCGAGTGTGAAAGTTCGGCGGTACATCAGTGGCAAATGCAGAACGTTTTCTGCGTGTGCGGATATCTG  
GAAAGCAATGCCAGGCAGGGGCGAGGTGGCCACCGTCTCTCTGCCCCCGCCAAAAATCACCACCCACTGG  
TGGCGATGATGAAAAAACCATTAGCGGCCAGGATGCTTACCCAATATCAGCGATGCCGAACGTATTTT  
TGCCGAACTTTTGACGGGACTCGCCGCCGCCAGCCGGGGTTCCCGCTGGCGCAATTGAAAACTTTCGTC  
GATCAGGAATTTGCCCAAATAAAACATGTCTTGCATGGCATTAGTTTGTGGGGCAGTGCCCGGATAGCA
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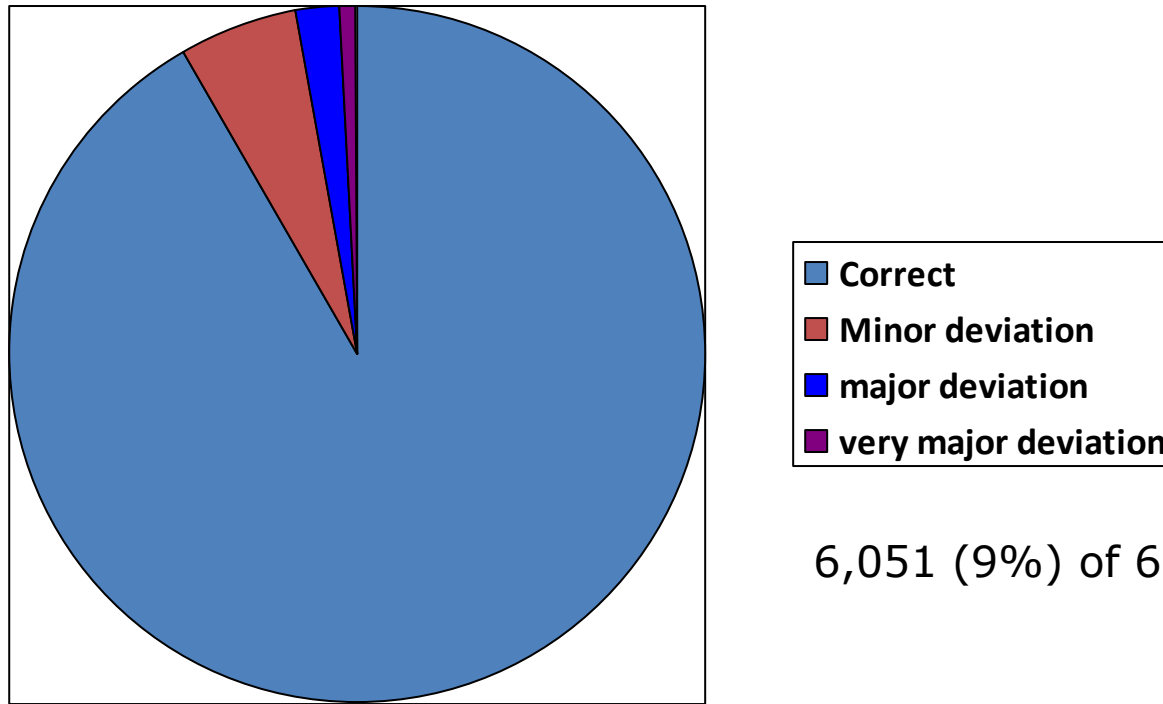
diffusion



dilution

Results of Use of WHO Global Salm-Surv External Quality Assurance System for Antimicrobial Susceptibility Testing of *Salmonella* Isolates from 2000 to 2007[∇]

Rene S. Hendriksen,^{1*} Anne Mette Seyfarth,¹ Arne B. Jensen,¹ Jean Whichard,² Susanne Karlsmose,¹ Kevin Joyce,² Matthew Mikoleit,² Stephanie M. DeLong,² François-Xavier Weill,³ Awa Aidara-Kane,⁴ Danilo M. A. Lo Fo Wong,⁴ Frederick J. Angulo,² Henrik C. Wegener,¹ and Frank M. Aarestrup¹

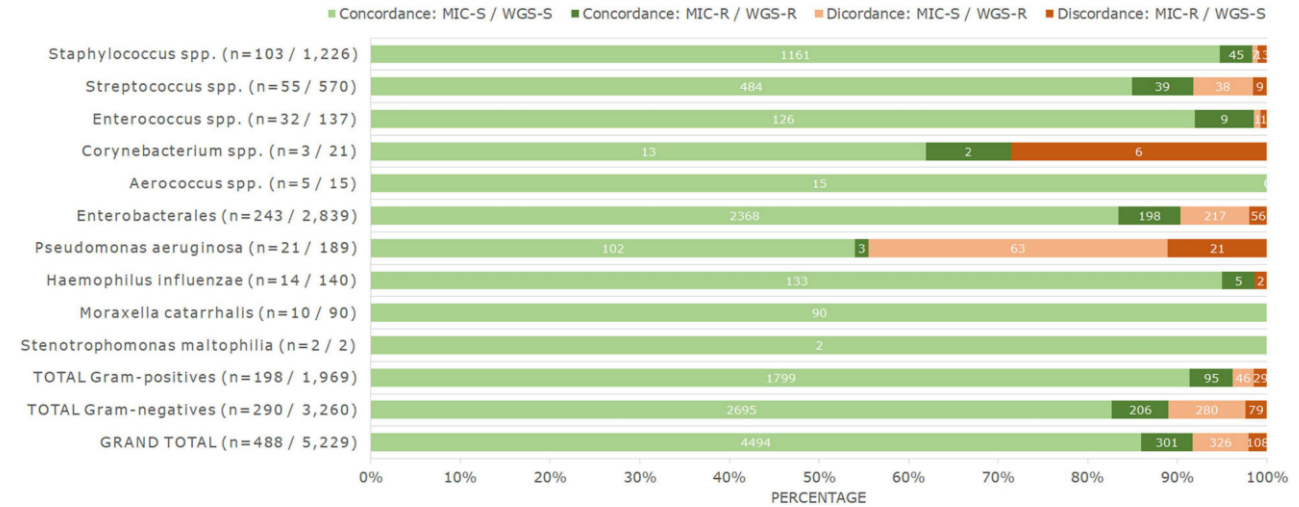


6,051 (9%) of 67,229 tests in-correct

And we were excited and very happy

NGS or phenotypes

- Phenotypes
 - Far from perfect
 - Problems with reproducibility
 - Difficult to compare between laboratories
- Phenotypes versus NGS
 - Zankari et al. 2013, JAC – 99.8% concordance
 - Stoesser et al. 2013 JAC – 97% concordance
 - Gordon et al. 2014. JCM – 99.3% concordance
 - McDermott et al. 2016, AAC – 99% concordance
 - Rebelo et al. Front Micro - 91% concordance
- **Susceptibility testing is an *in vitro* estimation of clinical *in vivo* efficacy**



Still some way to acceptance in clinical diagnostic – however beginning to be accepted in surveillance and epidemiology

Allows for rapid re-analyses

- Mcr-1 gene added to ResFinder database on Nov. 24
- Nov. 25 RAPID COMMUNICATIONS
isolate: Detection of mcr-1 encoding plasmid-mediated colistin-resistant *Escherichia coli* isolates from human bloodstream infection and imported chicken meat, Denmark 2015
- Nov. 25 to +3,0
- Nov. 29
- Dec. 2 Correspondence: Henrik Hasman (henh@ssi.dk)
- Dec. 9 publication

H Hasman¹, AM Hammerum¹, F Hansen¹, RS Hendriksen², B Olesen³, Y Agersø², E Zankari², P Leekitcharoenphon², M Stegger^{1,4}, RS Kaas², LM Cavaco², DS Hansen³, FM Aarestrup², RL Skov¹

1. Department of Microbiology and Infection Control, Statens Serum Institut, Copenhagen, Denmark

2. National Food Institute, Technical University of Denmark, Lyngby, Denmark

3. Department of Clinical Microbiology, Herlev and Gentofte Hospital, Copenhagen University Hospital, Herlev, Denmark

4. Pathogen Genomics Division, Translational Genomics Research Institute (TGen), Flagstaff, Arizona, USA

Correspondence: Henrik Hasman (henh@ssi.dk)

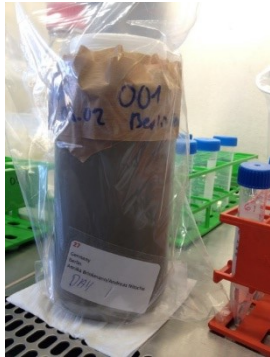
hits
extended

Metagenomics – One technology that takes all

Metagenomics is defined as the **sequencing-based analysis of genomes** contained within an **environmental sample**



Standardizing wet-lab methods



Sample storage. Poulsen et al. (2022) Microb. Spec.



DNA extraction from sample
BE Knudsen et al. (2016) mSystems



DNA sheared to 3-400 bp



DNA sequencing. Poulsen et al. (2021) Microb. Spec.

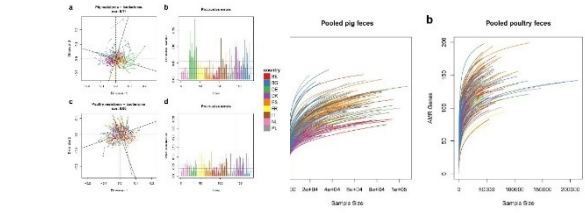


KAPA Hyper PCR-free library preparation

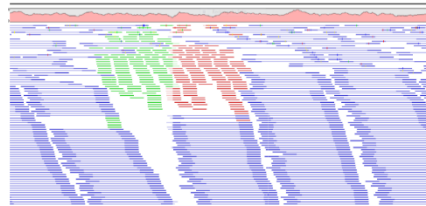


HiSeq 4000 (Poulsen et al. 2021) NovaSeq6000
2 x 35M 150bp fragments = 10 Gbp

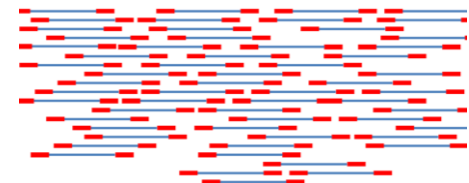
Dry-lab methods



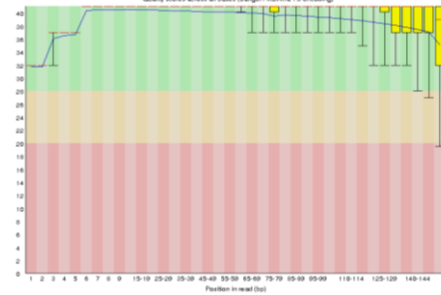
Quantitative analyses



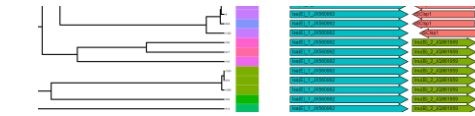
Alignment with **KMA** against **ResFinder** and **Silva**



PE DNA reads
>35M/ sample



QC, quality- and
adapter-trimming **bbduk2**

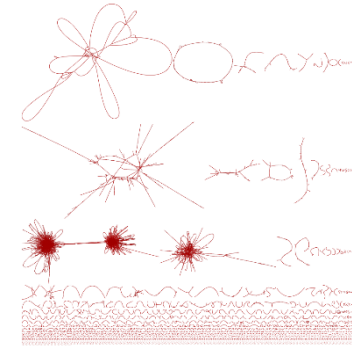


Flank-based analyses

Genome binning
with **MetaBat2**

Taxonomic
assignment
with **Kraken2**

Plasmid
identification
with **Kraken2**,
BLAST and
PPR-Meta



De novo metagenomic
assembly using **metaSpades**

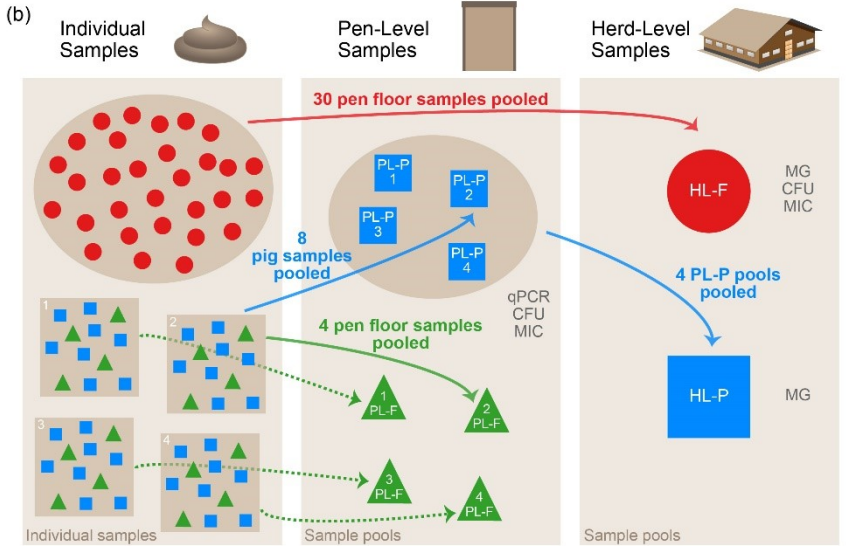
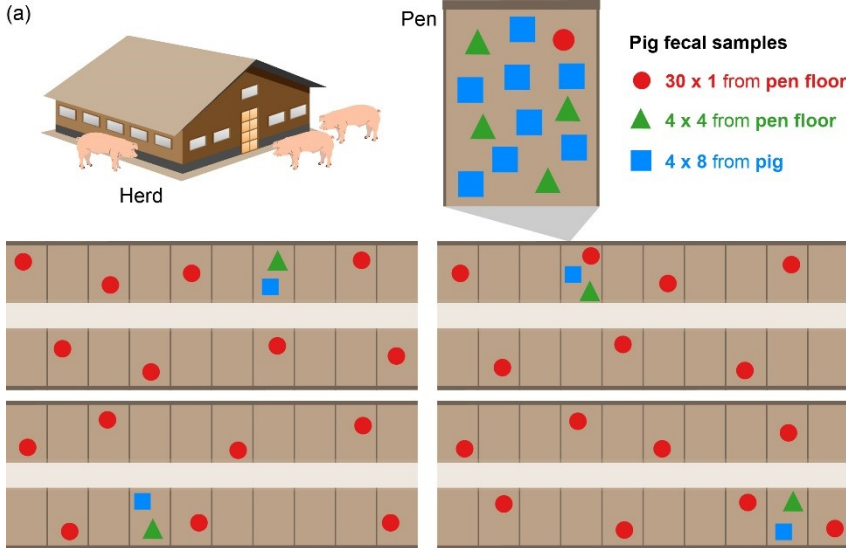
Munk et al. *Nat. Comm.* 2022
 Aytan-Aktug et al. *mSystems*. 2022
 Bortolaia et al. *JAC.* 2020
 Szarvas et al. *Commun Biol.* 2020
 Hendriksen et al. *Nat. Comm.* 2019
 Clausen et al. *BMC Bioinformatics.* 2018
 Munk et al. *Nat. Micro.* 2018
 Zankari et al. *JAC.* 2017
 Kaas et al. *PlosOne.* 2014
 Zankari et al. *JAC.* 2012
 Larsen et al. *J Clin Microbiol.* 2012

Identifying ARGs
with **ResFinder**

Finding and
annotating
genes with
Prokka

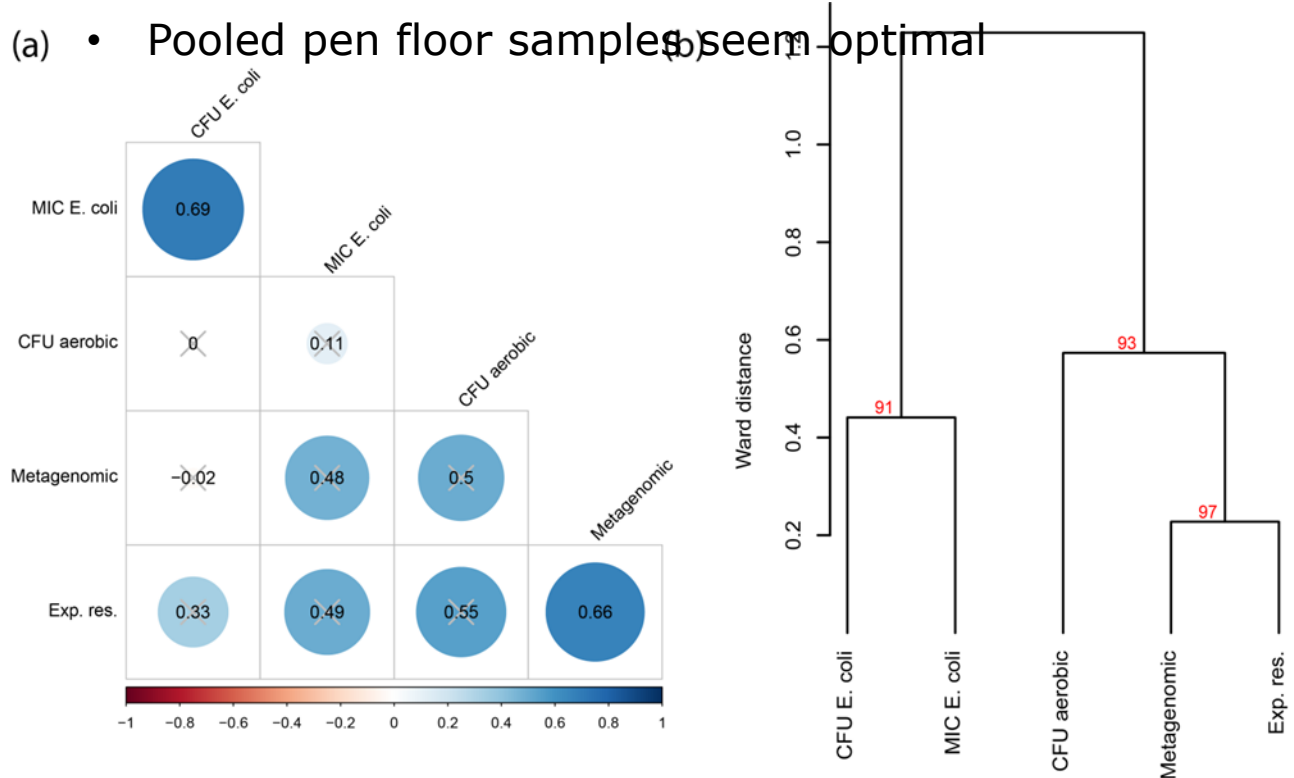
Evaluation: 10 Danish farms

- Different sampling procedures
- Different measure methods (isolates, CFU, qPCR, metagenomics)



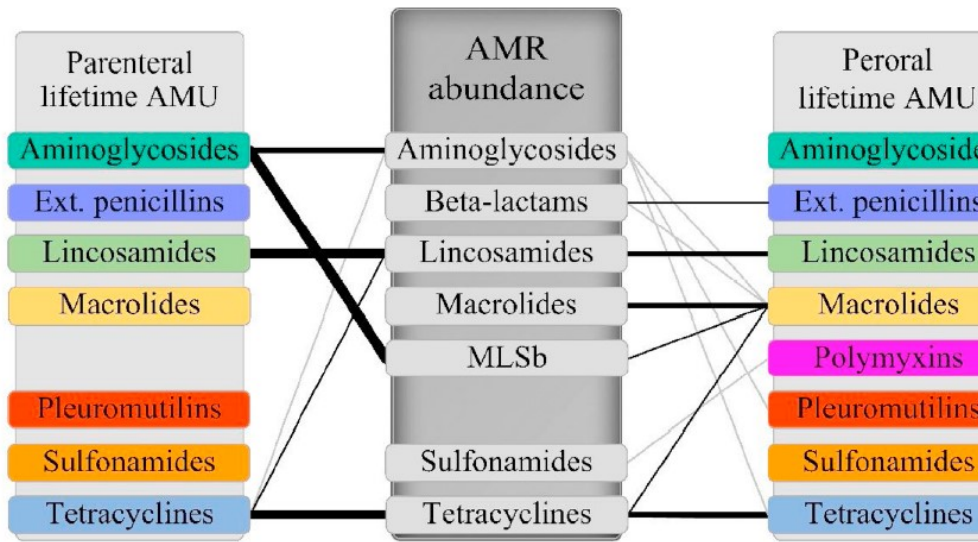
Result:

- Metagenomics correlates best with antimicrobial use
- (a) • Pooled pen floor samples seem optimal



Continued focus in Denmark

Predicting effect of interventions

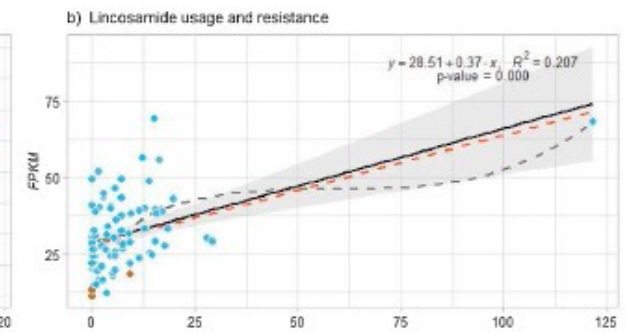
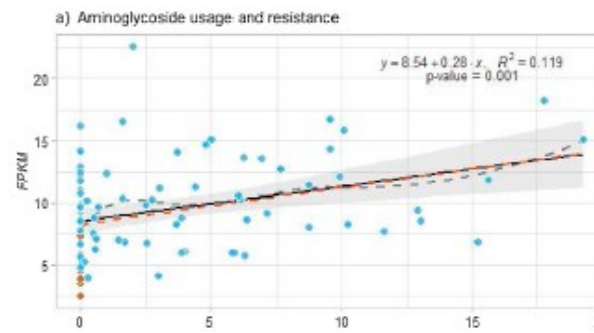


Munk et al. 2017. JAC 72: 385

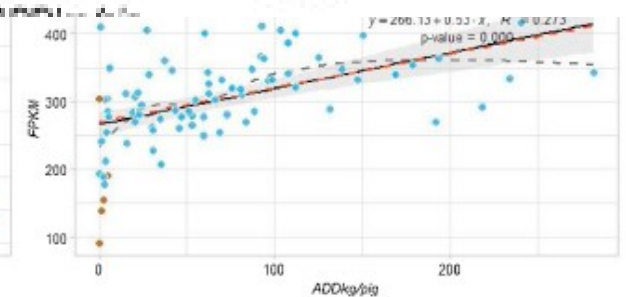
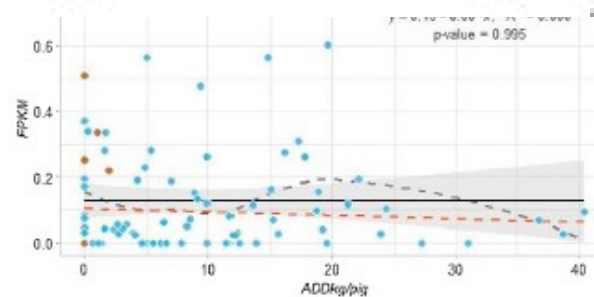
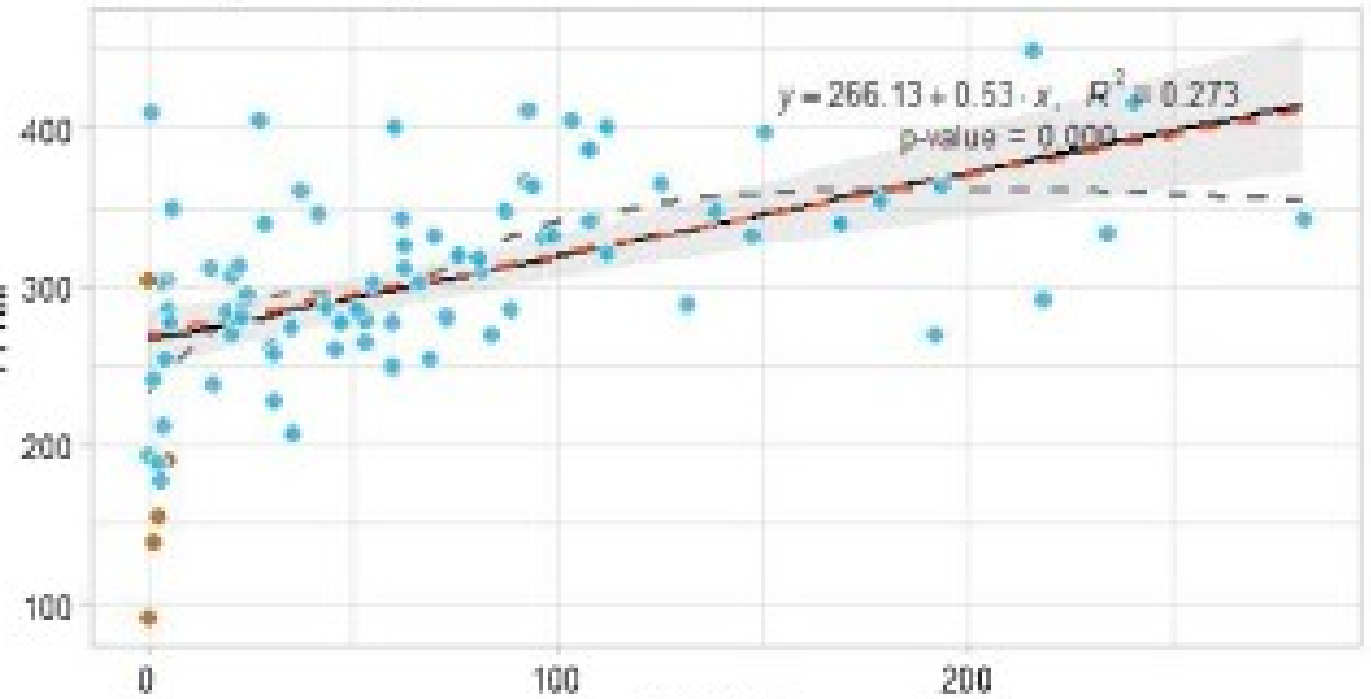
Andersen et al. 2017. Epidemiol. Infect. 145: 2827

Andersen et al. 2018. Epidemiol. Infect. 146, 515

Andersen et al. 2020. Prev. Vet. Med. 174: 104853



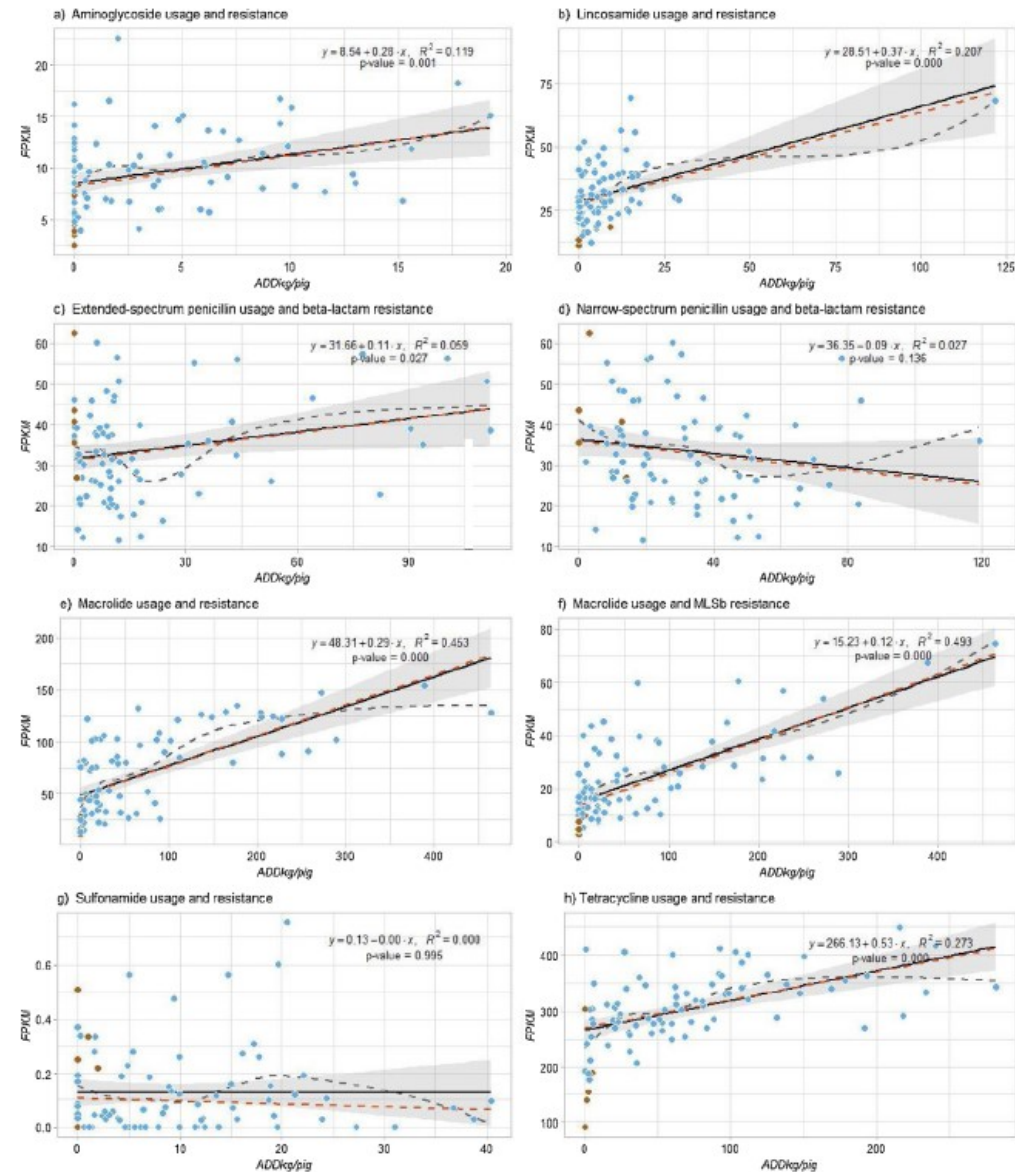
h) Tetracycline usage and resistance



Change of AMR following change in AMU

1) Rapid change from batch to batch

2) But 2-3 years before we see the full effect of changed AMU on AMR



Munk et al. 2017. JAC 72: 385

Andersen et al. 2017. Epidemiol. Infect. 145: 2827

Andersen et al. 2018. Epidemiol. Infect. 146, 515

Andersen et al. 2020. Prev. Vet. Med. 174: 104853

Substitution/Decreasement Increasement

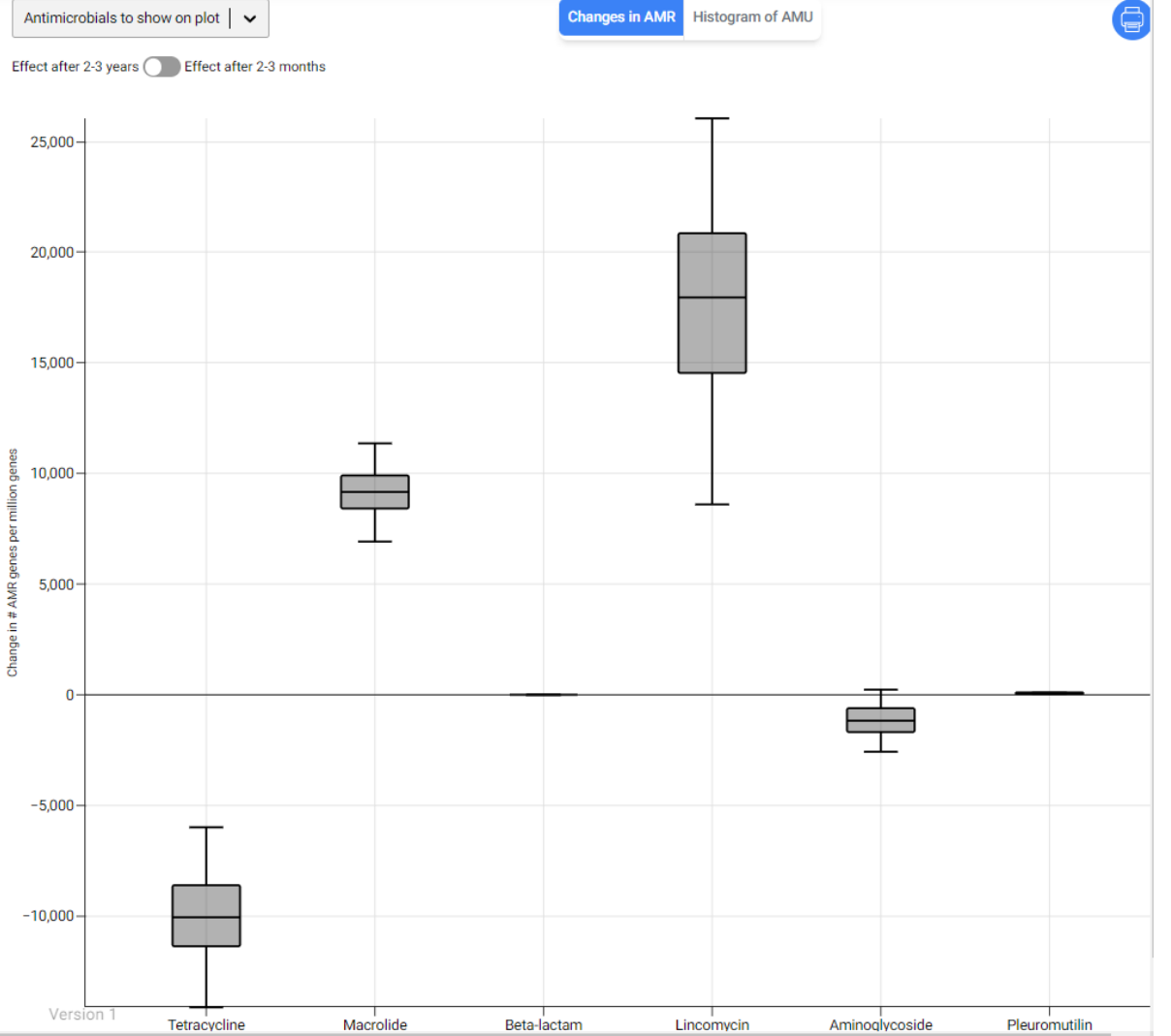
Administration	Current antimicrobial	Amount of reduction (%)	Replacement antimicrobial	Administration	Amount of substitution (%)
Peroral	Tetracycline	100	Macrolide	Peroral	100

Total reduction of current usage: 0 %

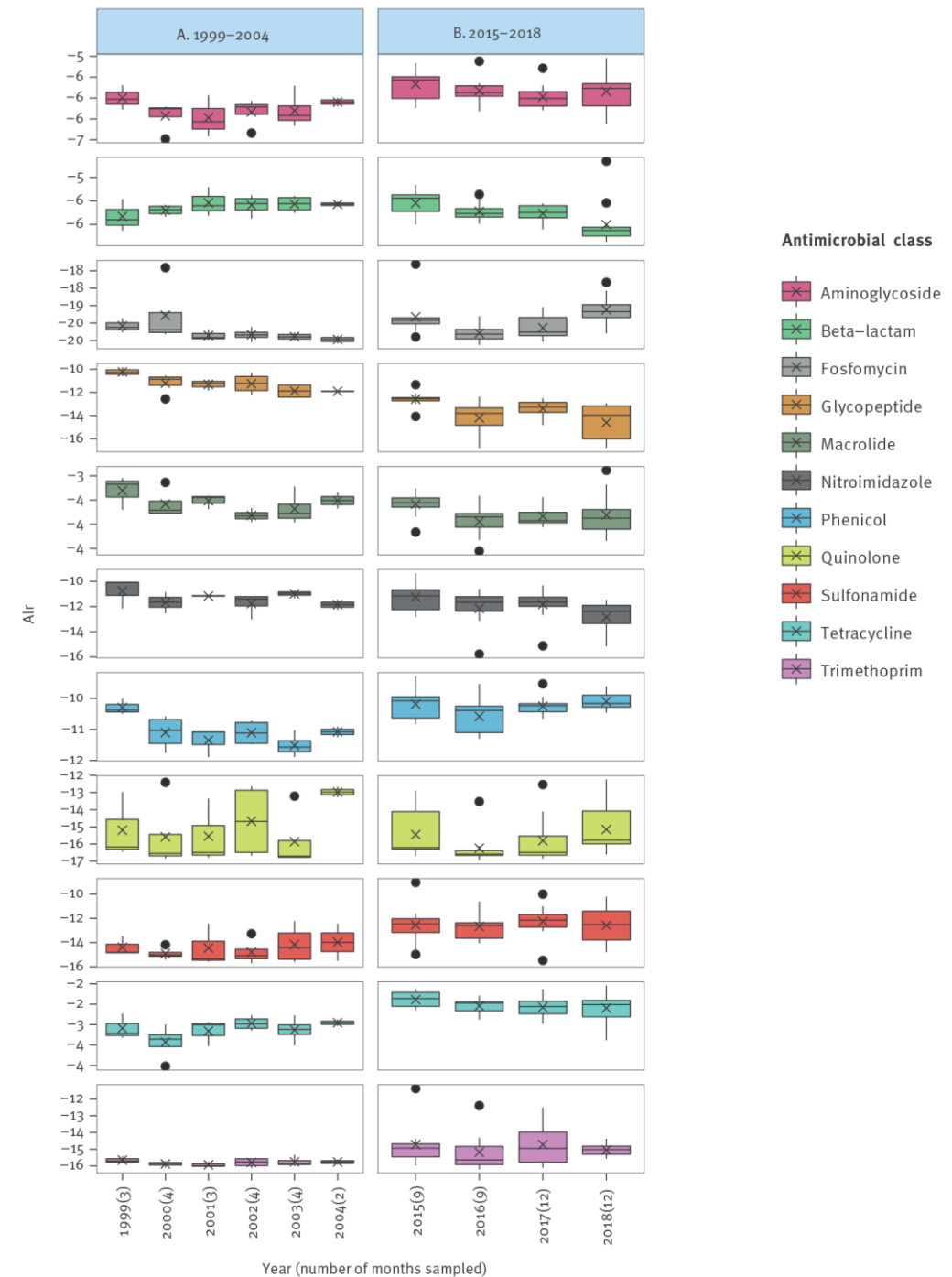
Reset Calculate

In this tab, you can predict the effects of a decreased usage of antimicrobials in pig production. In the dropdown menus on the left, you initially select which antimicrobial usage you want to reduce (antimicrobial class and way of administration) and the quantitative decrease in percentage. The decrease of one antimicrobial can (but doesn't have to) be substituted with one or several alternative antimicrobials. This is specified using the dropdown menus on the right side. You also have to specify how many % of the reduction (measured as ADD) you want to substitute. After you have pressed the icon "calculate" the tool performs 901 iterations to take the uncertainty in the effects of AMU -> AMR into account. The unit of the predictions is #genes coding for antimicrobial resistance per million genes. The box and whisker plots show the obtained predictions of the decrease/increase of antimicrobial resistance genes for the different antimicrobial classes. The red line indicates the median result from the iterations. The box ranges from the 25% percentile to the 75% percentile of the results. The whiskers range from the 5% to the 95% percentile of the results from the iterations, which is equivalent to the 90% confidence interval for the prediction.

Send feedback to DTU

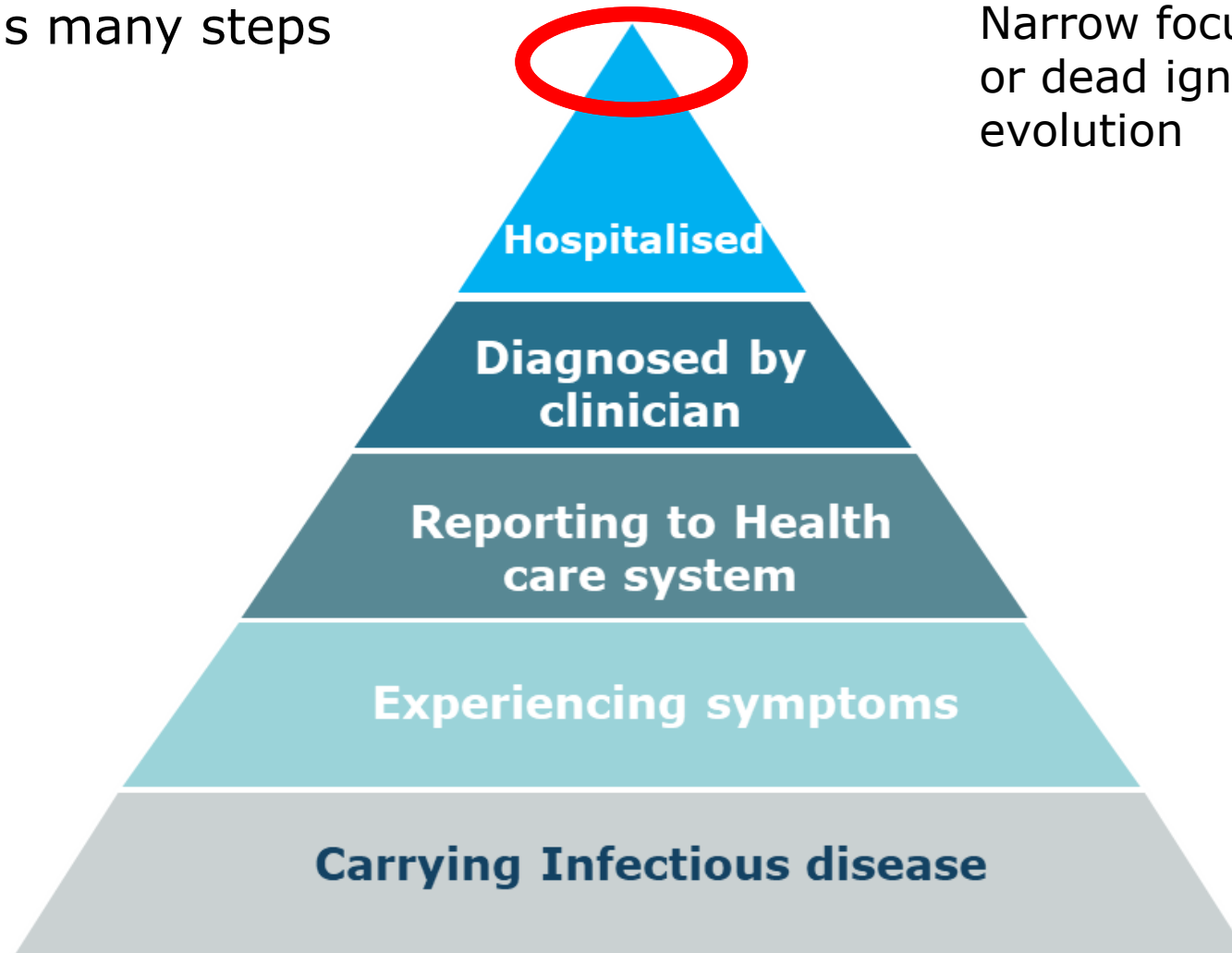


- Comparison of phenotypic (*E. coli* and *E. faecalis*) and resistome surveillance among Danish pigs 1999 – 2018
- Both measurements with good correlation to AMU, but resistome better
- Duarte et al. Eurosurveillance (accepted)



Where to sample?

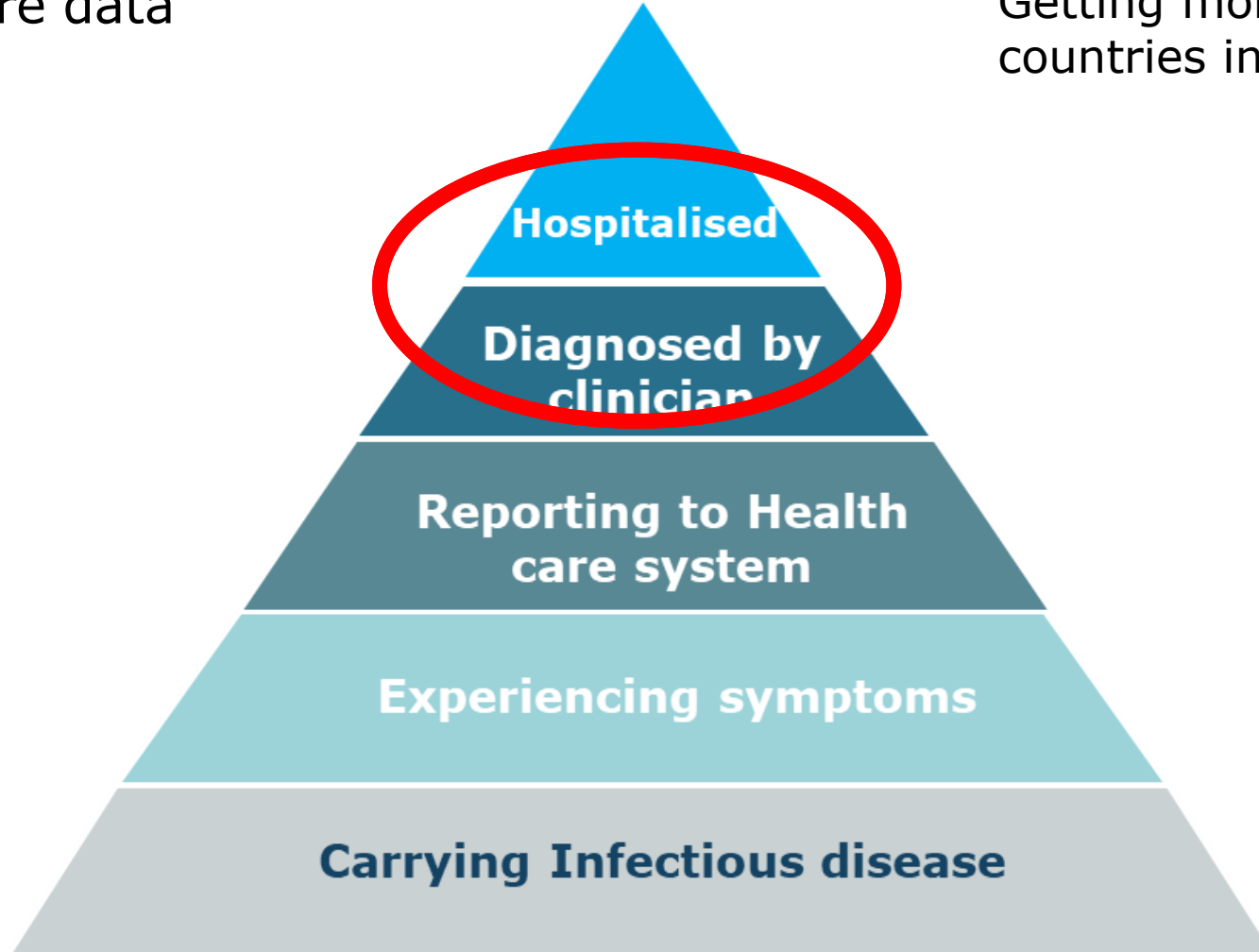
AMR epidemiology has many steps



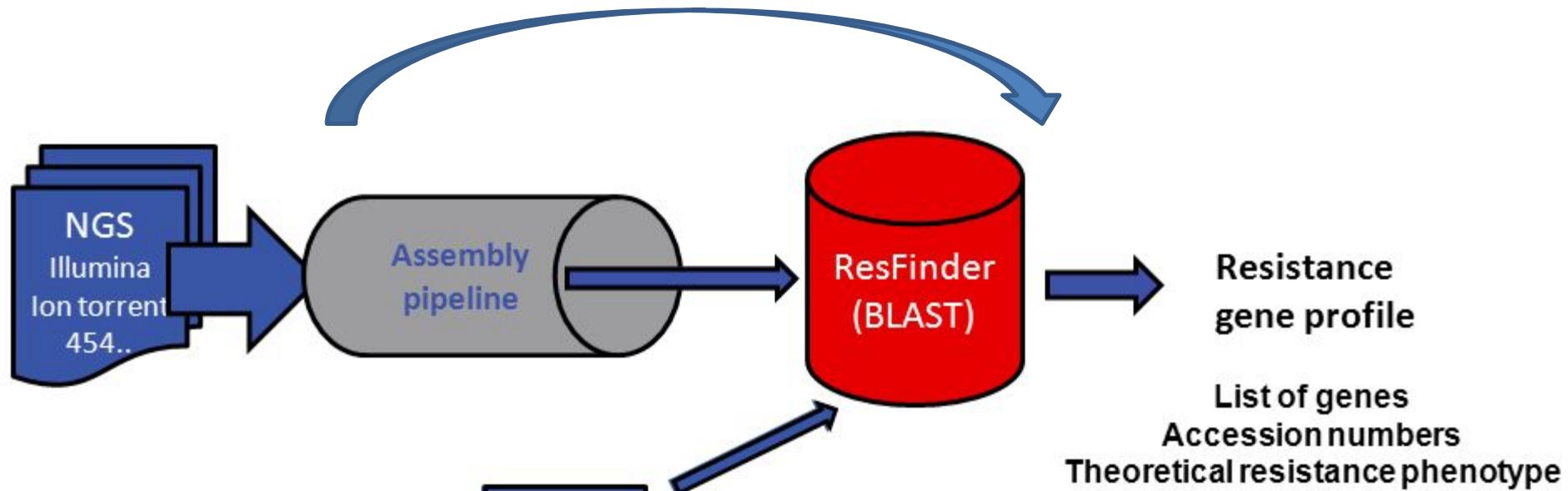
Narrow focus on the critically sick or dead ignores the early stage evolution

Enabling more to share data

Getting more laboratories and countries involved



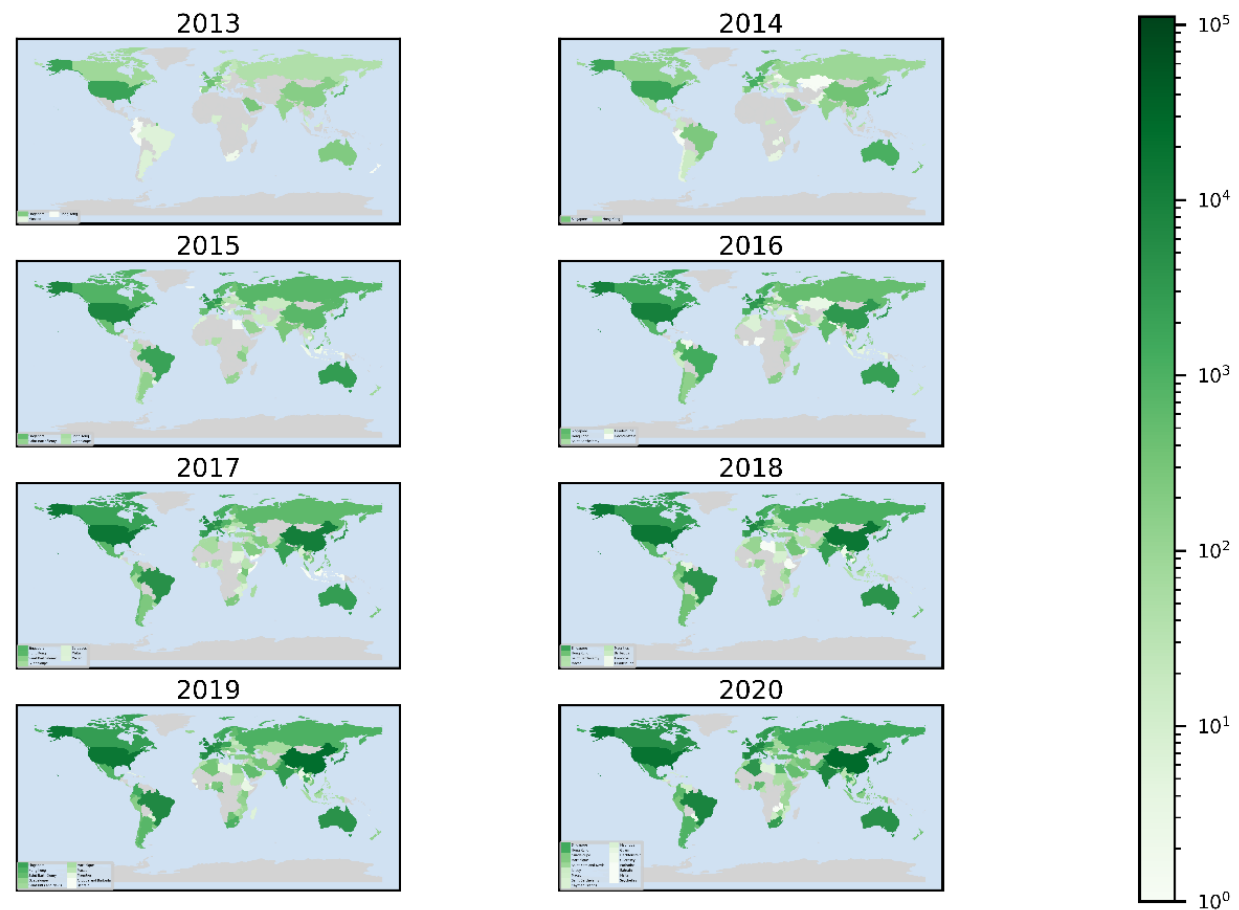
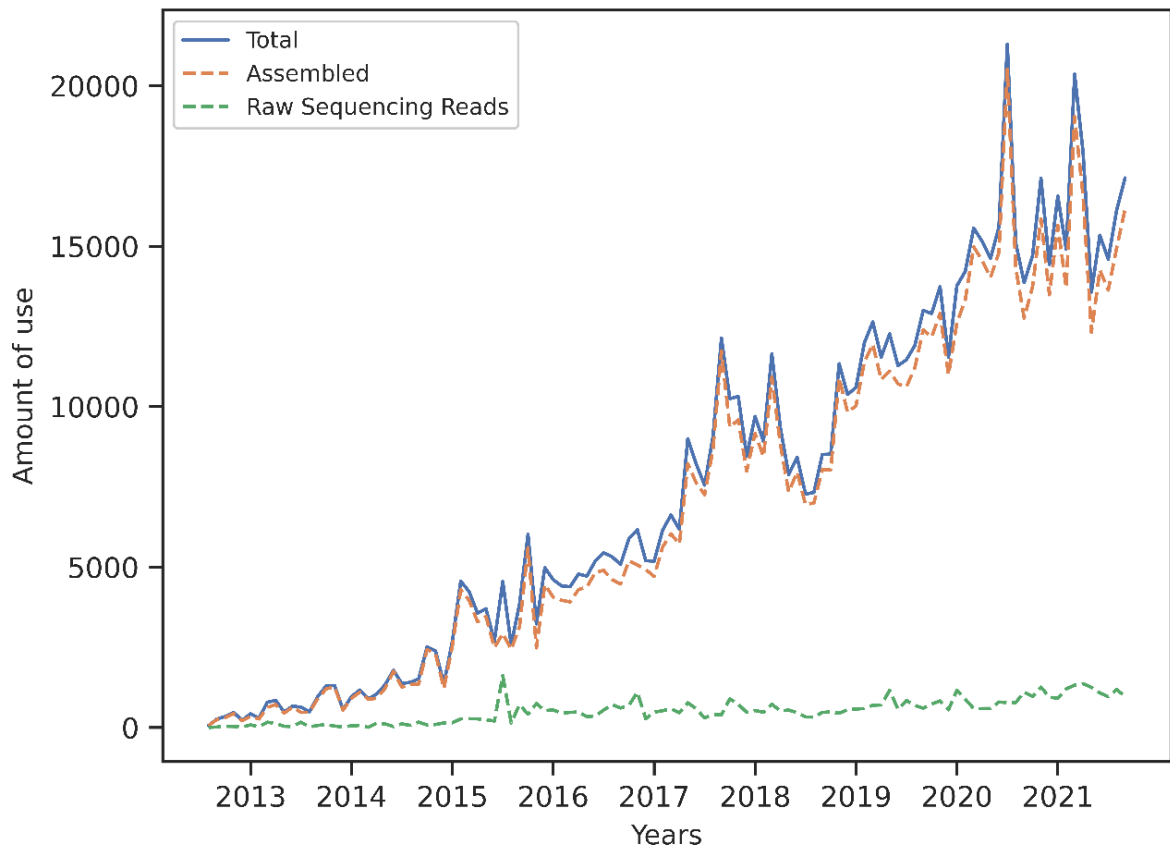
ResFinder



Implemented for surveillance by FDA, Public Health Canada and US-CDC

ResFinder 4.0 provides predicted phenotypes

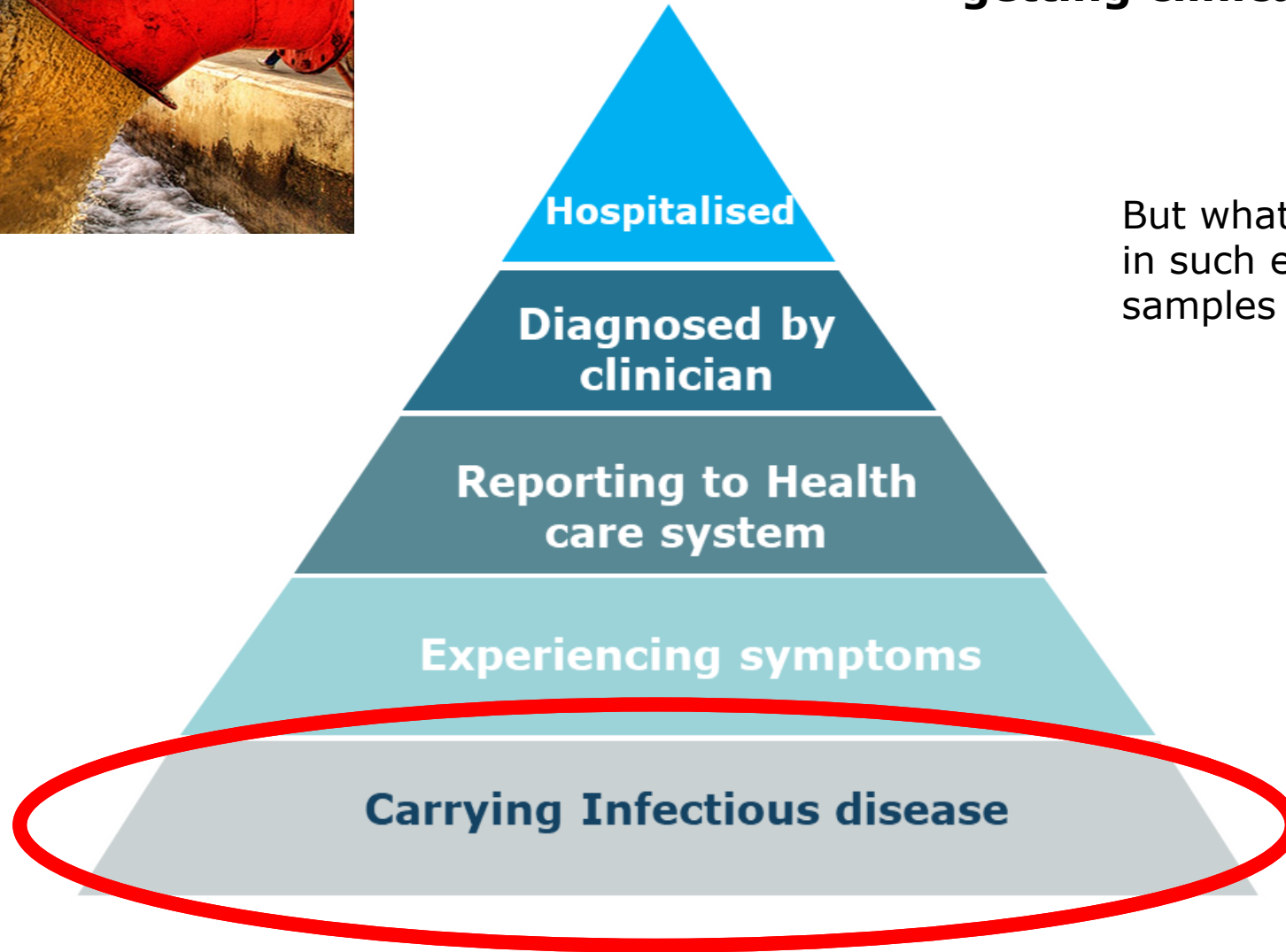
Aytan-Aktug et al. mSystems. 2022
Bortolaia et al. JAC. 2020
Szarvas et al. Commun Biol. 2020
Clausen et al. BMC Bioinformatics. 2018
Kaya et al. mSphere. 2017
Zankari et al. JAC. 2017
Kaas et al. PlosOne. 2014
Zankari et al. JAC. 2012
Larsen et al. J Clin Microbiol. 2012



Current use is around 15,000 analyses per month and LMICs have started to become visible

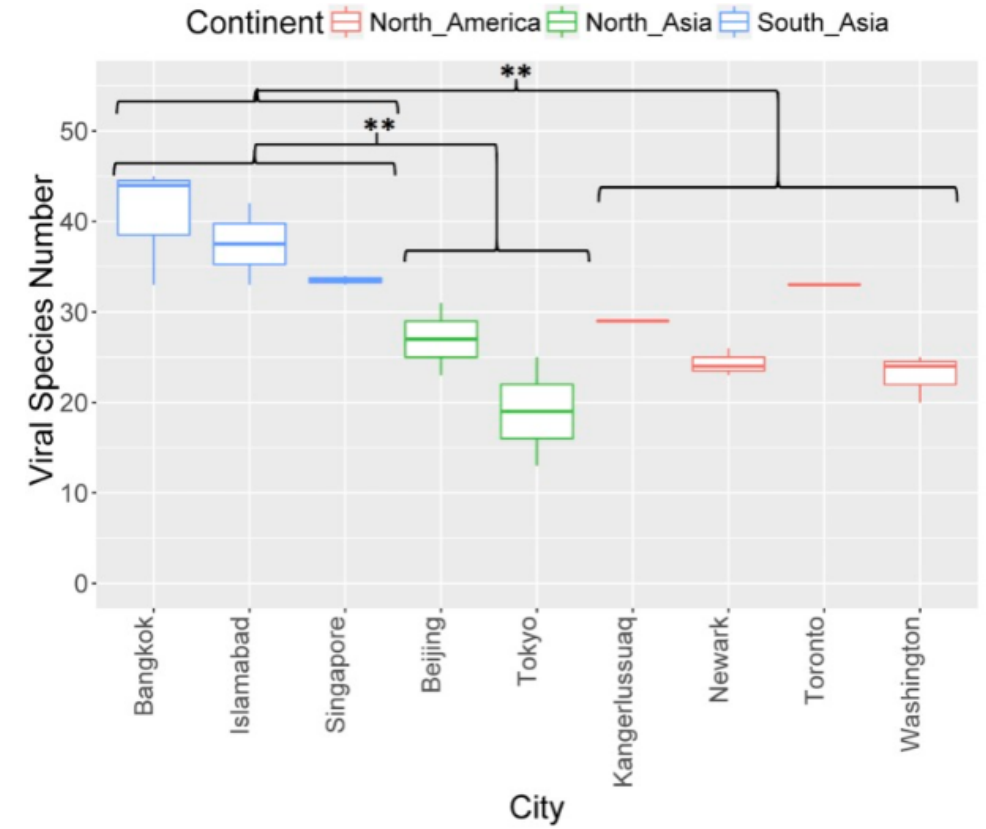
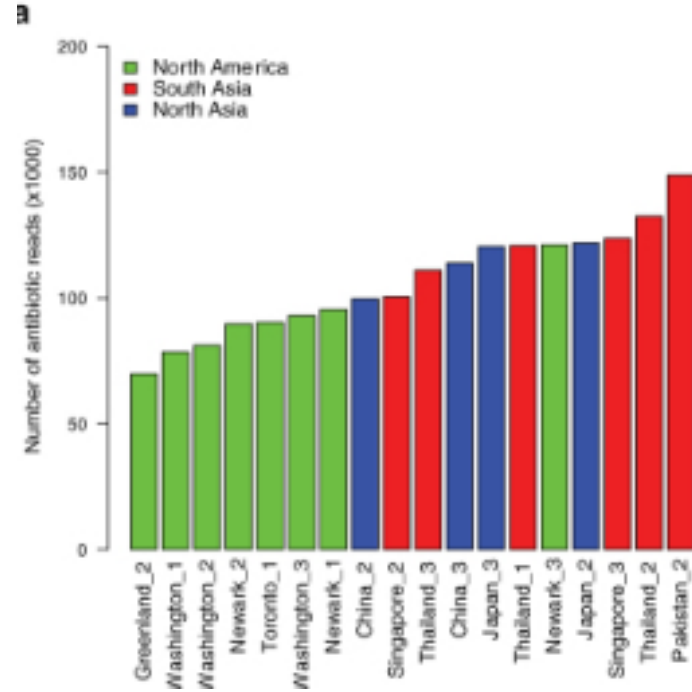
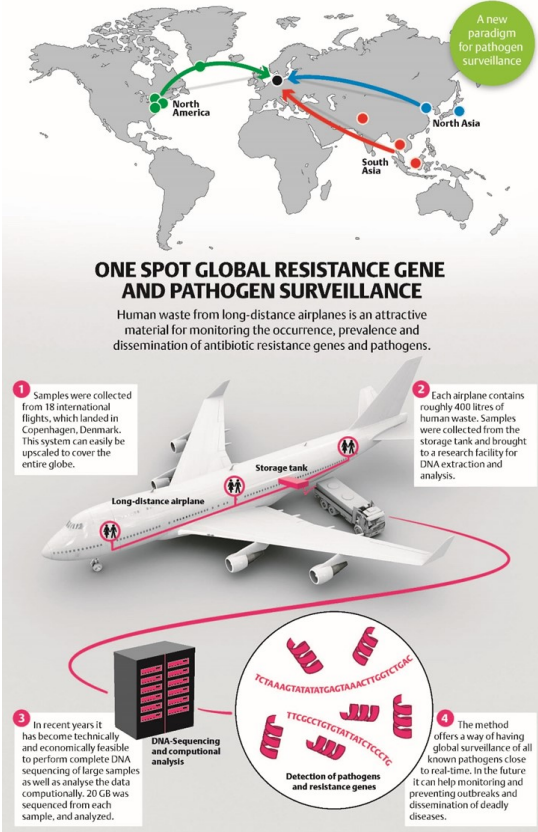


**Sewage
By-passing the problems of
getting clinical isolates**



But what should we measure
in such environmental
samples

Global hot-spots



Pathogens and AMR

Pedersen et al. 2015, Hjelmsø et al. 2019

Also global surveillance of medicine (and illicit drugs)

VOLUME 23 - No 1 - 2017 - Page 7-12 - doi: 10.1515/sjfs-2017-0003

Scandinavian journal of **FORENSIC SCIENCE** Nordisk rettsmedisin



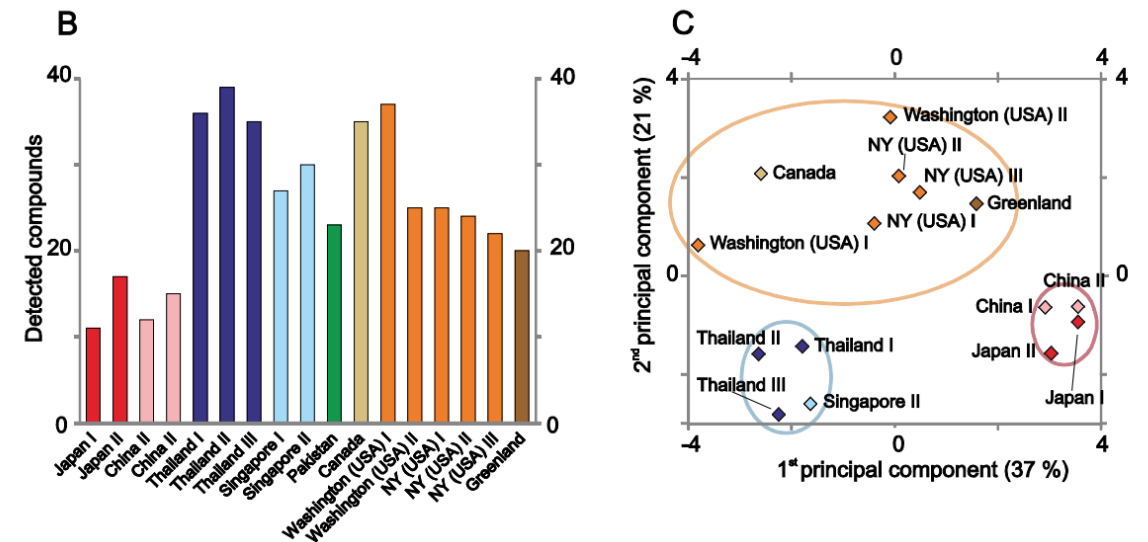
Analytical Profiling of Airplane Wastewater - a New Matrix for Mapping Worldwide Patterns of Drug Use and Abuse

Marie Mardal^{1*}, Frank Møller Aarestrup², Brian Schou Rasmussen¹, Christian Brinch Møllerup¹, Petur Weihe Dalsgaard¹, Kristian Linnet¹

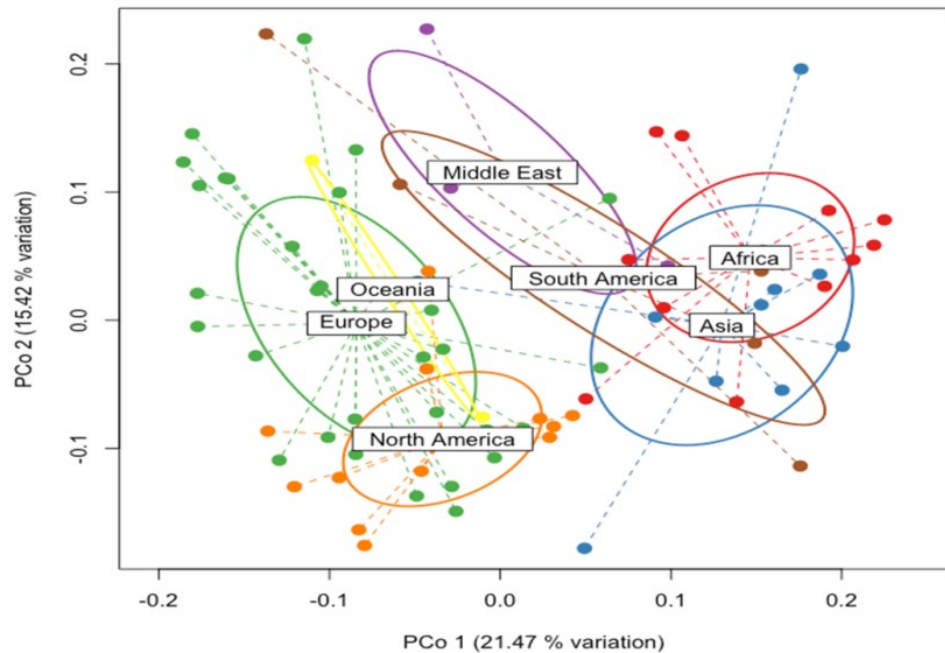
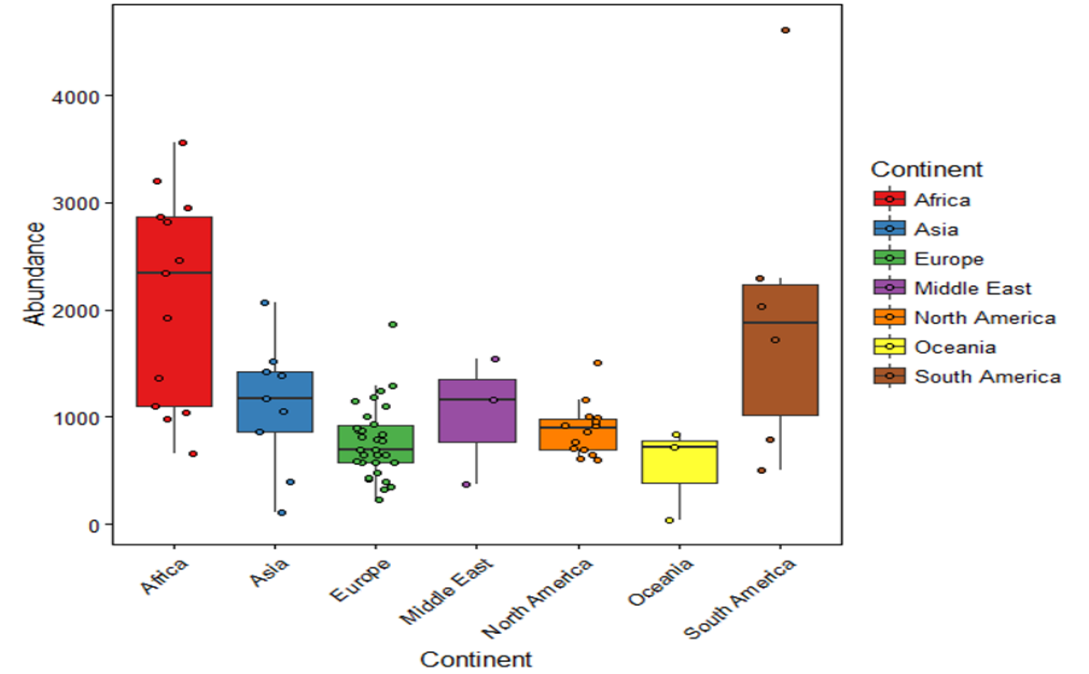
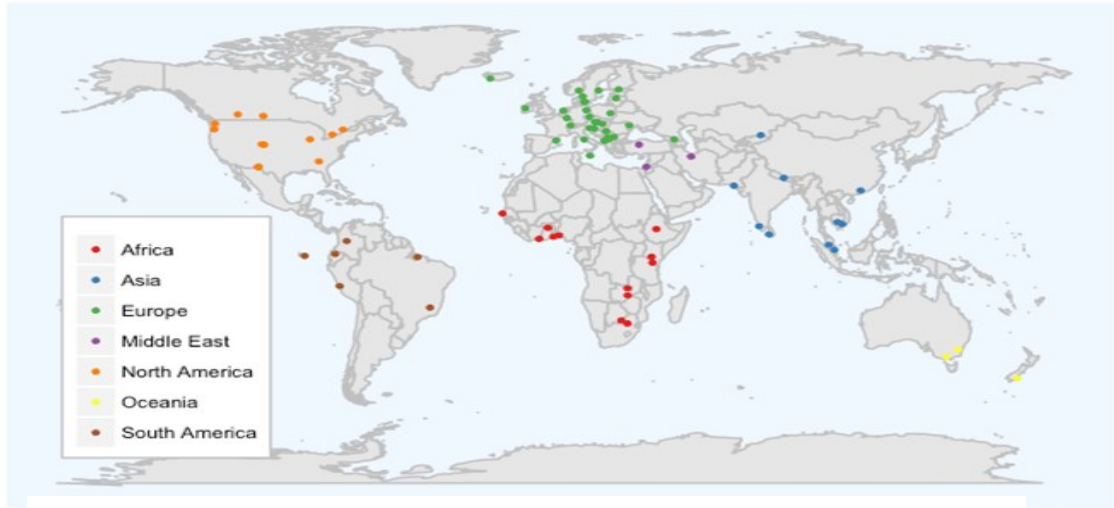
Table 2: Top-five detected compounds in the airplane wastewater samples.

Compound	ATC therapeutic main group	Number of detections
Paracetamol	N02	17
Pseudoephedrine	R01	16
Diphenhydramine	R06	16
Metformin	A10	15
Cetirizine	R06	15

N02: Analgesics, R01: Nasal preparations, R06: Antihistamines for systemic use, A10: Drugs used in diabetes



Global sewage surveillance - 2016



79 cities – 63 countries

1.500 Gb, large diversity + 30 million genes

Clear separation into:

Asia, Africa, Middle east, South Am.

Europe, North Am., Oceania

Comparing to socio-economic data from World bank

Predictors of higher AMR

- Open defecation
- Diarrhoea prevalence in children
- Mortality rate
- Death, by communicable diseases and maternal, prenatal and nutrition conditions
- Risk of maternal death
- Risk of impoverishing expenditure for surgical care
- Informal employment

Predictors of lower AMR

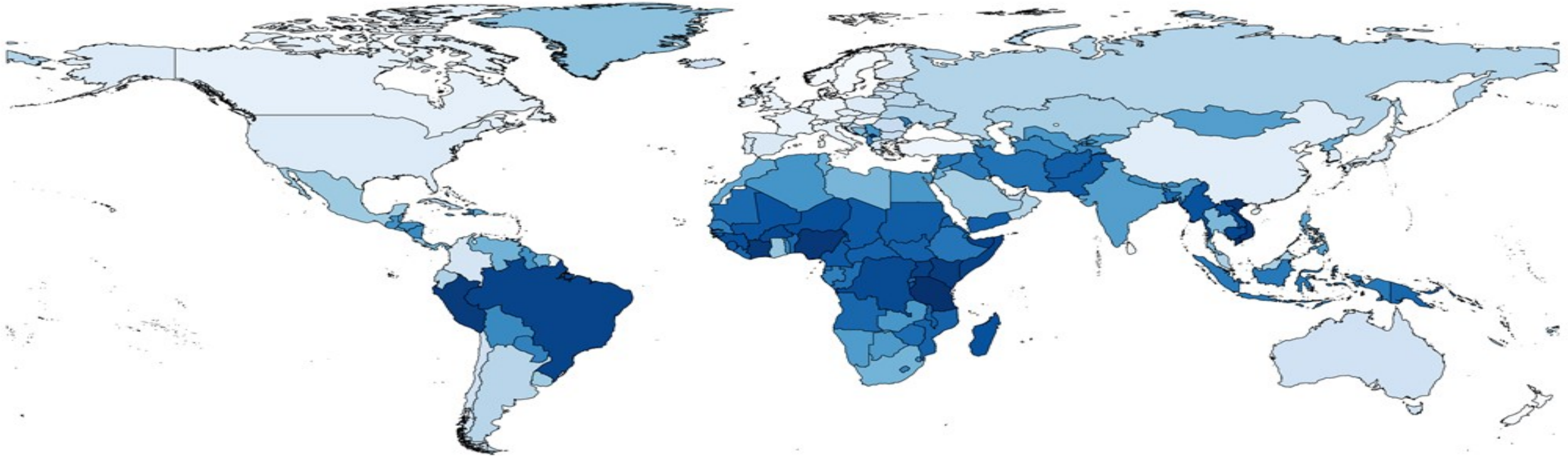
- Investment in water and sanitation
- Completeness of death reporting
- Educational attainment
- Number of surgical procedures
- Life expectancy at birth
- Number of Physicians
- Births attended by skilled health staff

Socio-economic parameters are more important than antimicrobial usage

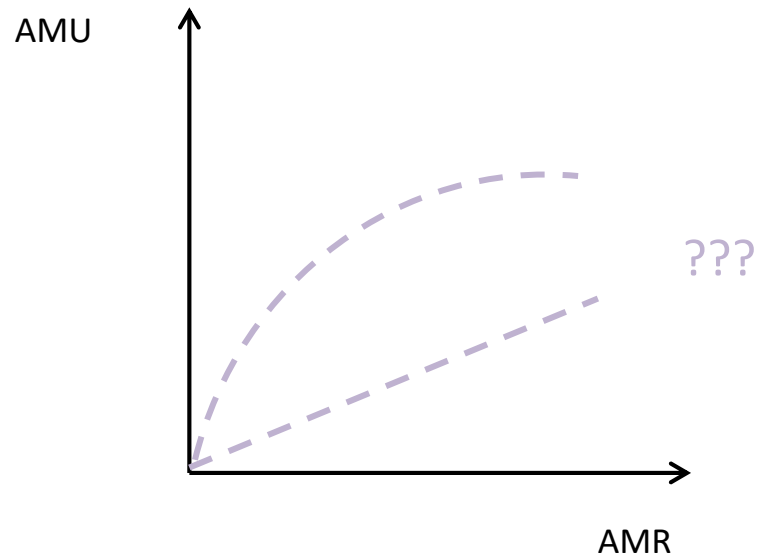
Direct impact on global policy



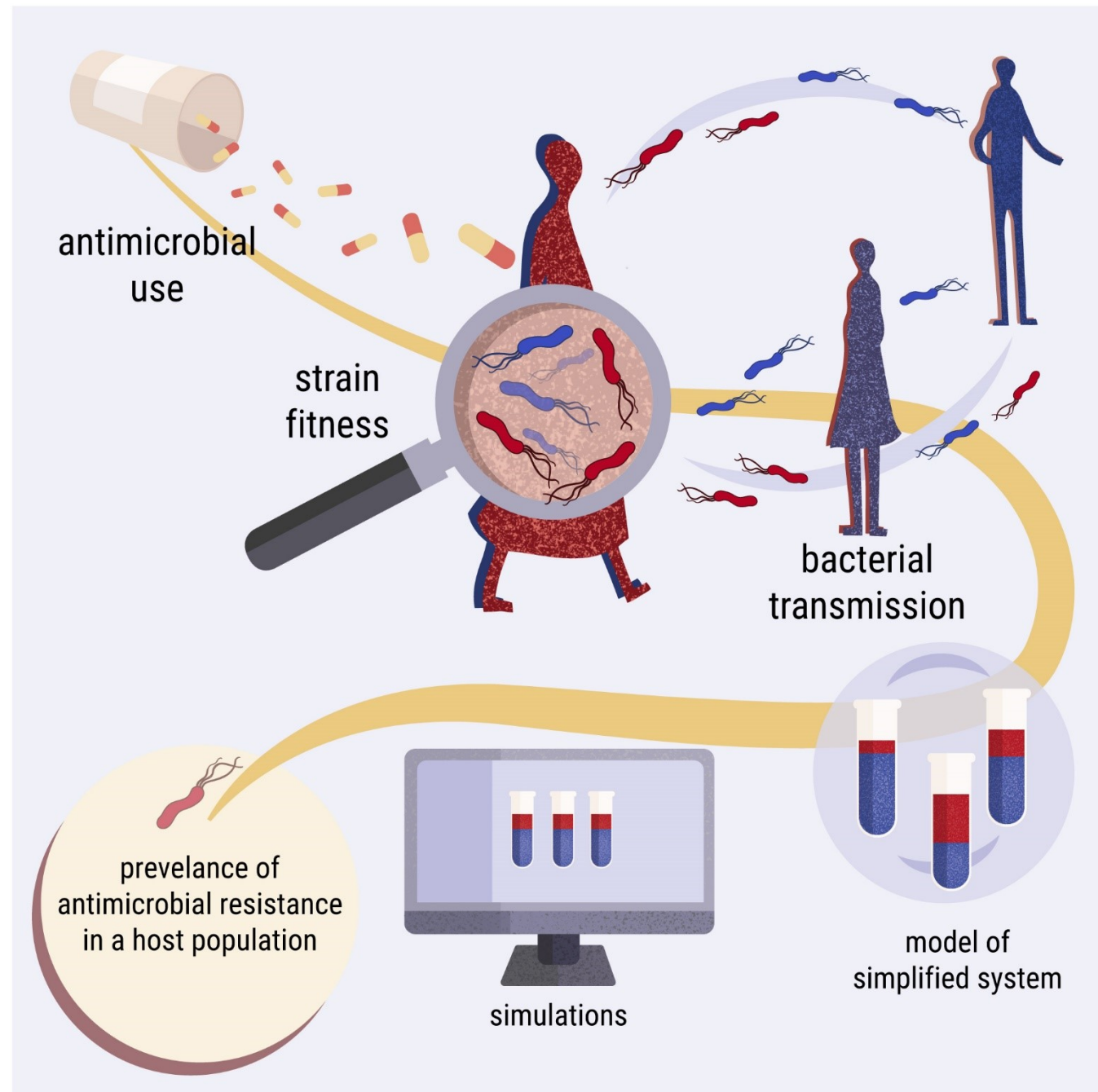
Global resistance prediction



Aim



What other factors
affect this relationship?



Bogri, A., Otani, S., Aarestrup, F. M. & Brinch, C. Interplay between strain fitness and transmission frequency determines prevalence of antimicrobial resistance.

Frontiers Ecol Evol **11**, 981377 (2023).

Results – between hosts

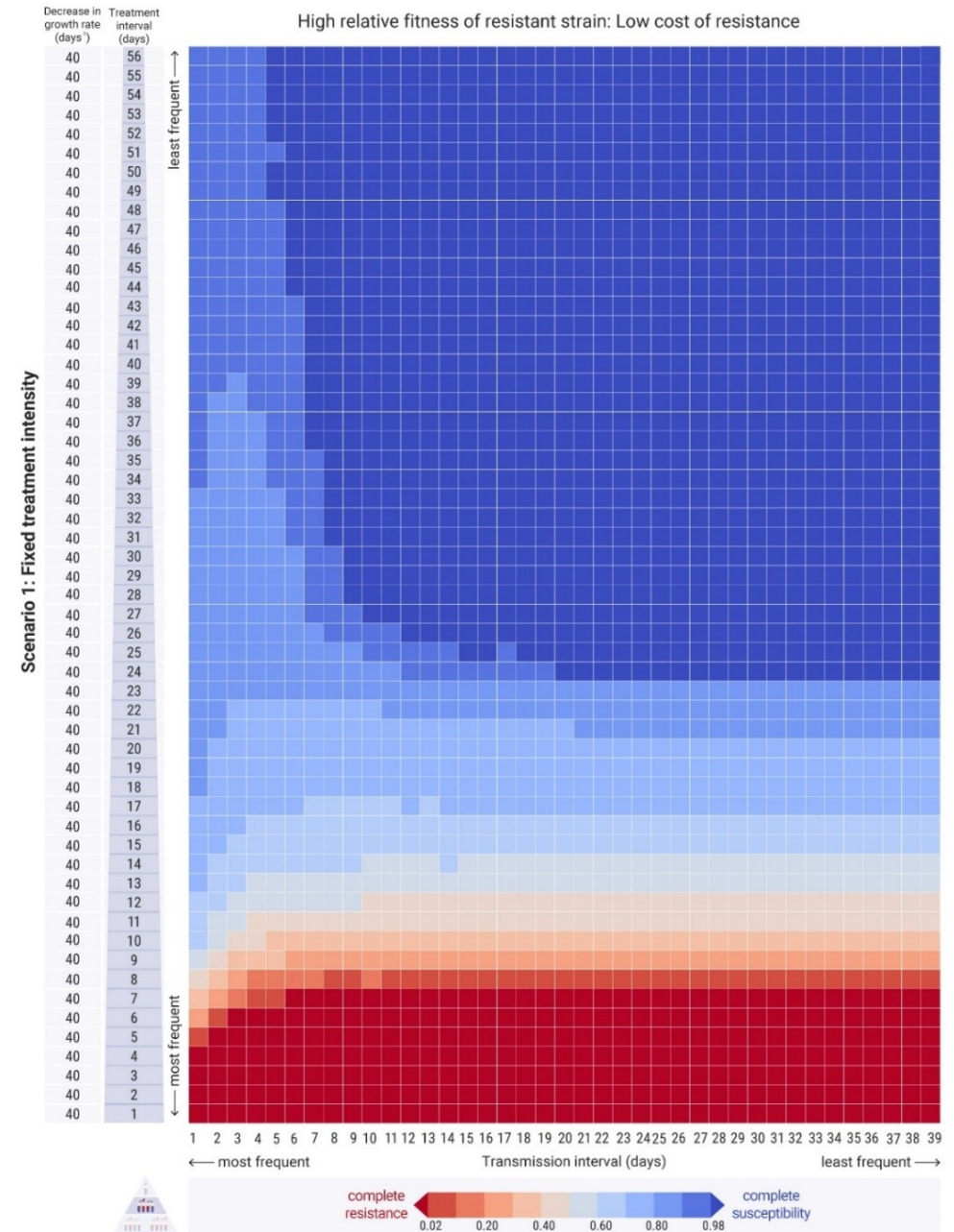
Treatment interval vs. transmission interval

Transmission benefits the less dominant strain.

So, when treatment frequency is high, transmission reduces resistance.

When treatment frequency is low, transmission increases resistance.

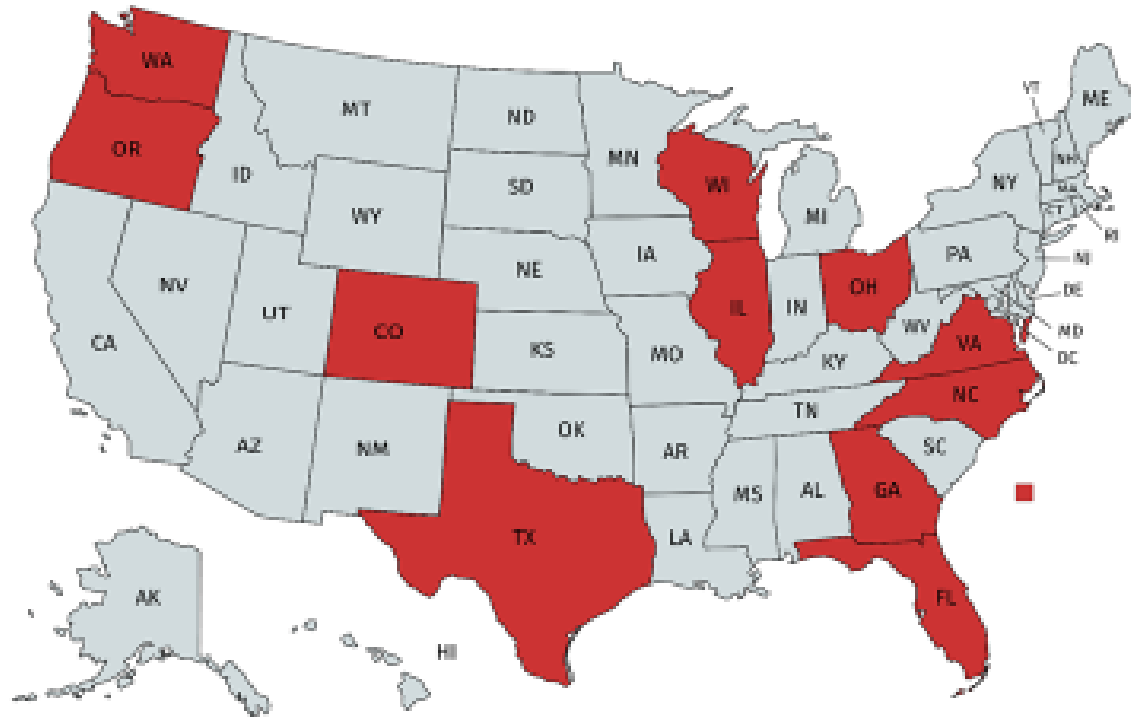
Low cost of resistance



Bogri, A., Otani, S., Aarestrup, F. M. & Brinch, C. Interplay between strain fitness and transmission frequency determines prevalence of antimicrobial resistance. *Frontiers Ecol Evol* **11**, 981377 (2023).

Expanding the global surveillance

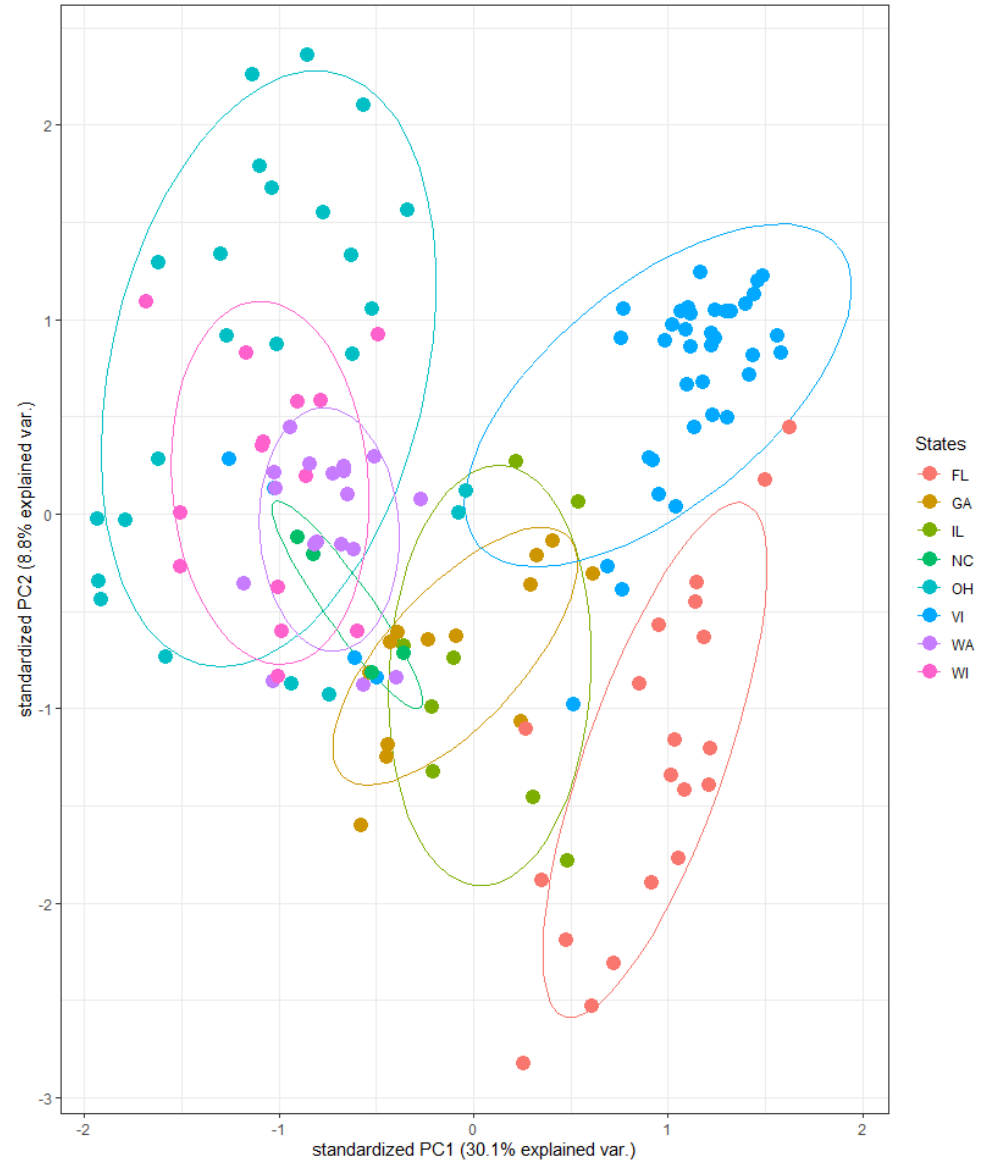
Inside USA – socio-economic drivers

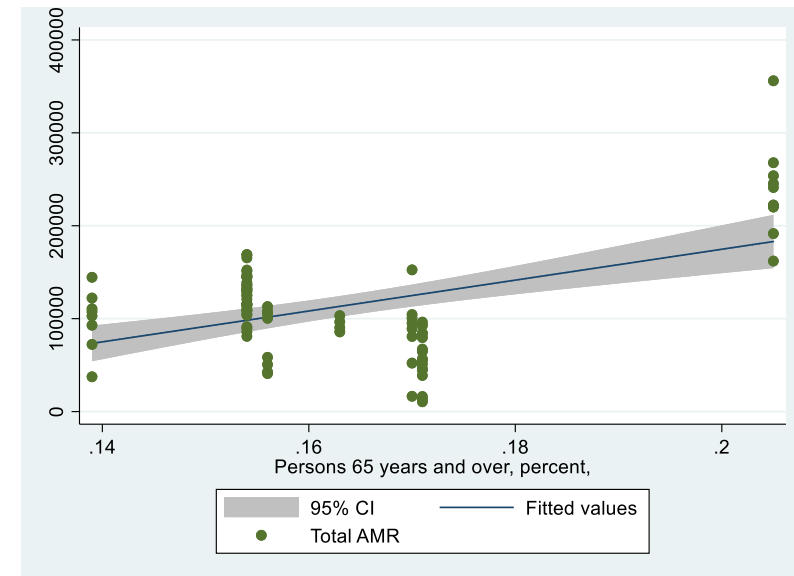
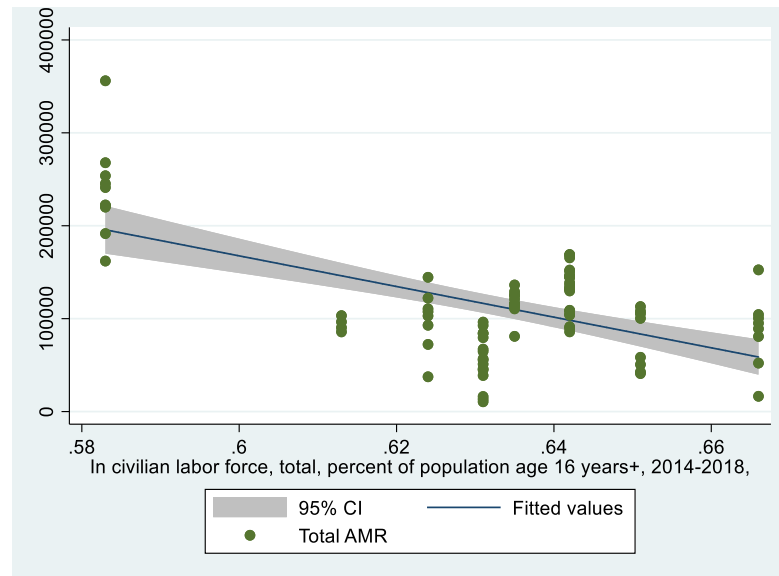
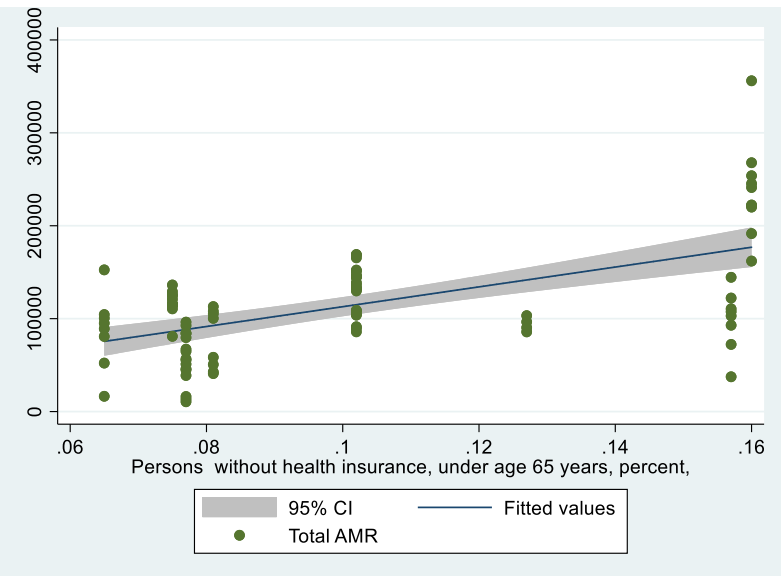


45 sites in 11 states

US sewage longitudinal – bacterial genera

- PCA plot by bacterial genera
- Colors are states
- Dots are samples



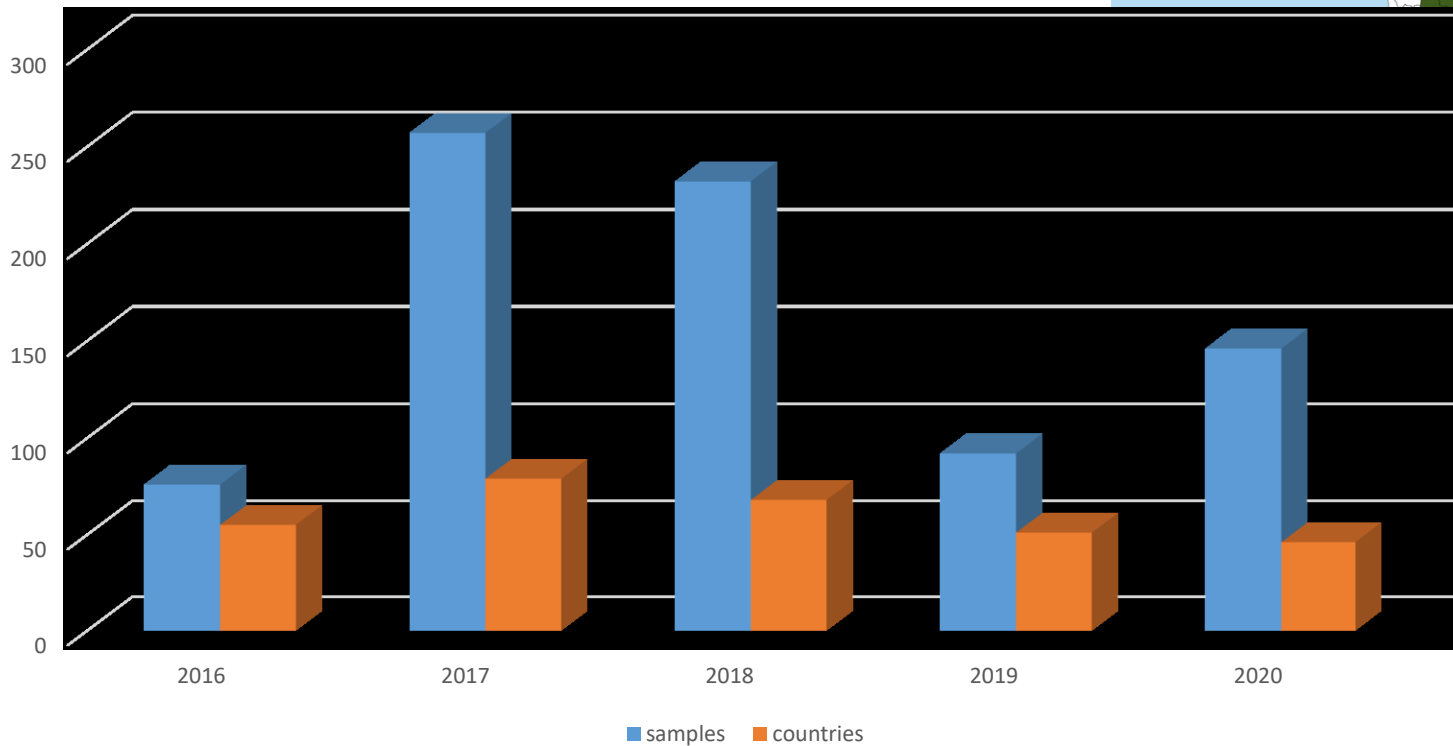
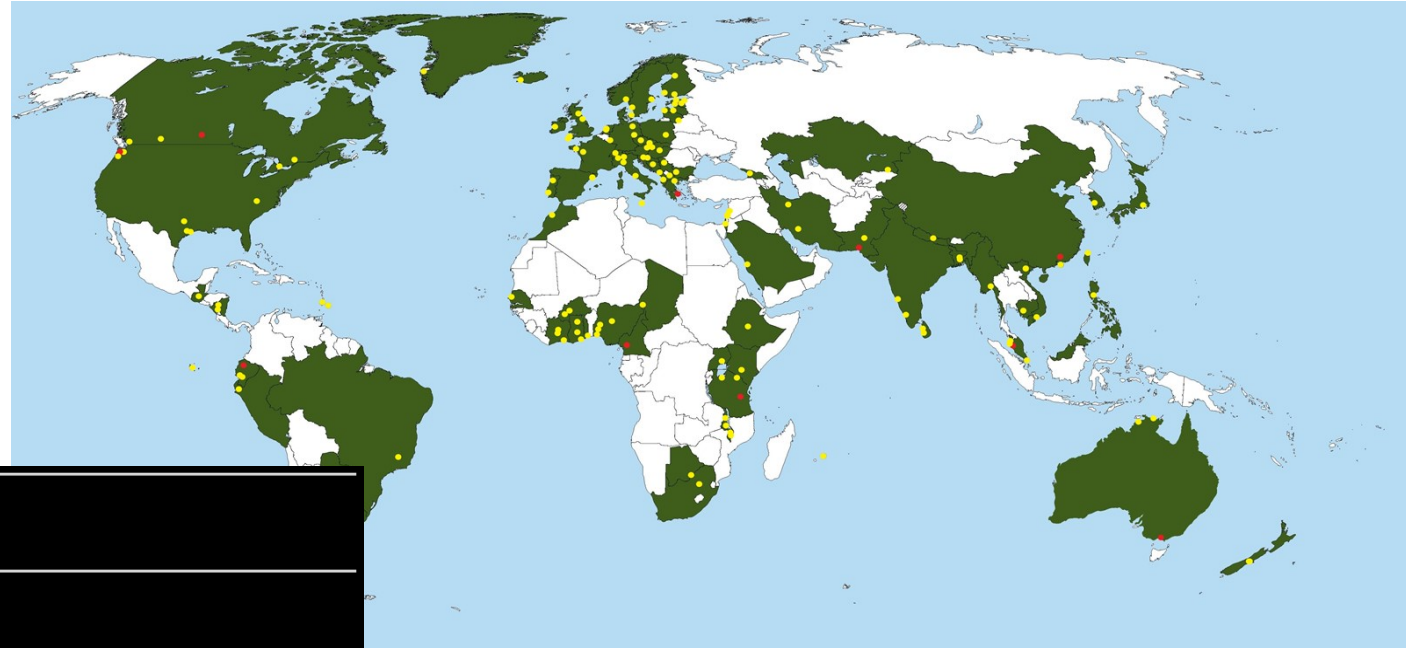


Continued global sewage surveillance

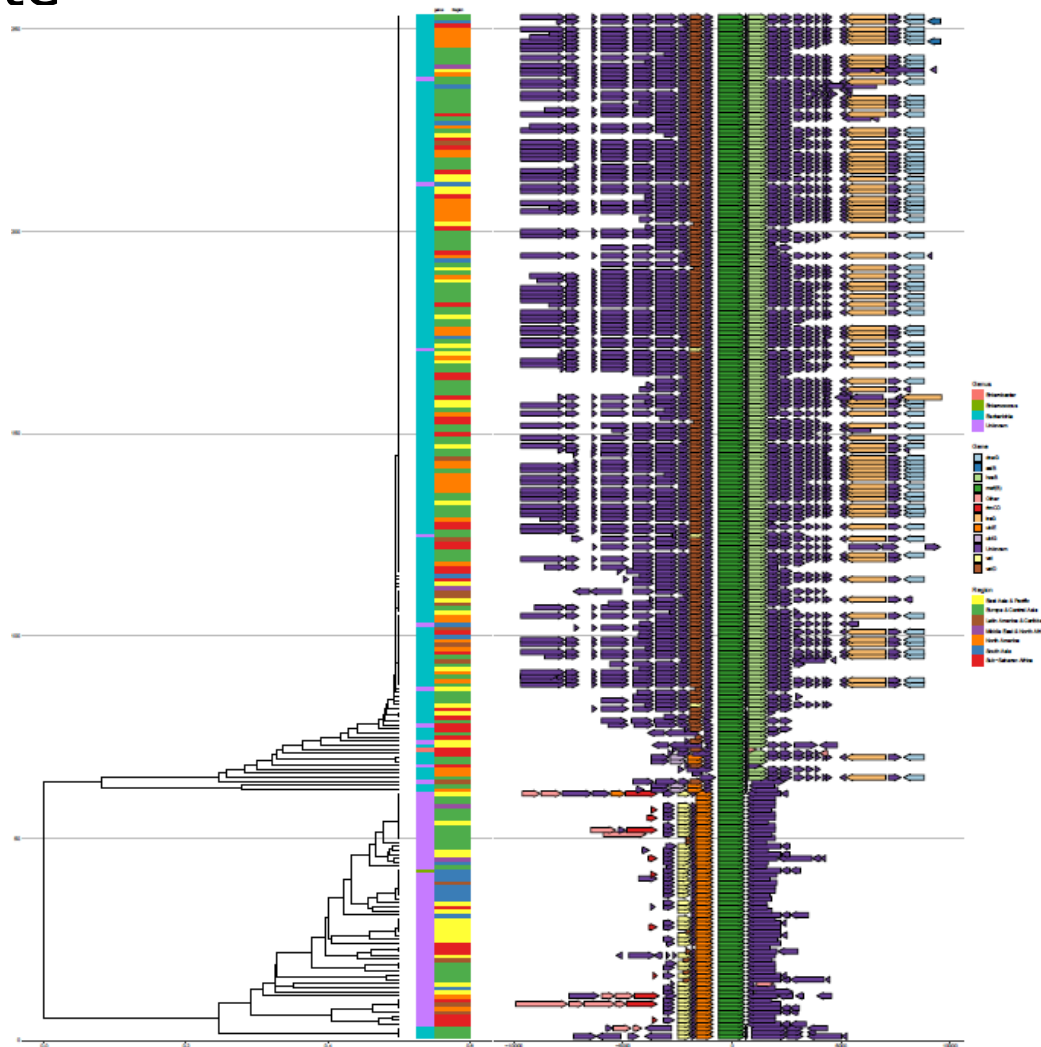
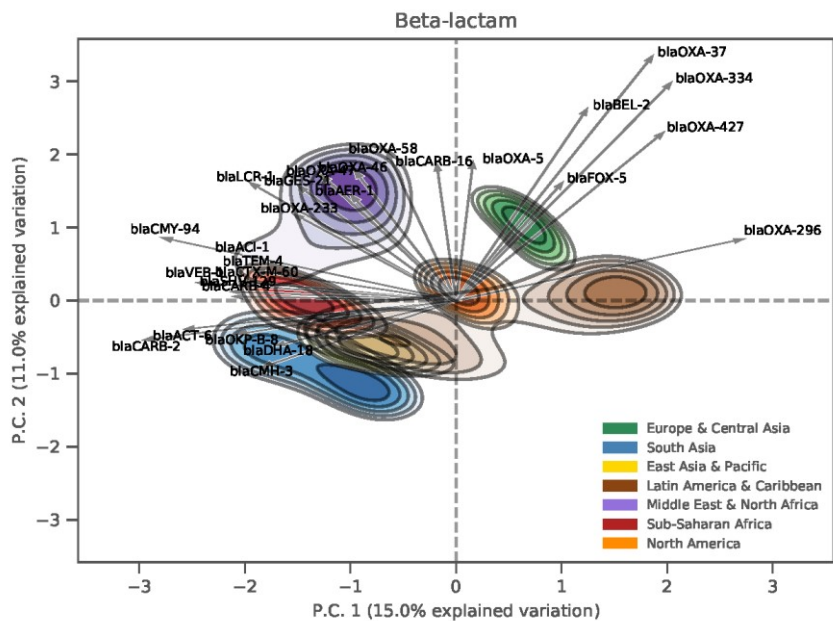
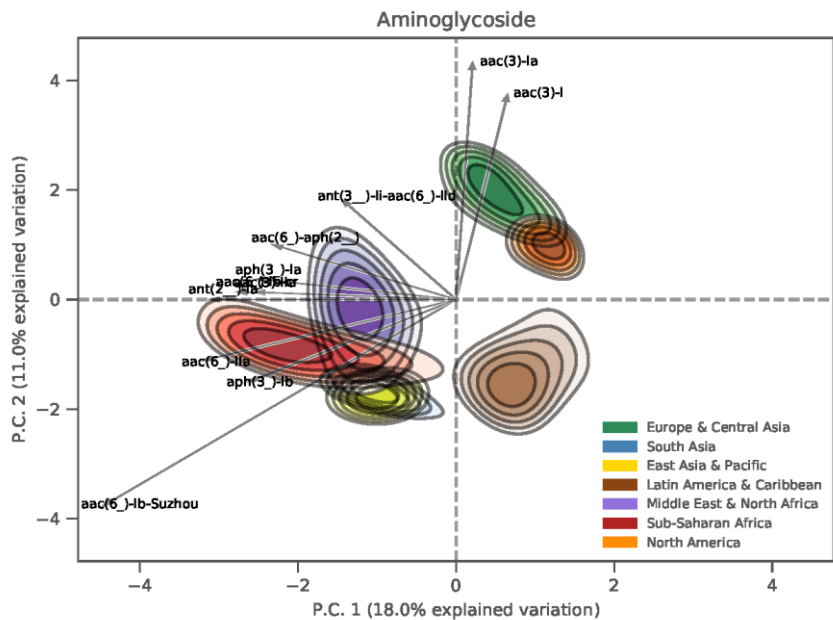
2016 – 2020 - > Up to 79 countries per time-point

➤ 800 samples

Longitudinal in 14 cities since March 2020



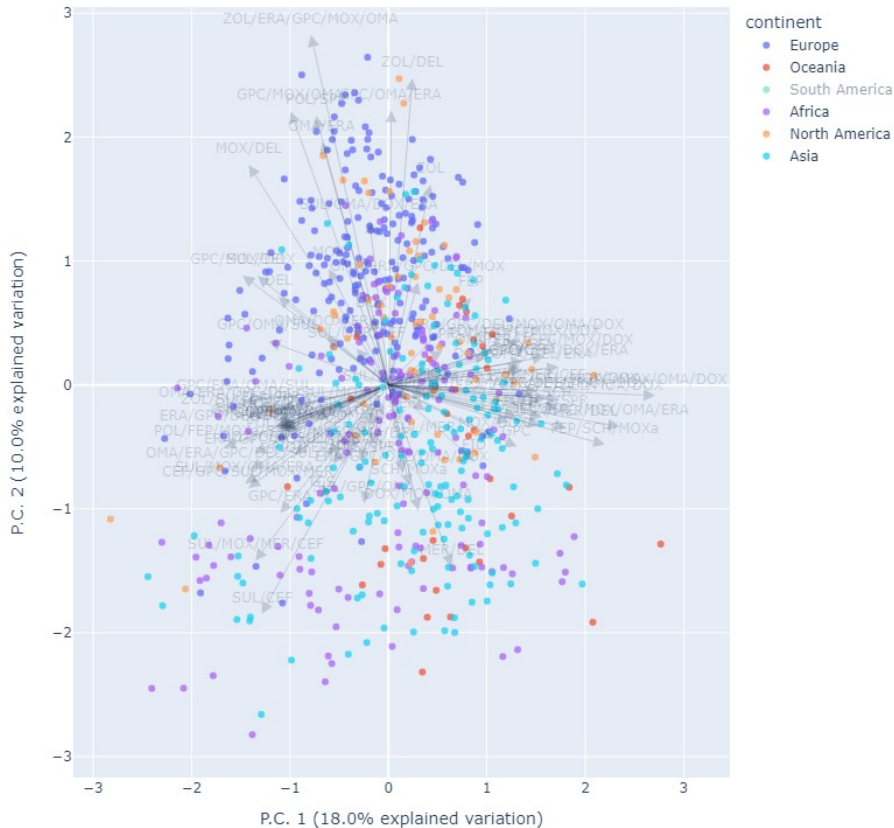
The different classes separate the world in different ways



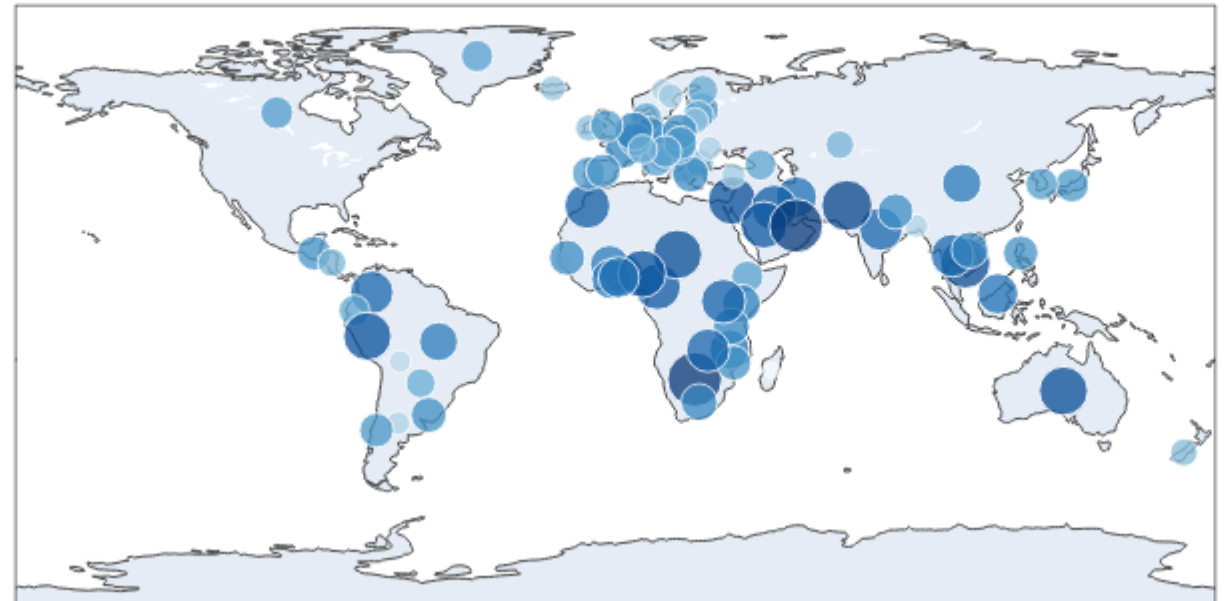
The functional resistome

- 21 antibiotics currently under development
- Novel ARGs identified by functional cloning

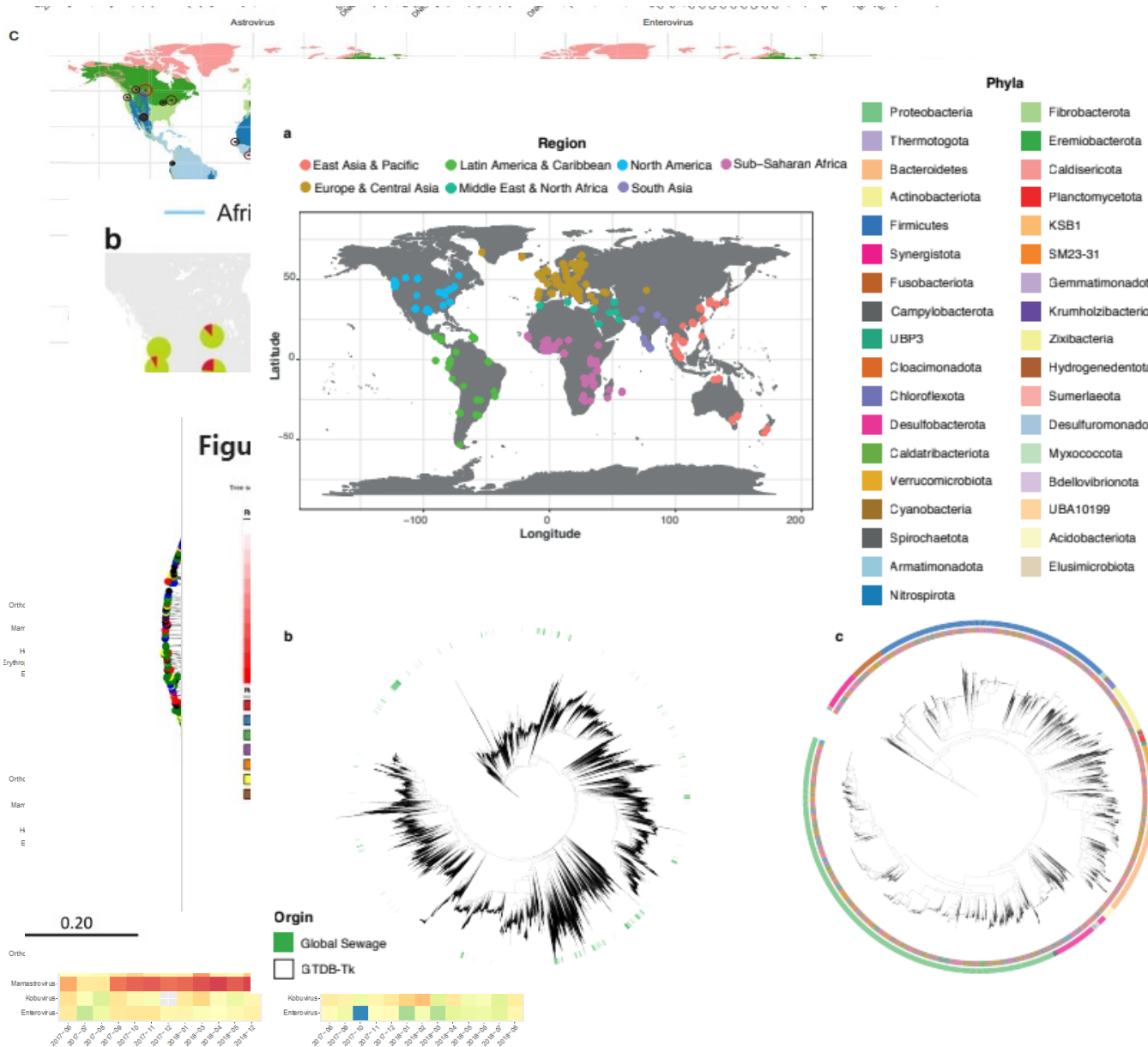
- Already wide distribution
- Seemingly not such a strong geographical clustering, but high abundance in the tropics



Total abundance of novel ARGs



Surveillance using wastewater – virus and everything else



Virus

Nieuwenhuijse et al. Sci. Rep. 2020.
Worp et al. in prep.

Human populations

Pipek et al. Sci. Rep. 2019

Plasmids

Teudt et al. mSystems. 2022

Bacteriophages

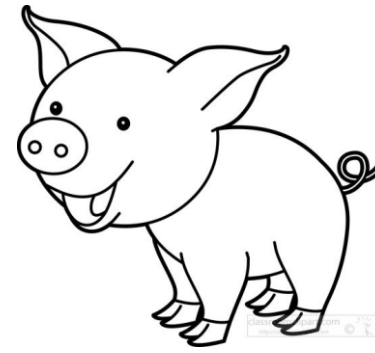
Edwards et al. Nature Microbiol. 2019

Bacterial phylogeny

Ahrenfeldt et al. Sci. Rep. 2020
Jespersen et al. in revision

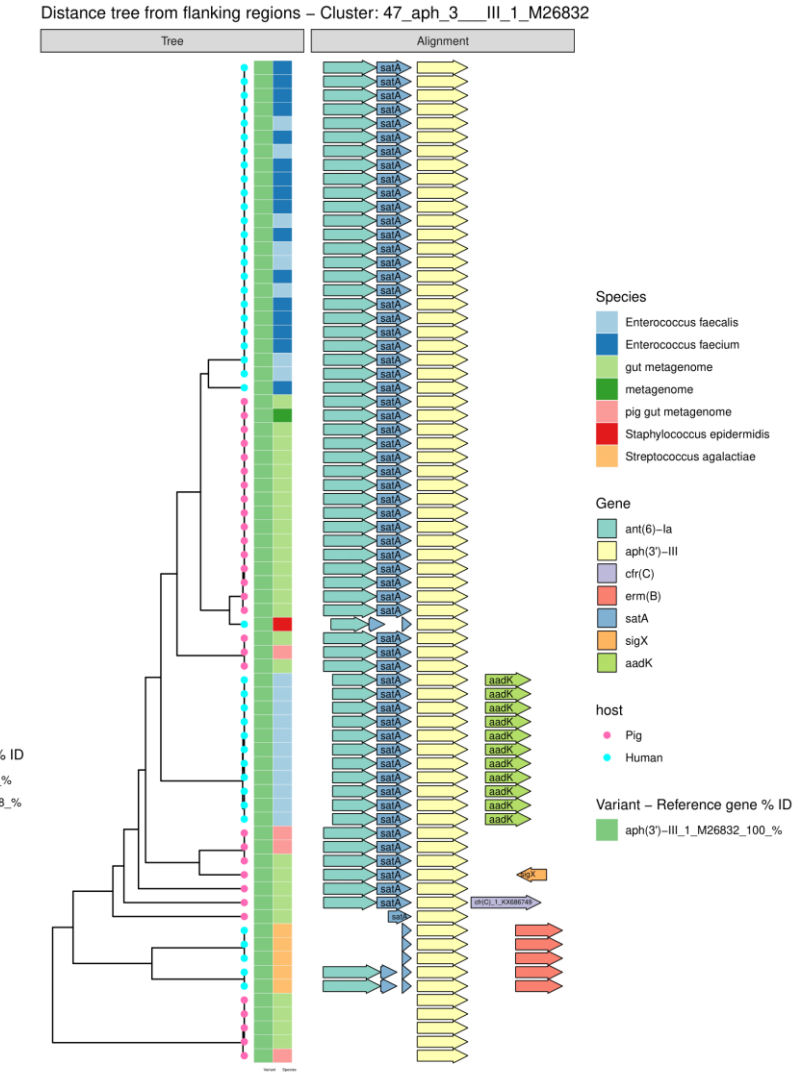
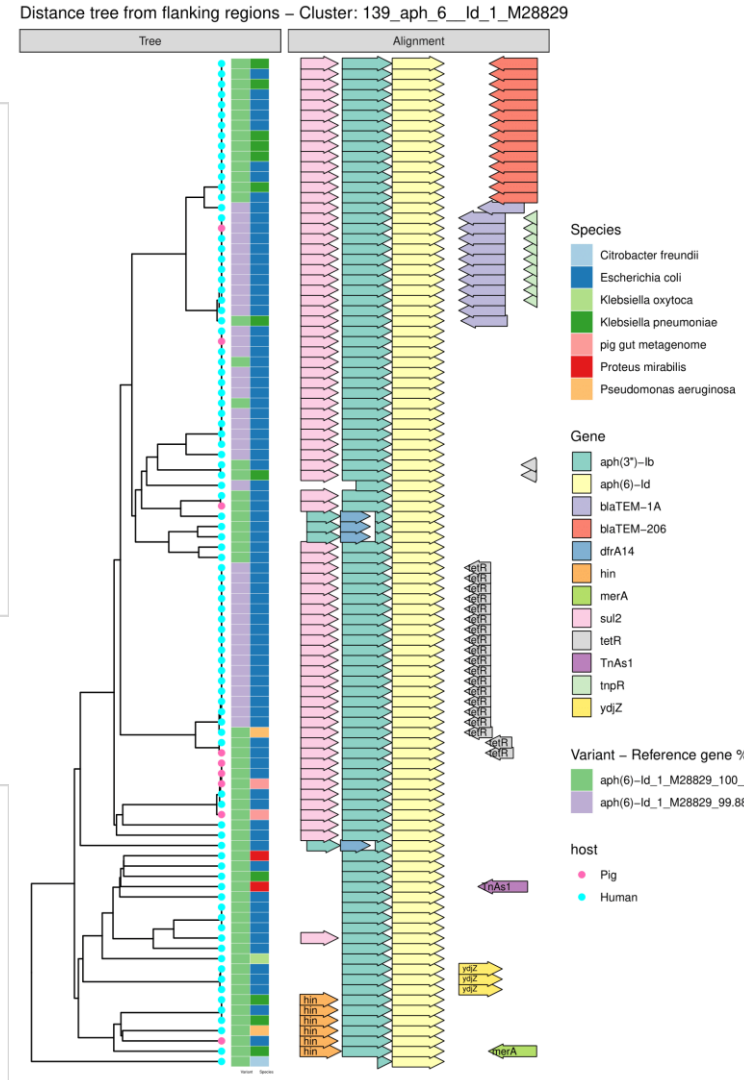
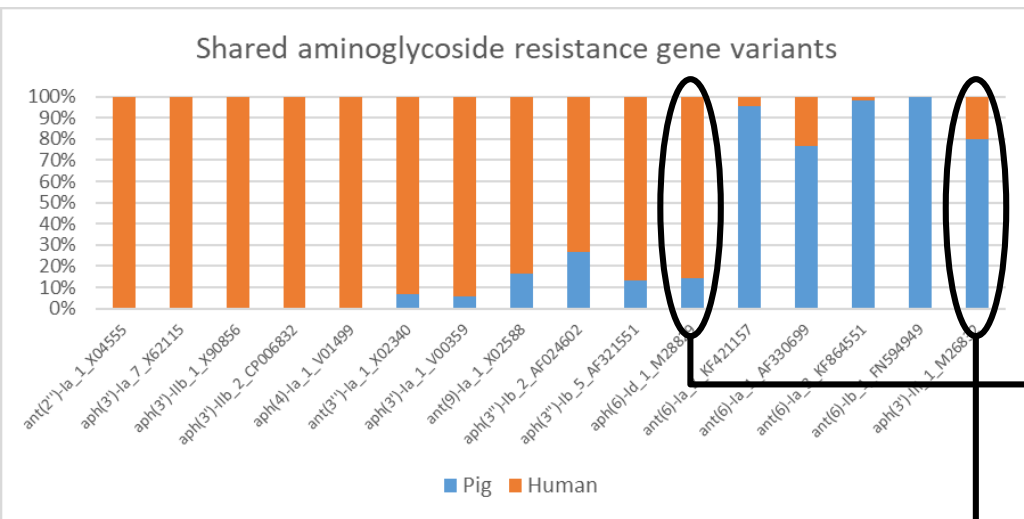
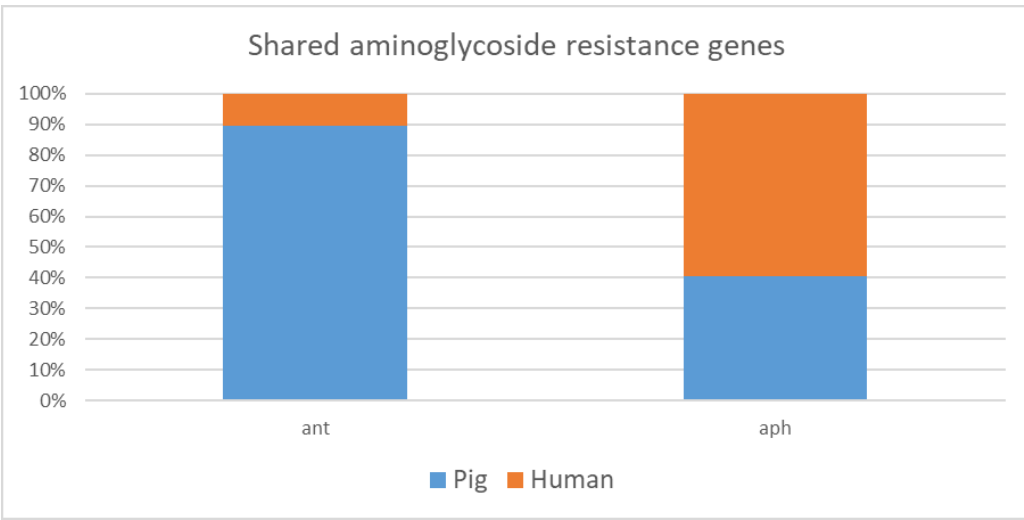
Shared resistome

but at what level(s)?



- Gene (*bla*CMY)
- Gene variant (*bla*CMY-34_1_EF394370)
- Genetic location (flanking regions)





From static to dynamic surveillance




Chaos, Solitons & Fractals

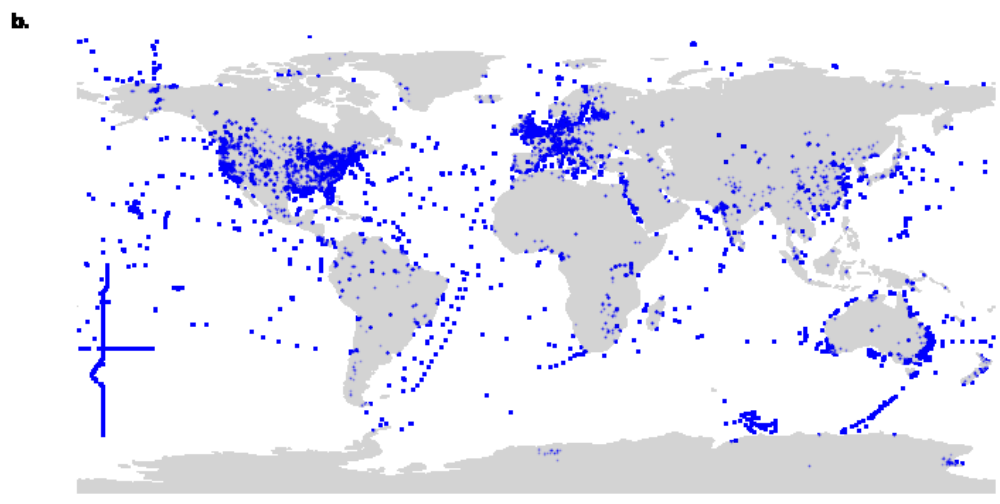
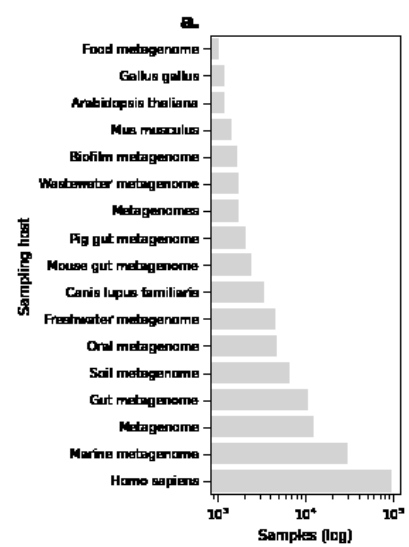
Volume 167, February 2023, 113093



Continuous adjustment of proportion of PCR positive samples from point-of-entry and the general population to be sequenced optimized the time to detect novel variants

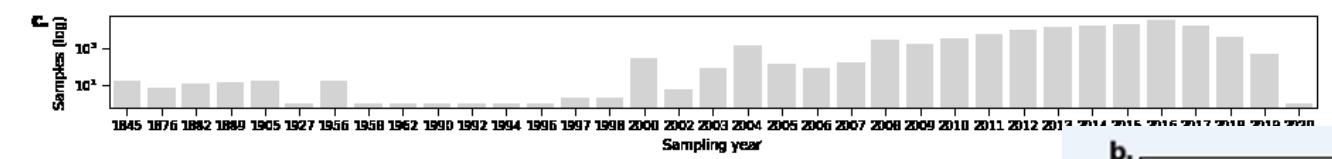
Model-based assessment of sampling protocols for infectious disease genomic surveillance

Sebastian Contreras^a  , Karen Y. Oróstica^b, Anamaria Daza-Sanchez^c,
Joel Wagner^a, Philipp Dönges^a, David Medina-Ortiz^d, Matias Jara^e,
Ricardo Verdugo^b, Carlos Conca^{c e}, Viola Priesemann^{a f}  ,
Álvaro Olivera-Nappa^{d g}  



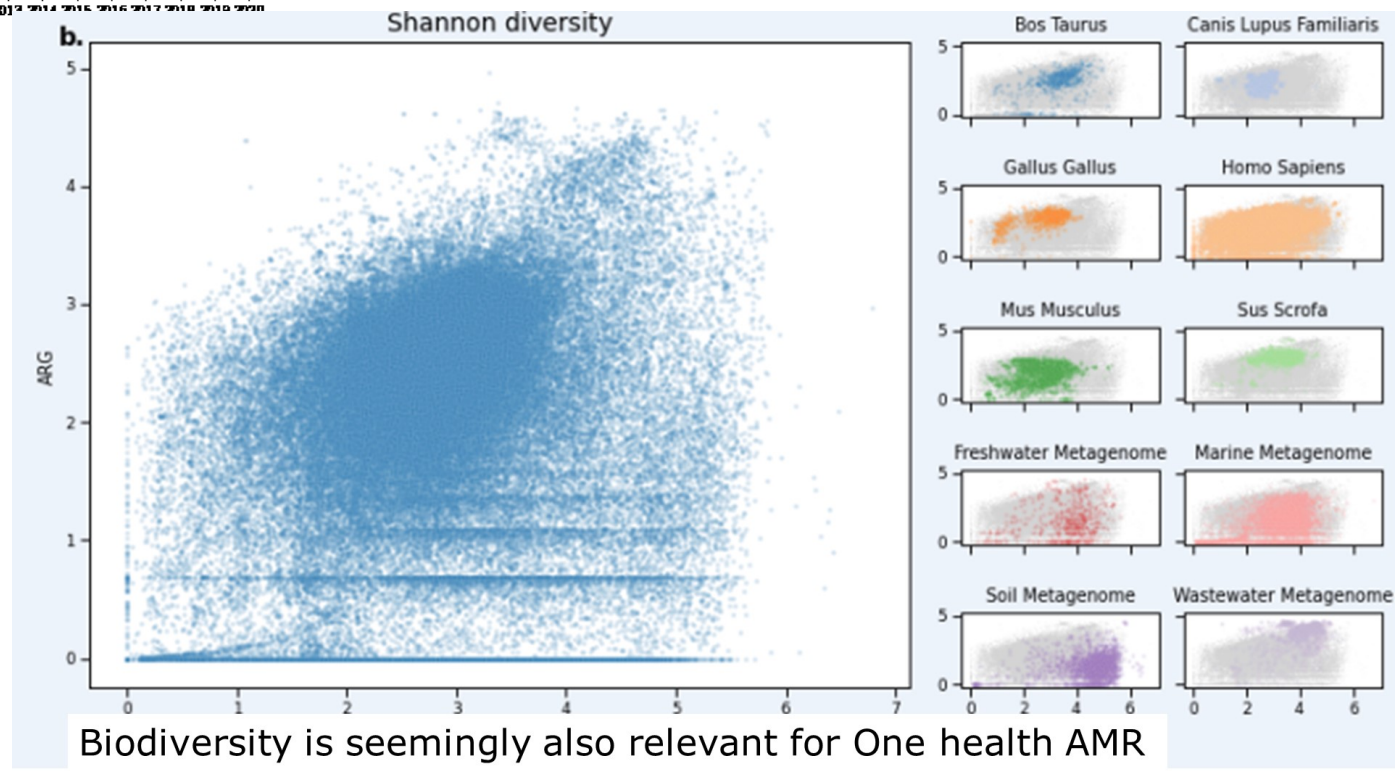
Martiny et al. Plos Biol. 2022.
 Martiny et al. mSystems. 2021

<https://zenodo.org/record/6919377#.Y3jYbnbMKUK>



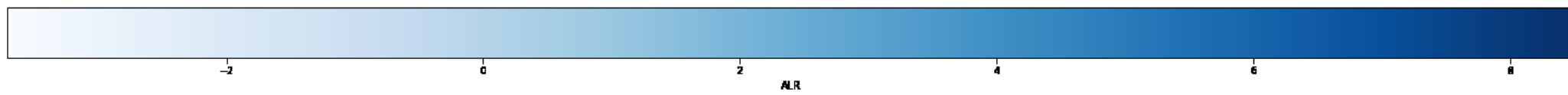
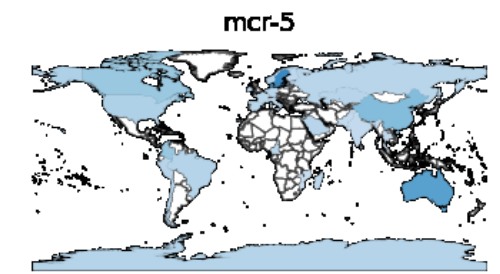
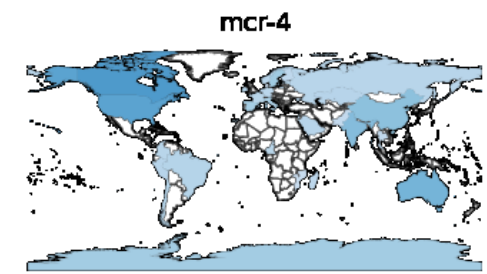
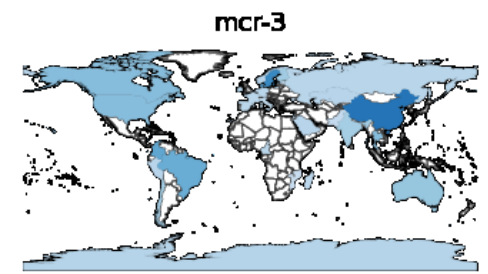
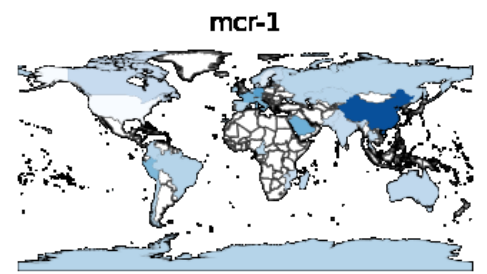
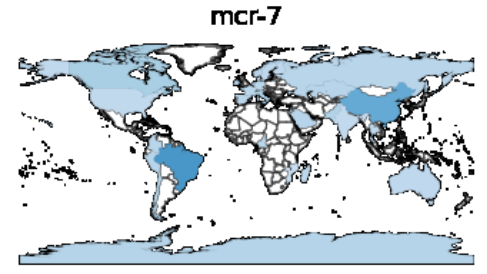
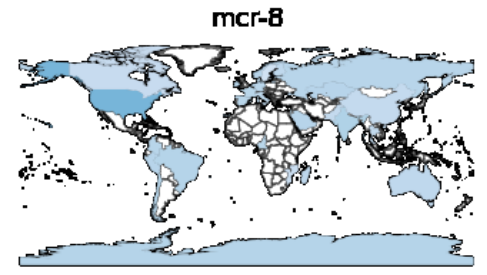
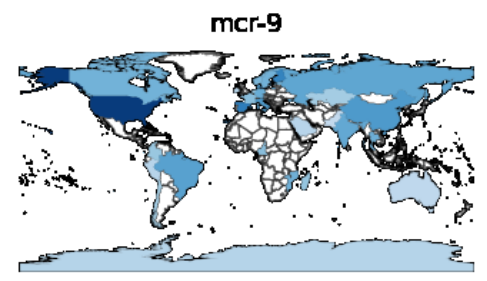
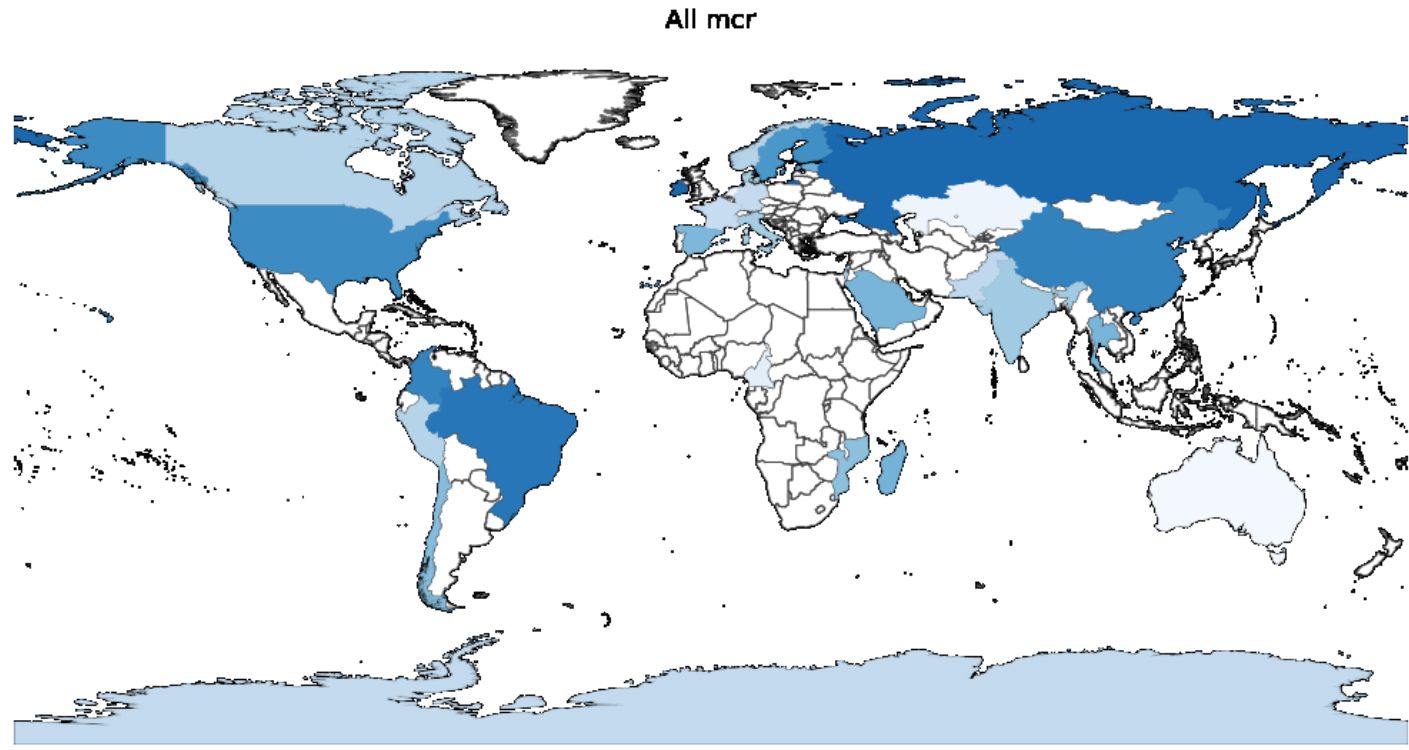
Analyse everything:

- Download of all available metagenomic data
- 214,095 metagenomics datasets - 442 Terabytes -
- Download time 3 months
- Mapped to all known ARGs - 2 months on a 40,000 core HPC
- 50 Gb table



mcr-gene variants
from the World

Clear geographical
differences in
variants



Concluding remarks

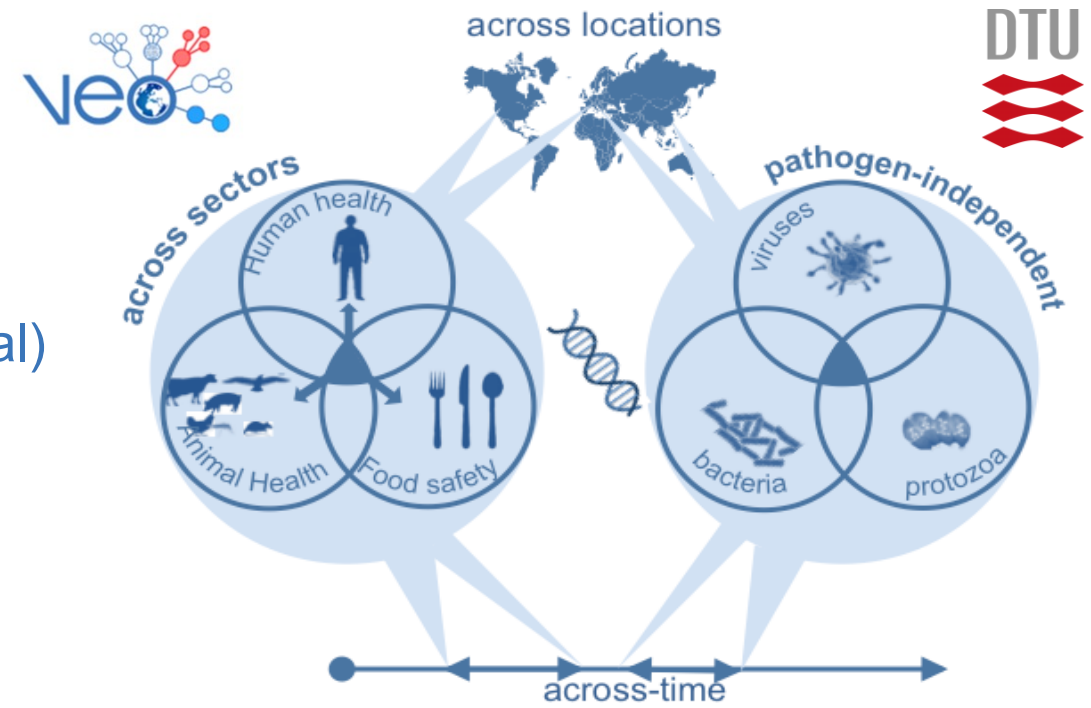
- Surveillance is the basis of everything, and without it we are flying blind
 - Easy to say – difficult to conduct
 - Perhaps time to consider novel options
 - Sewage surveillance could be a valuable contribution and can be implemented immediately
 - Perhaps time to rethink and not wait until all countries can do everything?
- NGS technologies offer several novel options for global surveillance
 - Comparing single isolates and metagenomes
- Perhaps also time to rethink other options
 - Dynamic rather than static surveillance
 - (re) use of public and unstructured data
- (raw) Data-sharing is essential and should be a mandatory part of any funding
- Prevention is better than cure (but fame and fortune is in the cure)
(Bian Que 401-310 BC)



Our vision: one system serves all

Guiding principles:

- Cross sector, cross domain, open source (not commercial)
- Interaction with the rest of the world (all inclusive)
- Data for action (actionable outputs)
- Central repository (ENA, DDJ, NCBI)



VILLUM FONDEN



novo nordisk fonden