Using novel methodologies to support burden of disease estimates

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On behalf of the FOCAL project team
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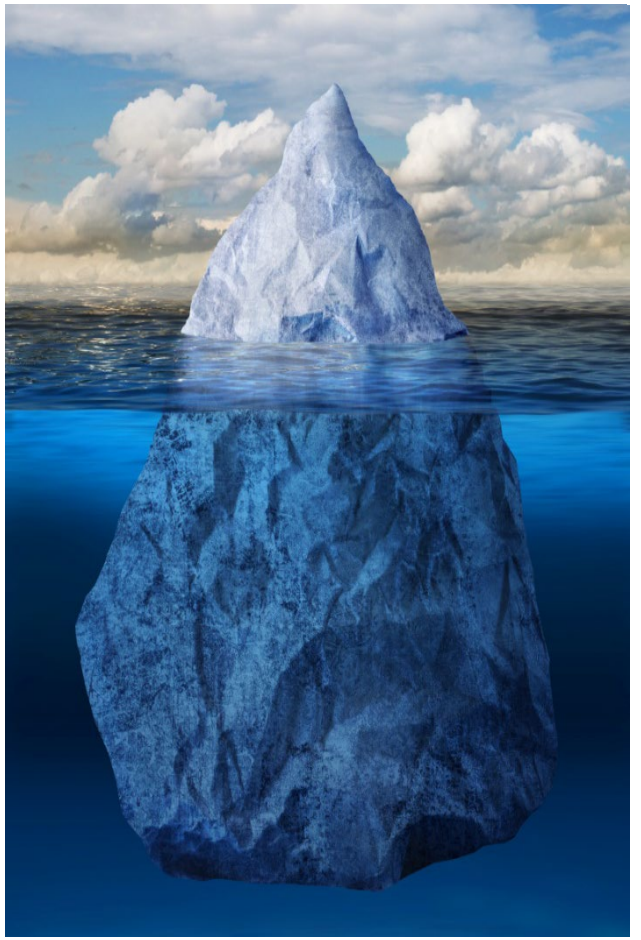
The burden of diarrheal infectious illnesses

WHAT WE KNOW

Surveillance data – reported cases

WHAT WE WANT TO KNOW

The true number of cases



Burden of illness approach in FOCAL

HEALTH-CARE SURVEILLANCE DATA

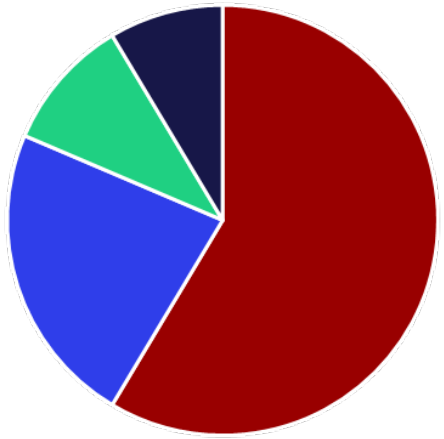
DATA FROM POPULATION SURVEYS

DATA FROM SEWAGE

Approaches for estimating etiology proportions

- Systematic review of peer-reviewed inpatient, outpatient and community studies
- Review of available health-care surveillance data incl. hospital records
- Relative abundance of pathogen-specific genes in untreated sewage samples from the same populations (i.e. using MG analysis).

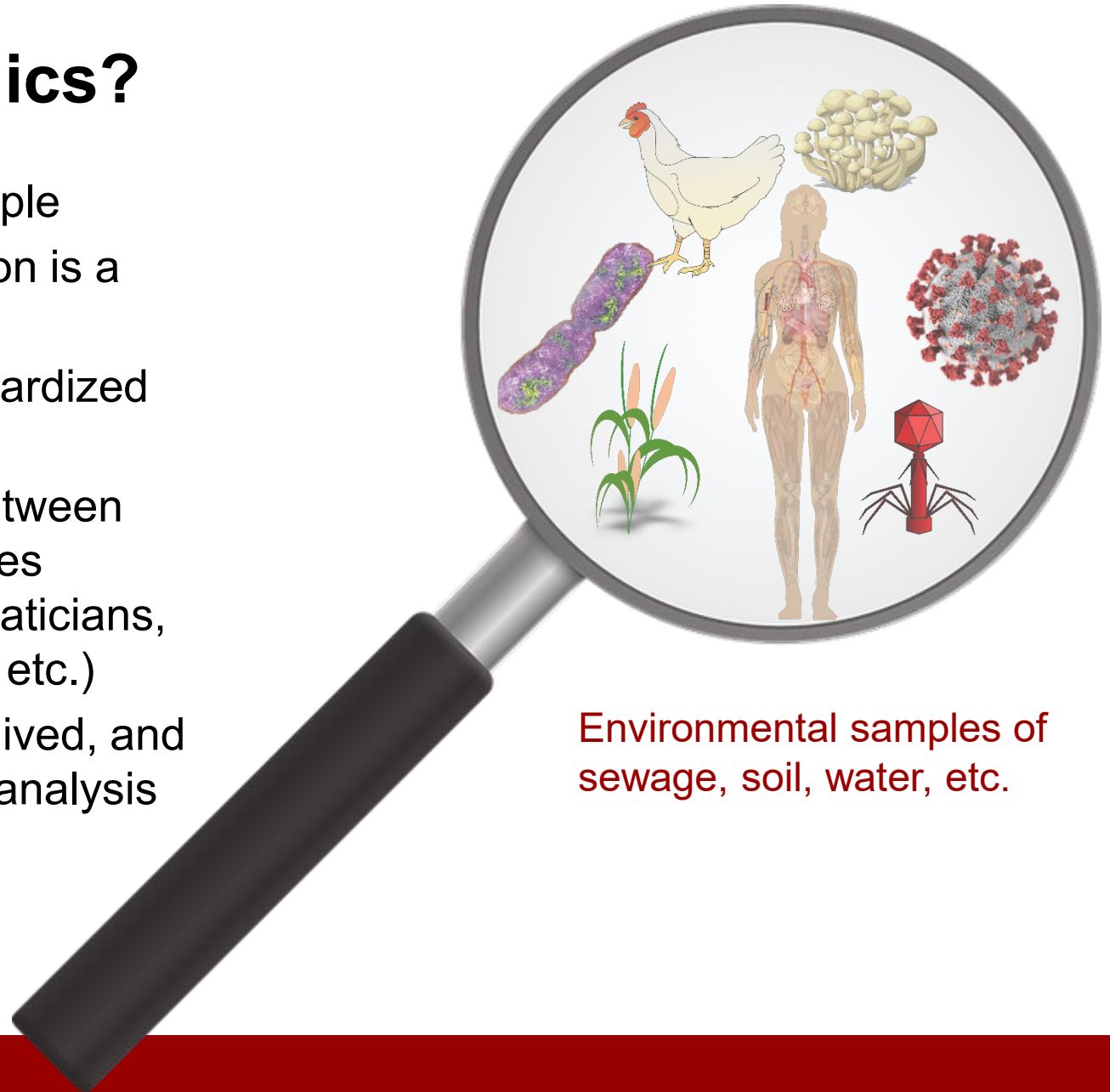
Etiology proportions



What is the relative contribution from each diarrhoea-causing microorganism to the total burden of diarrheal illness?

Why metagenomics?

- Detects all DNA in a sample
- DNA sequence information is a universal ”language”
- Can be provided in standardized electronic format
- Can be readily shared between laboratories and disciplines (microbiologist, bioinformaticians, epidemiologists, doctors, etc.)
- DNA sequences are archived, and thus available for further analysis



Environmental samples of sewage, soil, water, etc.

Metagenomics – wet lab

Wastewater, fecal or pooled fecal sample

DNA extraction from sample

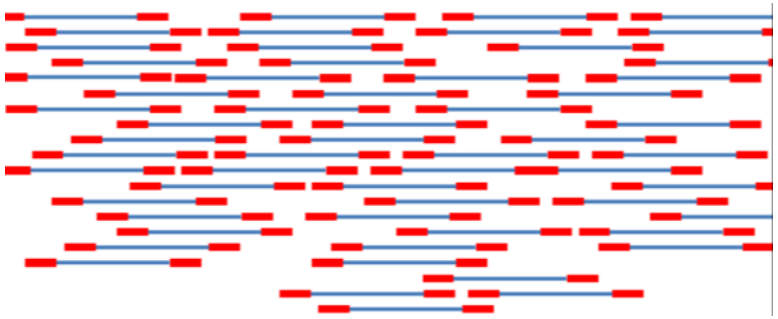
DNA sheared to 3-400 bp

DNA library preparation

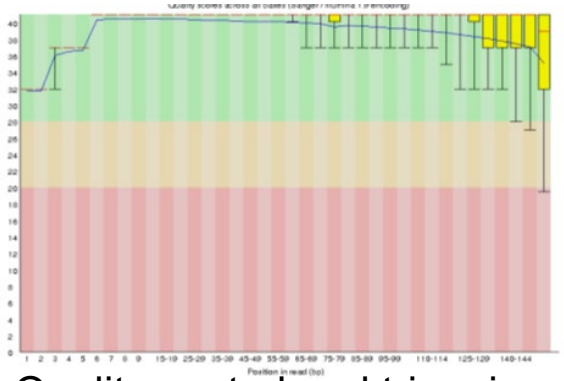
HiSeq2500/3000/Novaseq

2 x 35M 150bp fragments = 10 Gbp

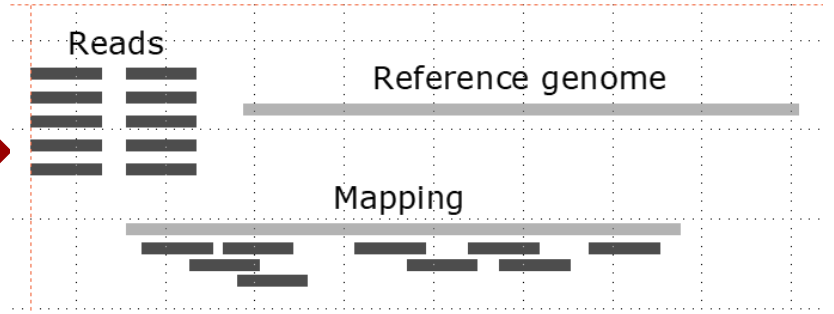
Metagenomics – dry lab



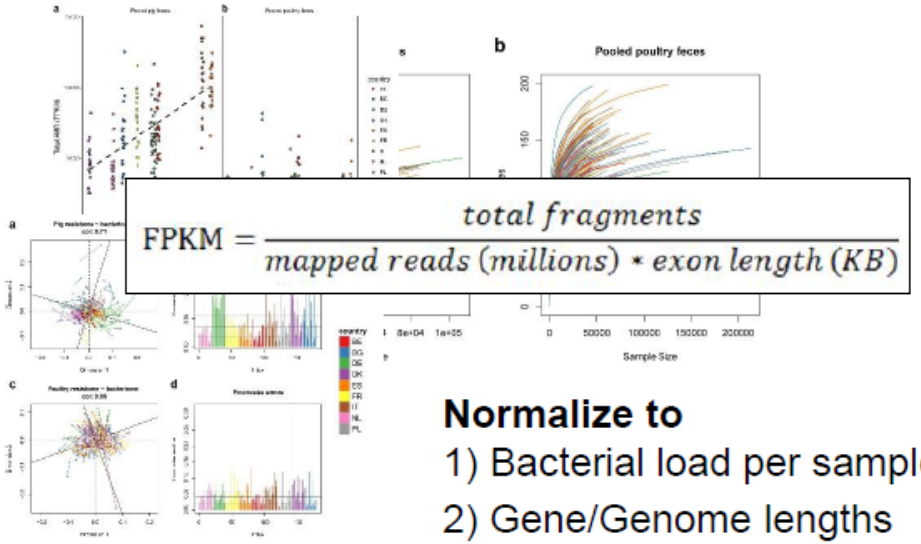
Paired-end DNA reads



Quality control and trimming



Mapping reads to genomes of interest e.g. bacterial genomes or antimicrobial resistance genes.



$$\text{FPKM} = \frac{total\ fragments}{mapped\ reads\ (millions) * exon\ length\ (KB)}$$

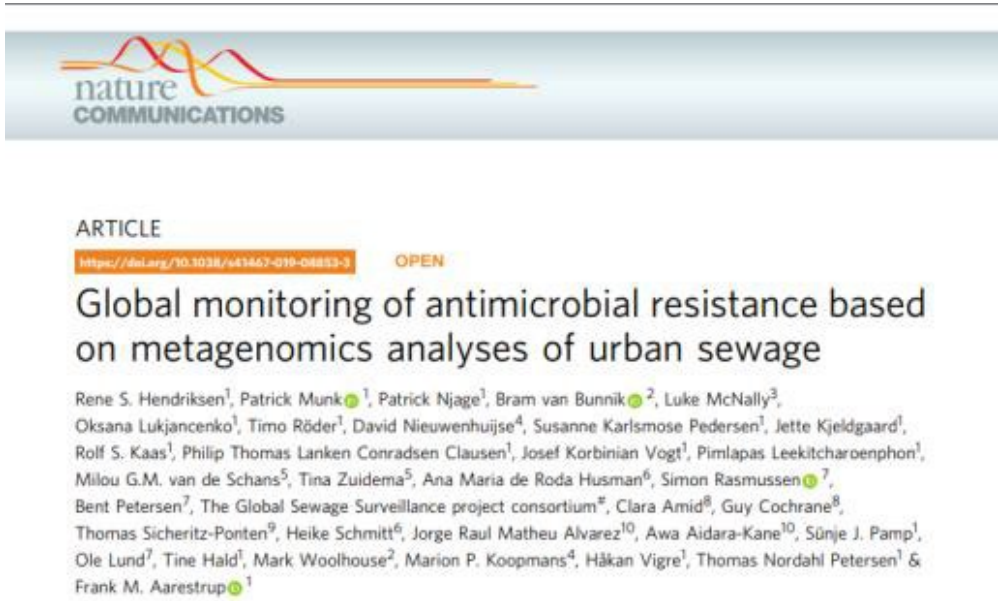
Normalize to

1) Bacterial load per sample
2) Gene/Genome lengths

	Sample 1	ample 2	Sample 3	Sample 4
Ref 1	23	56	0	45
Ref 2	15	0	11	2
Ref 3	6	17	33	0

Global Surveillance of AMR using sewage

- 60 countries across all continents
- 79 samples of urban sewage



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ARTICLE

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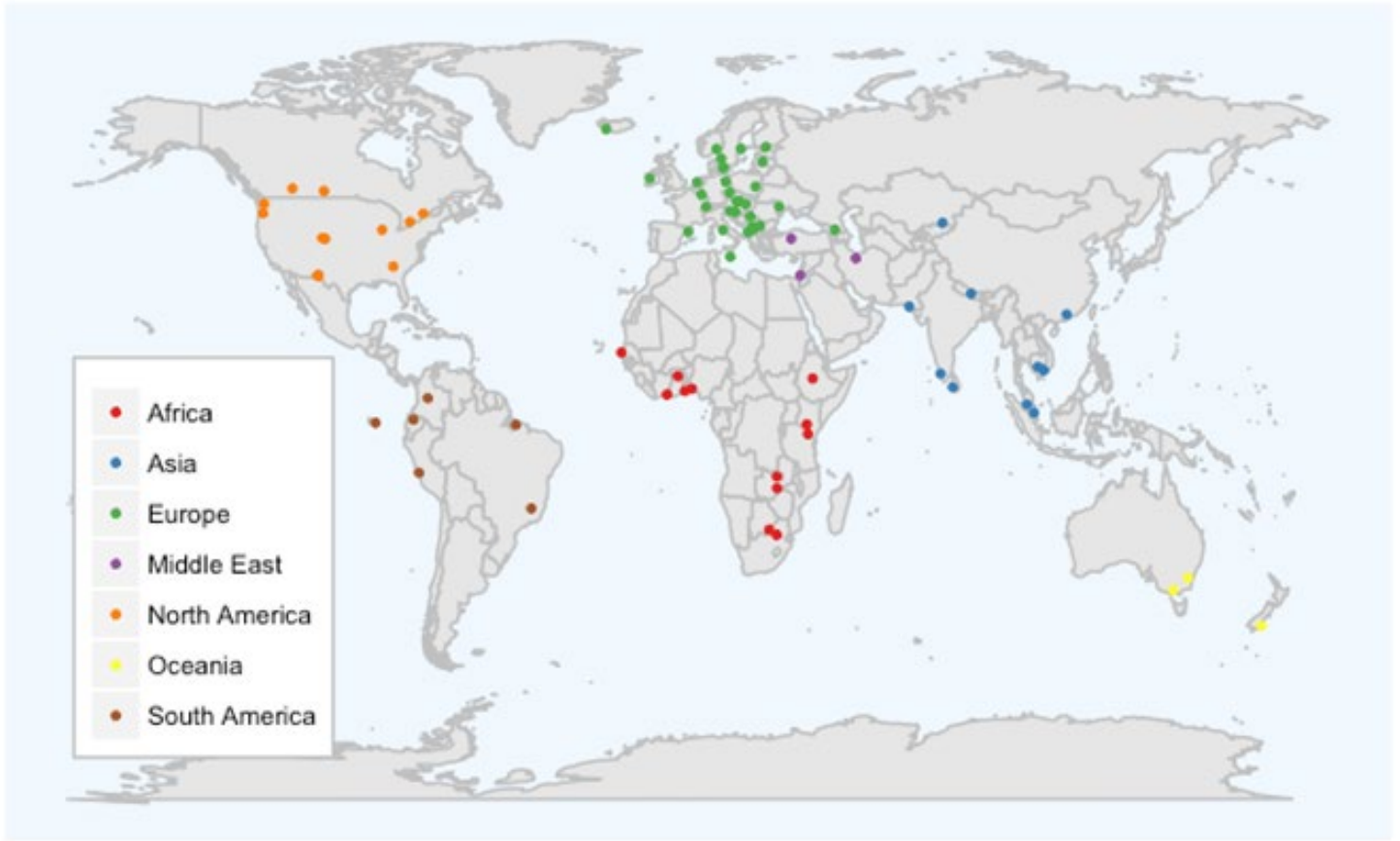
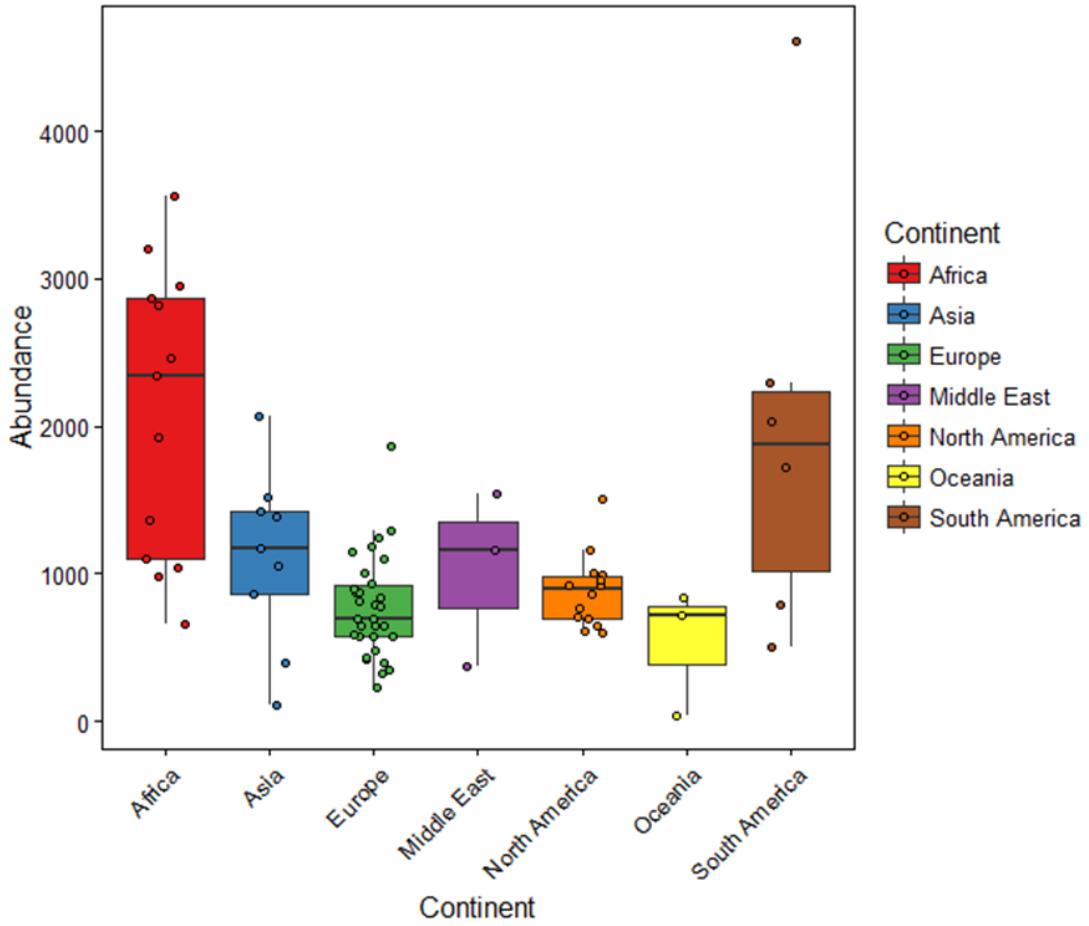
Global monitoring of antimicrobial resistance based on metagenomics analyses of urban sewage

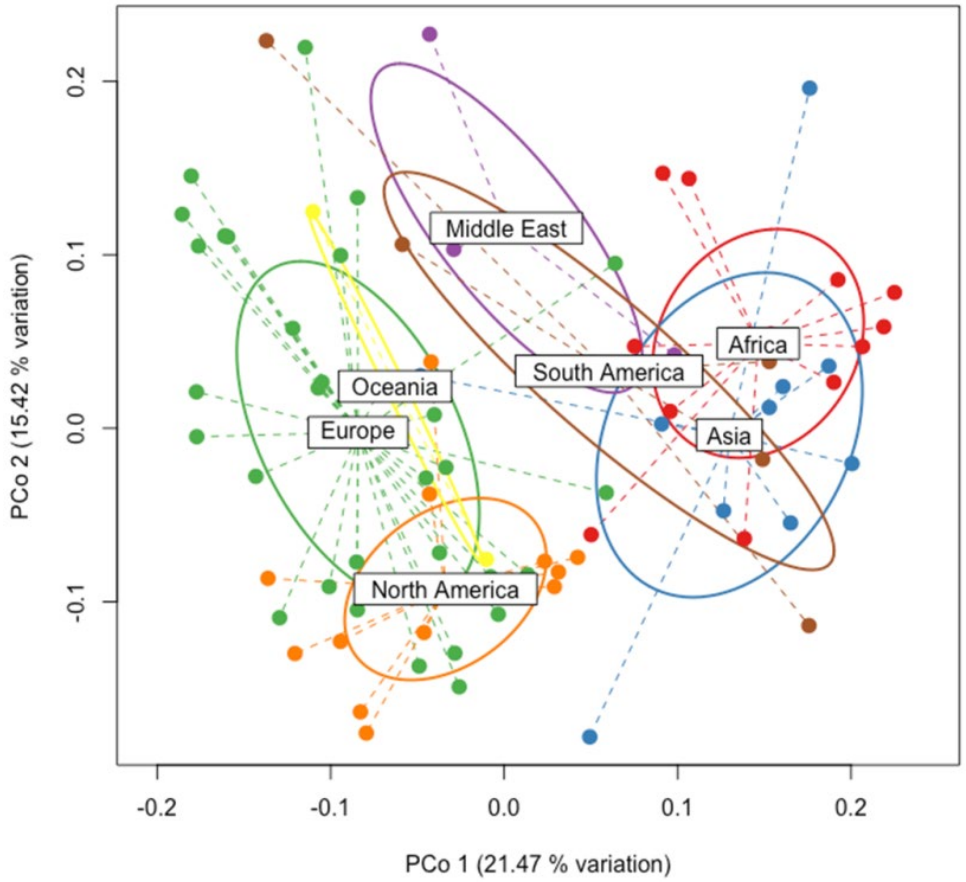
Rene S. Hendriksen[1], Patrick Munk[1], Patrick Njage[1], Bram van Bunnik[2], Luke McNally[3], Oksana Lukjancenko[1], Timo Röder[1], David Nieuwenhuijse[4], Susanne Karlsmose Pedersen[1], Jette Kjeldgaard[1], Rolf S. Kaas[1], Philip Thomas Lanken Conradsen Clausen[1], Josef Korbinian Vogt[1], Pimlapas Leekitcharoenphon[1], Milou G.M. van de Schans[5], Tina Zuidema[5], Ana Maria de Roda Husman[6], Simon Rasmussen[7], Bent Petersen[7], The Global Sewage Surveillance project consortium*, Clara Amid[8], Guy Cochrane[8], Thomas Sicheritz-Ponten[9], Heike Schmitt[6], Jorge Raul Matheu Alvarez[10], Awa Aidara-Kane[10], Sünje J. Pamp[1], Ole Lund[7], Tine Hald[1], Mark Woolhouse[2], Marion P. Koopmans[4], Håkan Vigre[1], Thomas Nordahl Petersen[1] & Frank M. Aarestrup[1]



Hendriksen et al., Nature Coomunications, 2019

Global sewage resistomes





Hendriksen et al., Nature Communications, 2019

Conclusions

- Sewage-based surveillance using metagenomics is flexible, scalable, and easy to quickly implement and standardize
- Surveillance of sewage for AMR occurrence works pretty well; able to quantify resistance occurrence.
- For pathogen detection, sewage sampling can provide a qualitative snapshot of pathogens occurrence in a population
  - When refined, it may also provide a reliable quantitative snapshot
- Sewage-based surveillance may complement and support
  - The detection and surveillance of ‘silent’ epidemics
  - Clinical, isolate-based surveillance
  - Burden of illness studies

Acknowledgement

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