Metagenomic sequencing for resistome analysis

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Disclosures

- Chairman of EUCAST, previous member of the EUCAST subcommittee for next generation sequencing as a tool for antmicrobial susceptibility testing
- No other disclosures pertaining to the subject
- I am not a bioinformatician – not even close…

Key topics

- Can presence and absence of resistance genes predict phenotypic resistance on a bacterial strain level?
- Resistome analysis carried out from shotgun sequencing data
- What is the potential utility of resistome analysis
  → In the clinical setting
  → In the environmental setting

Classical scenario

- Culture negative
- A pathogen (or several) are found with shotgun metagenomics or 16S rDNA sequencing
- Clinician is happy to have the name of the species BUT
- Still doesn't know which antimicrobial to select
- Is the lesson that cultureomics will always prevail?

Prediction of phenotypic resistance from WGS data (strain sequencing)

Role of WGS in AST recently reviewed

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Review

The role of whole genome sequencing in antimicrobial susceptibility testing of bacteria: report from the EUCAST Subcommittee

M.J. Ellington [1, †], O. Ekelund [2, †], F.M. Aarestrup [3], R. Canton [4], M. Doumith [1], C. Giske [5], H. Grundman [6], H. Hasman [7], M.T.G. Holden [8], K.L. Hopkins [1], J. Iredell [9], G. Kahlmeter [2], C.U. Köser [10], A. MacGowan [11], D. Mevius [12, 13], M. Mulvey [14], T. Naas [15], T. Peto [16], J.-M. Rolain [17], Ø. Samuelsen [18], N. Woodford [1, *]

The resistome on a strain level

- BLAST analysis of draft genomes
- Mapping of individual reads to downloadable or web-based tools
- Second option: able to identify <100% identity, truncated genes because of suboptimal assembly
  → But: has to assessed to be translated into a predicted phenotype
- Sequence deep: >=30 x coverage
- Standardized and intensively curated open-access databases are needed
- Long-read sequencing with improved assemblies may solve some problems over time
- **In general easiest to predict wild-type vs non wild-type, not the level of resistance**

Current status for clinically important species

S. pneumoniae, N. gonorrhoeae, P. aeruginosa, A. baumannii and *C. difficile* more studies needed even to define the knowledge gaps

Enterobacteriaceae (including *Salmonella*), to some extent promising, but challenges related to chromosomal resistance

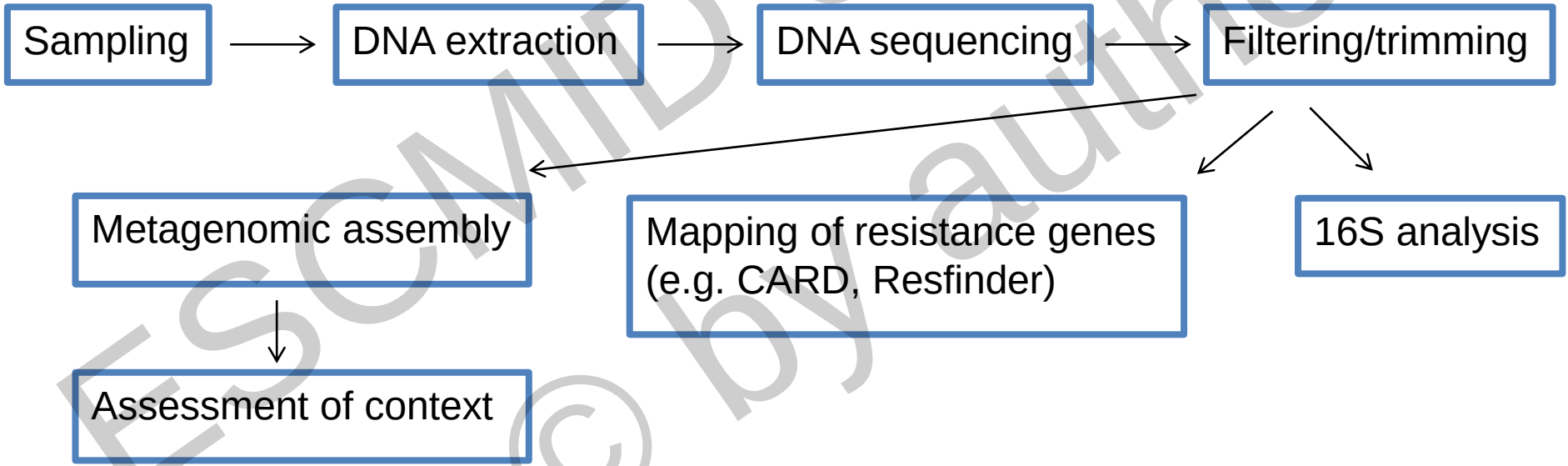
S. aureus and *M. tuberculosis*: promising results

Conclusion regarding strain resistome

- Clinical decision-making not feasible
  → Additionally lack of speed and too high cost
- *M. tuberculosis* may happen relatively soon
- Could be important for molecular surveillance
- End users will have to learn how to relate to results
- Curated databases with public access is pivotal
- So does this mean we can skip shotgun sequencing resistome, since not even feasible on a strain level?

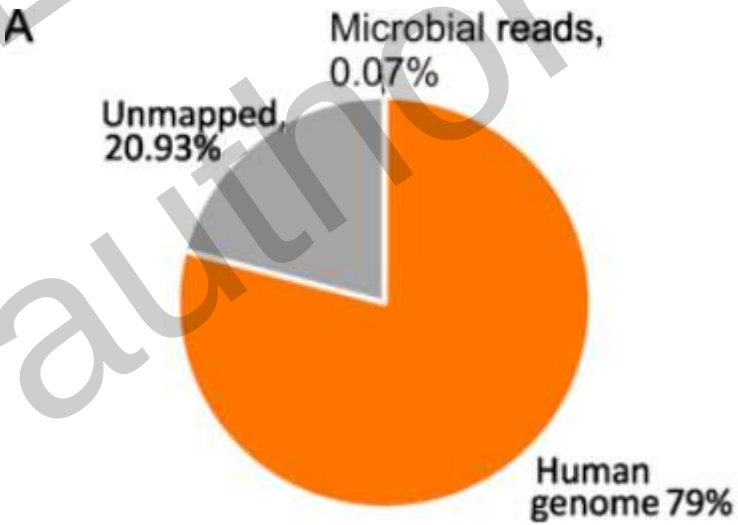
Clinical resistome analysis

Procedure for resistome analysis

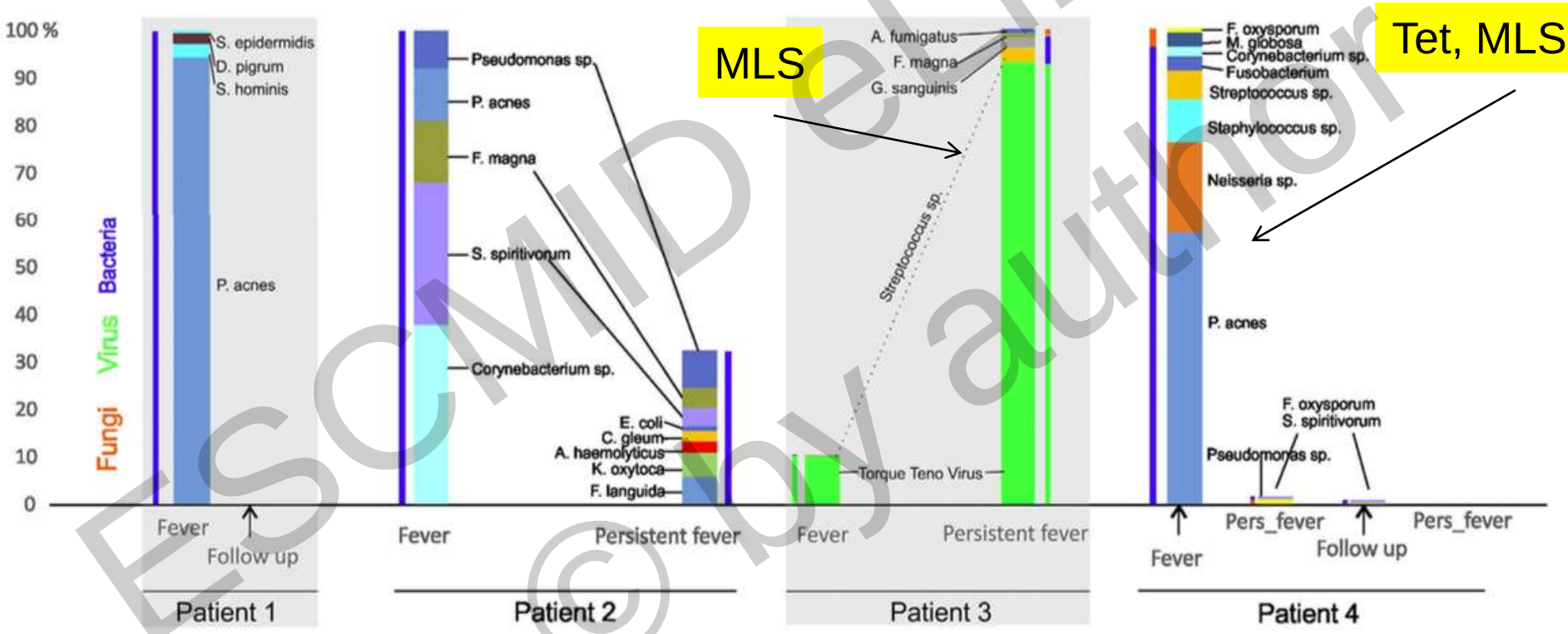


Microbiome and resistance in BSI, neutropenic fever

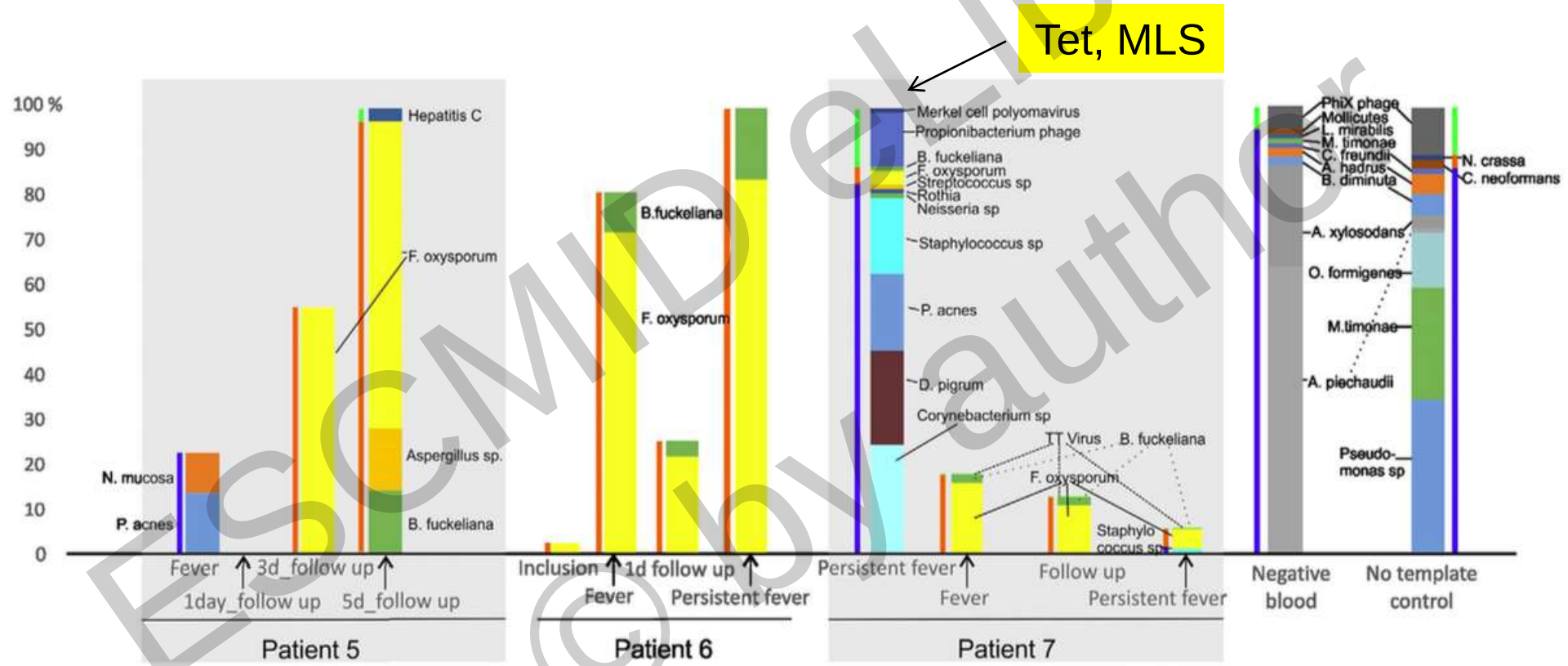
- Gyarmati P et al. Sci Rep 2016: 6:23532:
- Patients with hematological cancer and fever episodes 4.5 mL blood directly from the patient without enrichment
- MolYsis and NebNext microbiome enrichment
- Sequencing with HiSeq
- Assay validated with spiked *E. coli* (detection limit 10 cfu/mL)
- NCBI Microbial Genomes and ARG-ANNOT
- 33.5 million reads per sample on average



Microbiome and resistance in BSI, neutropenic fever (cont.)



Microbiome in BSI, cont.



Further on sequencing in sepsis patients

- Grumaz S et al. Genome Med. 2016;8(1):73
- Plasma was prepared from blood samples by centrifugation for 10 min at 292 × g and 4 °C, snap frozen, and stored at −80 °C until further processing
- Nucleic acids were isolated from thawed plasma after a centrifugation step of 5 min at 1000 × g with the circulating Nucleic Acid Kit (Qiagen)
- Septic patient with liver transplant: 100 % identity to *vanB,* confirmed by culture (in *E. faecium*)
- Detection of *mecA* from methicillin-resistant *S. aureus* in other patient plasma samples

Urinary tract infection resistome analysis

- Ten heavily infected (>10^7 cfu/mL) clinical urines from patients at the Norfolk and Norwich University Hospital
- Urine from healthy volunteer spiked with MDR *E. coli*
- Bacterial DNA enriched prior to sequencing
- MinION (long-read) sequencing carried out on extracted DNA and analysed with BLAST (assignment of taxa) and CARD (assignment of resistance genes)
- Metrichor WIMP (taxa) and ARMA (resistance) software was adopted late in the study and allowed real-time analysis
  → Almost 7x depth of coverage after 1 h (covers 99.905% of *E. coli* genomes
- Schmidt K et al. JAC 2017; 72: 104–114

UTI resistome analysis (cont.)

4-10 mL urine centrifuged for 2 min at 300 g (deplete human cells)

↓

Supernatant re-centrifuged at 12300 g for 5 min

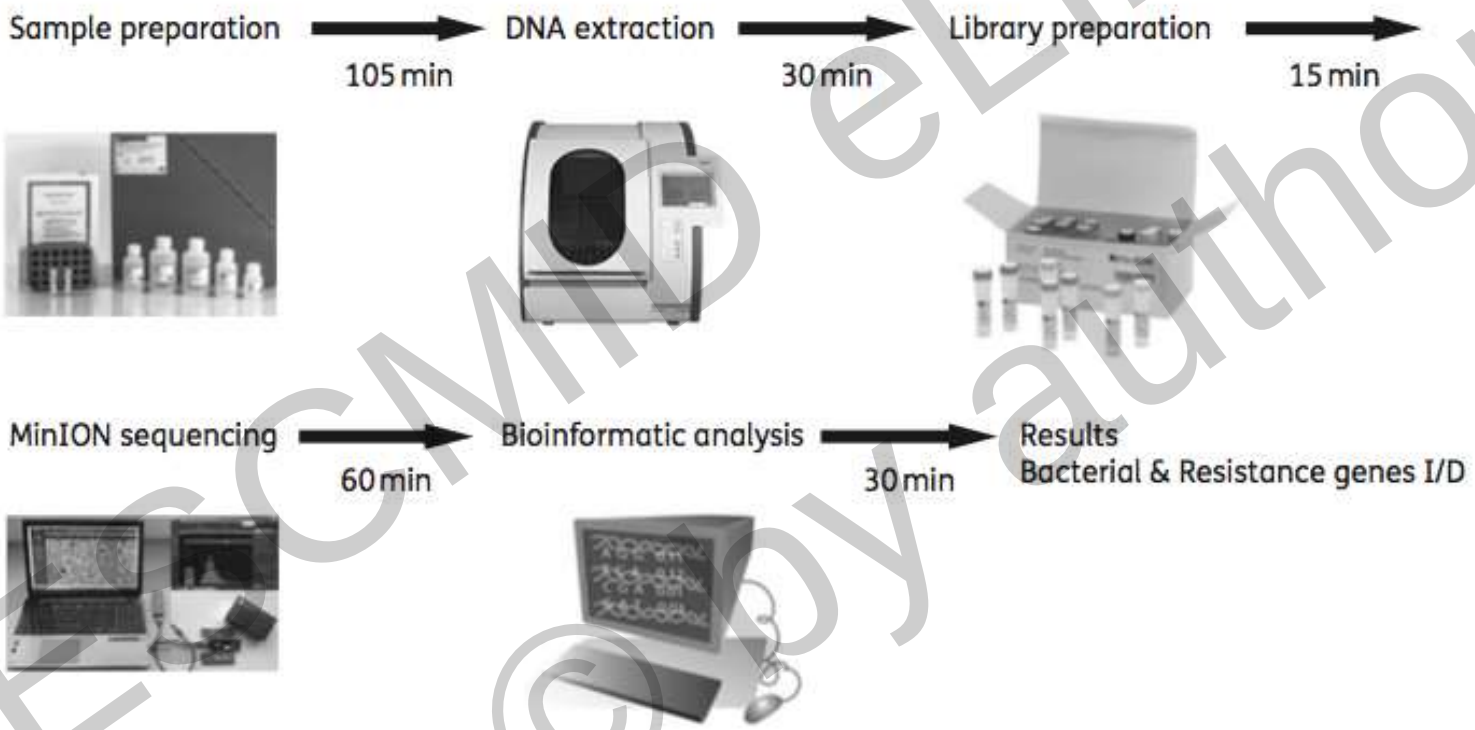
↓

Bacterial pellet resusp. in 1 mL PBS and processed with MolYsis

↓

Proteinase K treatment and MagNA Pure DNA extraction

UTI resistome analysis (cont.)



UTI resistome analysis (cont.)

Phenotypic resistance	Genotypic resistance
KP: CTX, ESBL+, CIP, GEN, TOB, TRI	*bla*$_{\text{CTX-M-15}}$, *bla*$_{\text{SHV-12}}$, *qnrB*, *aac(6')-Ib-CR*, *aacC2*, *dfrA14*
EC: CTX, ESBL+, CIP, GEN, TOB, TRI	*bla*$_{\text{CTX-M-15}}$, *bla*$_{\text{AmpC}}$, *aac(6')-Ib-CR*, *gyrA*, *parC*, *aacC2*, *aadA5*, *dfrA17*
EC: CTX, ESBL+, CIP, GEN, TOB, TRI	*bla*$_{\text{CTX-M-15}}$, *bla*$_{\text{AmpC}}$, *aac(6')-Ib-CR*, *gyrA*, *aacdA1*, *aadA3*, *strA*, *strB*, *dfrA1*
EC	*bla*$_{\text{CMY}}$
E. cloacae	
KP: CTX, ESBL+, CIP, GEN, TOB, TRI	*bla*$_{\text{CTX-M-15}}$, *bla*$_{\text{SHV-28}}$, *qnrB*, *aac(6')-Ib-CR*, gyrA, parC, *aacA4, aacC2*, *strA*, *strB*, *dfrA14*

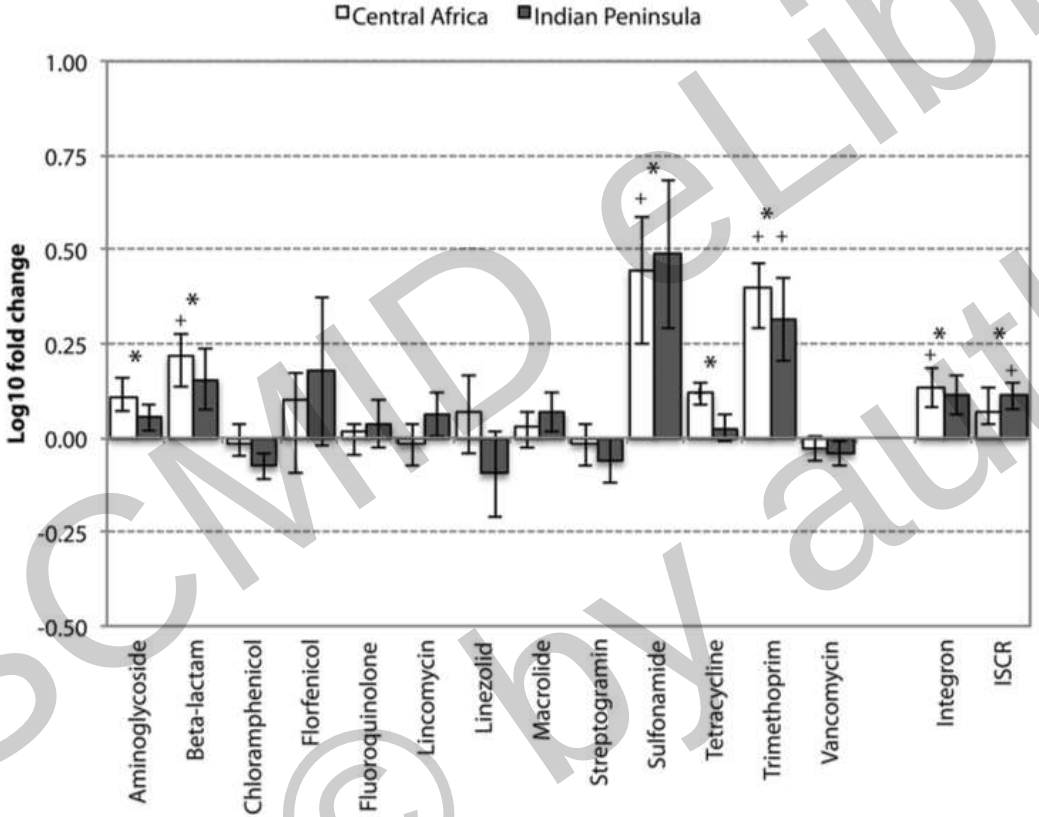
Urine resistome – conclusions

- Heavy load of bacteria
  - → Minimizing effect of contaminating bacteria
  - → Optimizing DNA yield
- Limitation
  - → Not tried with lower bacterial loads
  - → Expensive
  - → Distinction between allelic variants
  - → Silent genes

Fecal resistome in travellers

- Fecal samples from 35 Swedish students before and after travel to India or Central Africa (exchange programme)
- Self-submission of fecal samples – sequenced with Illumina HiSeq 2000
- Screening for ESBL-producing Enterobacteriaceae done with chromogenic culture media
- Quality filtering and trimming followed by analysis of resistance genes with Resqu and small-subunit rRNA with Metaxa2
- Resistance gene abundances normalized for gene length and number of bacterial 16S rRNA sequences in each library
- Bengtsson-Palme J et al. AAC 2015; 59:6551–6560.

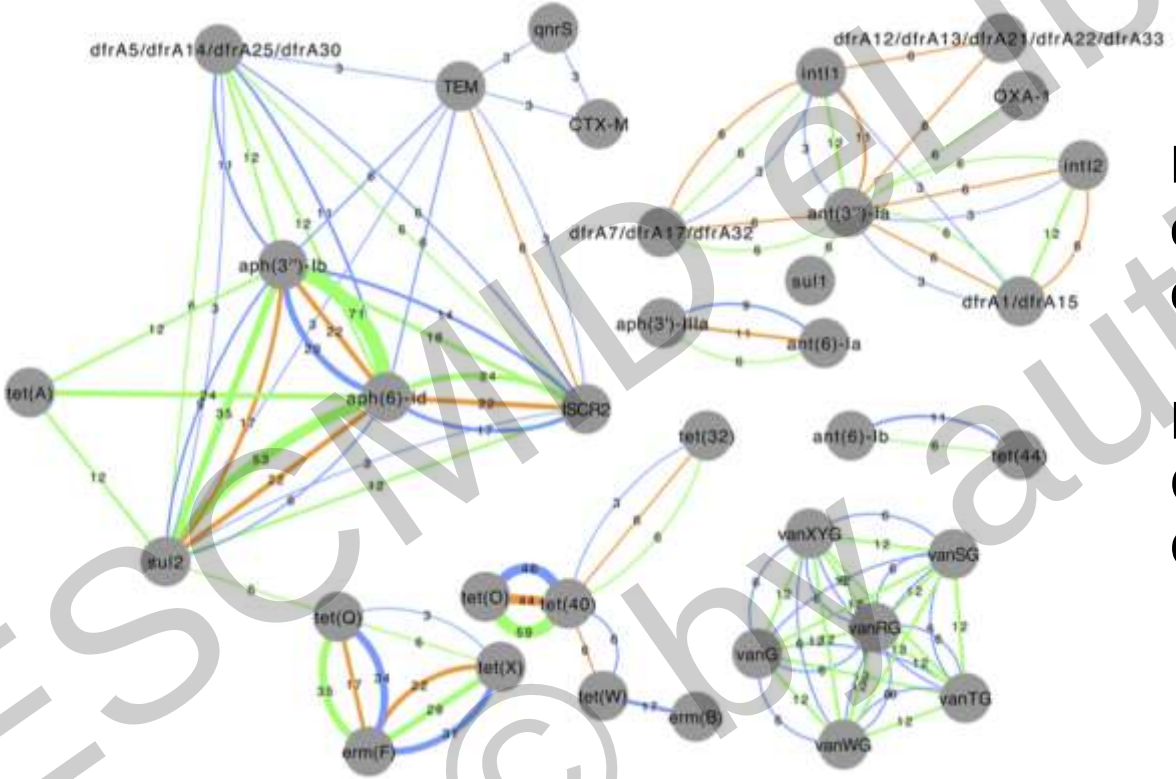
Average fold change of resistance genes



* Significance entire cohort

+Significance India vs Central Africa

Co-localization of resistance genes



Resistance genes on the same contigs

Blue=before
Green=Central Africa
Orange=India

Comparison with culture

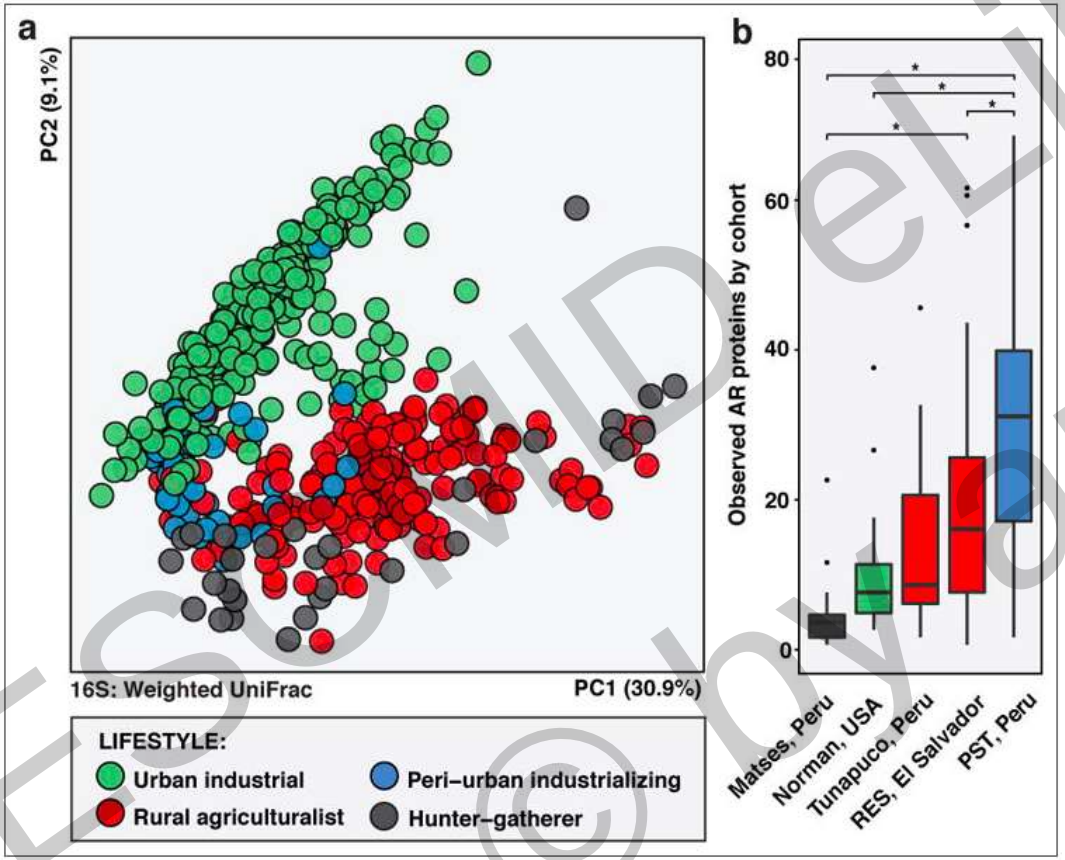
- 12/18 travellers to India carried ESBL-producing *E. coli* after returning (0/18 prior to travel)
- Whole-genome strain sequencing revealed CTX-M-15 in all strains
- Just 4/12 of the culture positive were positive with the metagenomic approach
- 1/6 culture negative had a low abundance of CTX-M with metagenomics
- CTX-M was thus missed frequently despite using sequencing able to detect a resistance gene present in one out of 100,000 bacterial cells

High-throughput comparisons between populations

Population level assessment of AMR

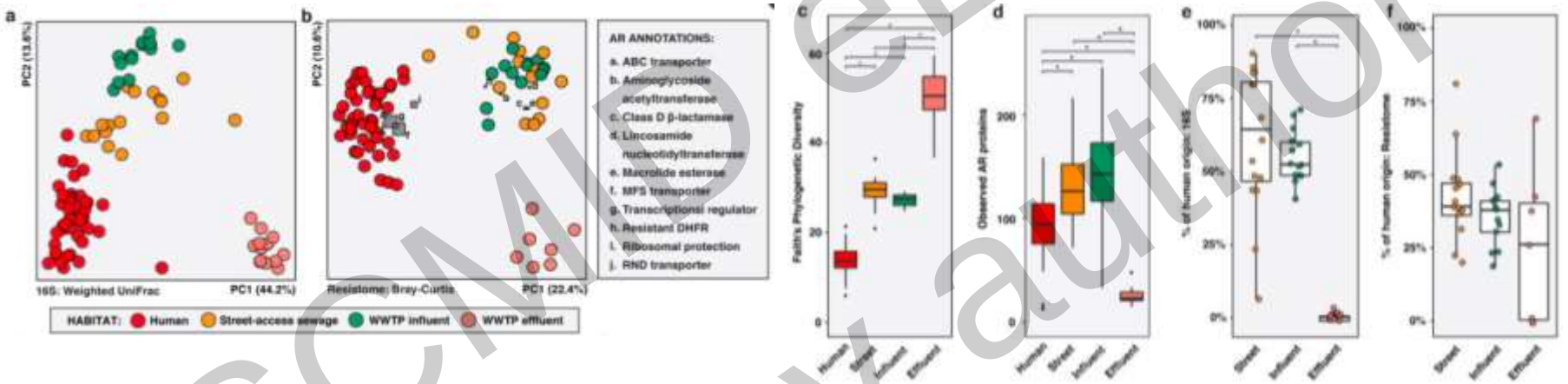
- Culture-based methods labour-intensive
- Non-comparative studies (not comparing with culture) can generate data to compare different populations and lay grounds for quantitative risk assessments and surveillance in different habitats
- 263 fecal samples from 115 individuals in 27 houses over two years from rural village in El Salvador (RES) and peri-urban shanty town in Lima, Peru (PST), as well as 209 environmental samples (animals, soil, water, wastewater plants) from donor households and surrounding areas in these communities
- Sequencing was done with Illumina HiSeq and resistance genes were searched for using CARD and quantified with ShortBRED
- Pehrsson EC et al. Nature. 2016; 533: 212–216.

RES and PST resistomes vs global



- Fecal microbiota composition clustered by host lifestyle, despite differences in geographic origin and study
- PST had the greatest number of AR proteins per person

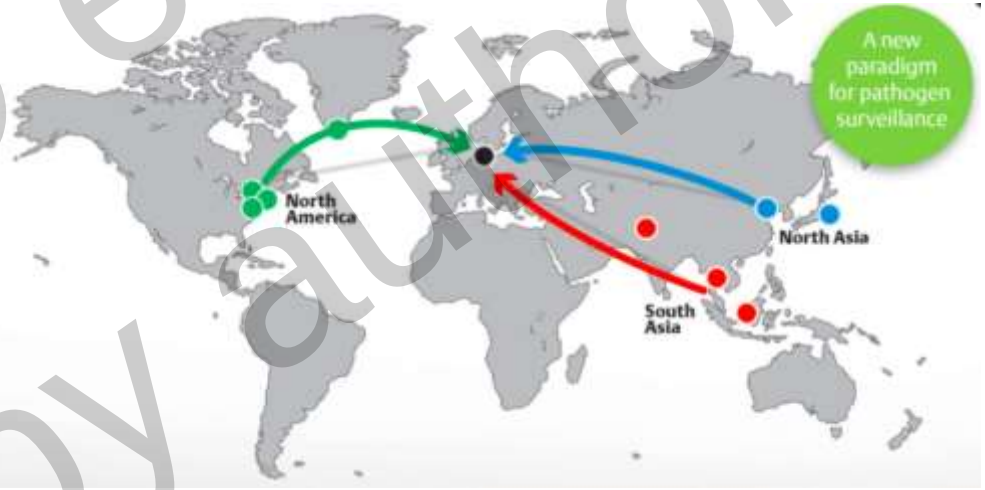
Peruvian peri-urban slum human fecal and sewage microbiota and resistomes



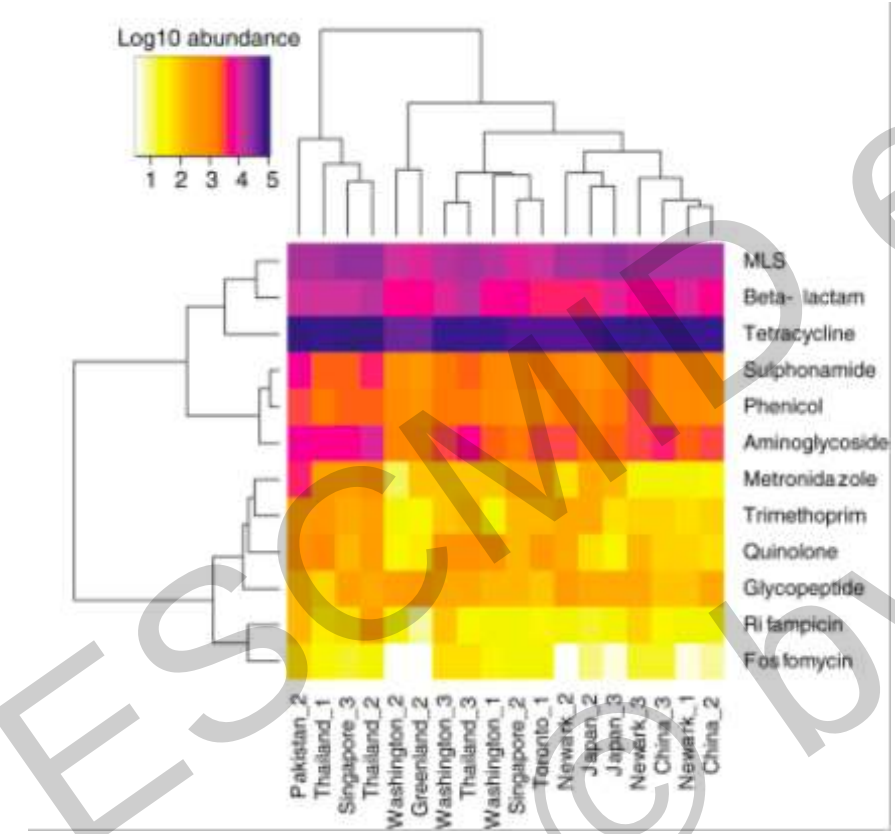
- Sewage resistomes decreased in similarity to PST human fecal resistomes at each treatment stage
- Street-access sewage and WWTP influent had both higher phylogenetic diversity and more AR proteins per sample than PST human feces

Resistome in toilet waste from long-distance flights

- Petersen TN et al. Sci Rep. 2015; 5: 11444
- Emptying of waste toilets, removal of large cells and debris, DNA extraction with 2 rounds of phenol/chlorophorm
- Illumina HiSeq sequencing
- Resfinder was used for detection of resistance genes

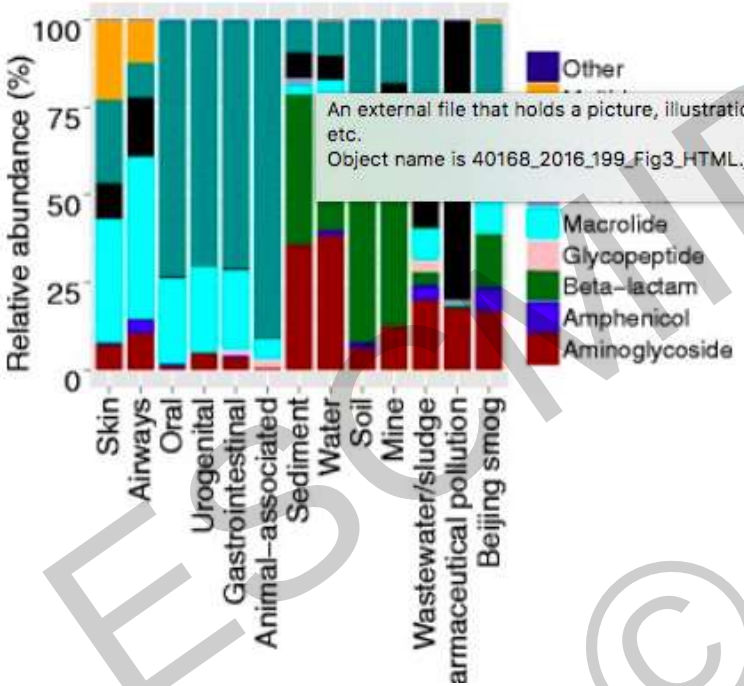


Abundance of resistance genes



- Compared to North America significantly higher abundance of antibiotic resistance genes among samples from South Asia, North Asia and all Asian samples combined
- Resistance gene richness was higher in samples from South Asia and all Asian samples combined, compared to North America

Human vs animal vs enviromental



- Large-scale metagenomic survey and quantitative comparison using 864 deeply sequenced metagenomes (Illumina), from humans, animals and a range of external environments
- Environmental samples generally contained a wider distribution of resistance genes to a more diverse set of antibiotics classes
- Pal C. Microbiome. 2016; 4: 54.

Predictions for future use

So what would be applications then?

“It is time to close the book on infectious diseases, and declare the war against pestilence won”
(not Surgeon Gen William H. Stewart, but rather urban legend. Spellberg et al. Inf Dis Pov 2013.)

Some suggested areas

- Environmental microbiome investigations
- Clinical microbiome investigation – population risk assessments and comparisons or molecular surveillance
- TB-diagnostics including detection of resistance genes
- Culture negative samples sent for metagenomic sequencing
- Diabetic wounds?
- Abdominal abscesses?
- Screening for resistance genes for infection control use?

Thanks for your attention!

Photographer: Dr A-M Örmälä-Odegrip