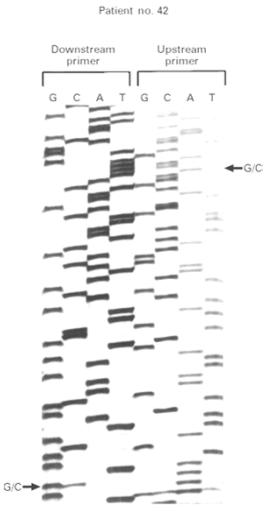
Community Reference Laboratory Antimicrobial Resistance

WHO Collaborating Centre for Antimicrobial Resistance in Foodborne Pathogens
www.antimicrobialresistance.dk/who

National Food Institute
Technical University of Denmark

DTU

Clinical metagenomics in urinary tract infections



Frank M. Aarestrup
DTU – Food
www.genomicepidemiology.org
www.compare-europe.eu

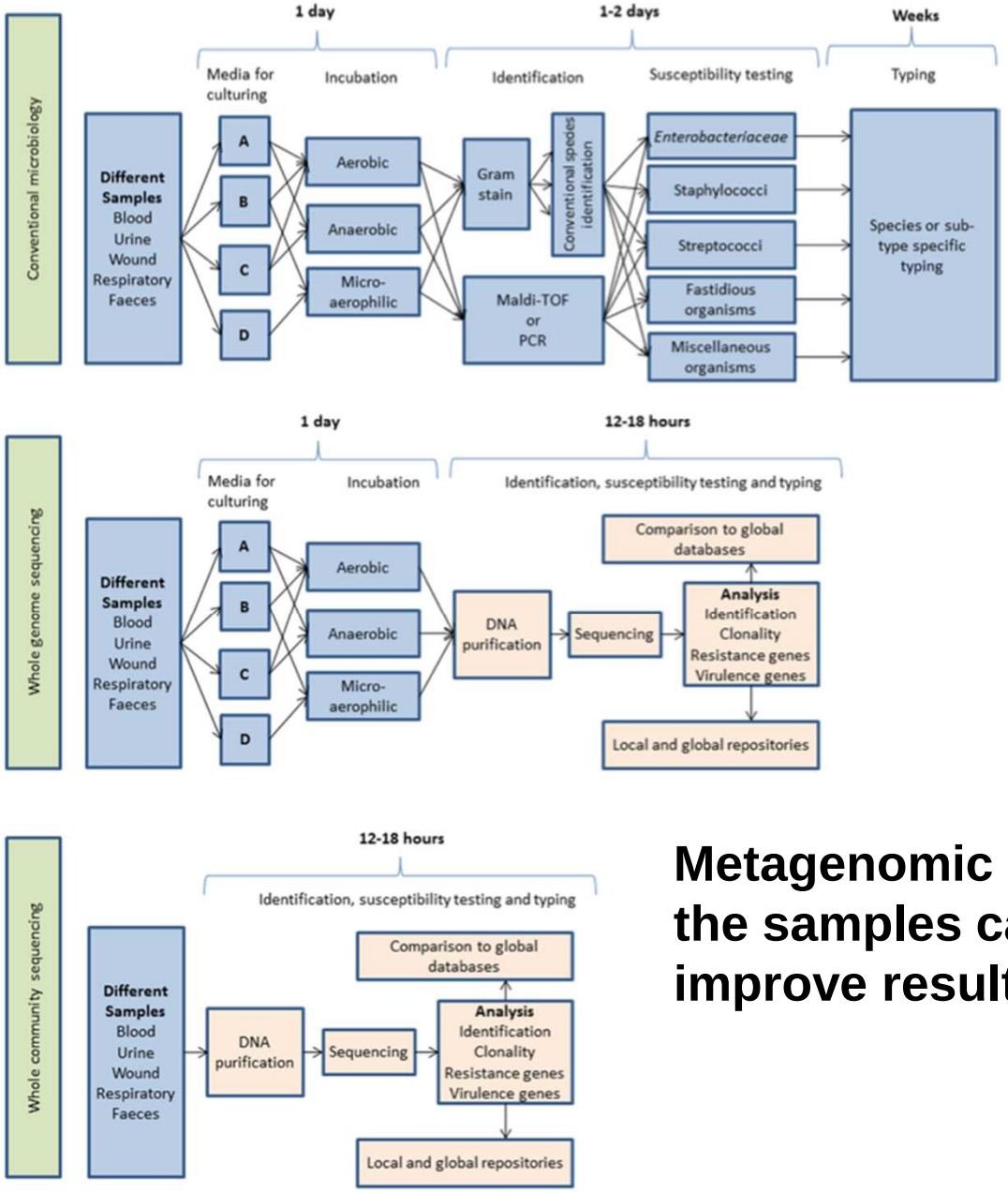


FIG 2 Schematic representation of the workflow anticipated after adoption of whole-genome sequencing used either on cultured isolates or directly on the clinical samples, with an expected time scale.

Metagenomic sequencing directly on the samples can reduce time and improve result

Rapid whole genome sequencing for the detection and characterization of microorganisms directly from clinical samples. Hasman H, Saputra D, Sicheritz-Ponten T, Lund O, Svendsen CA, Frimodt-Møller N, Aarestrup FM. J Clin Microbiol. 2014 Jan;52(1):139-46

JCM
JournalsASM.org

Rapid Whole-Genome Sequencing for Detection and Characterization of Microorganisms Directly from Clinical Samples

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Whole-genome sequencing (WGS) is becoming available as a routine tool for clinical microbiology. If applied directly on clinical samples, this could further reduce diagnostic times and thereby improve control and treatment. A major bottleneck is the availability of fast and reliable bioinformatic tools. This study was conducted to evaluate the applicability of WGS directly on clinical samples and to develop easy-to-use bioinformatic tools for the analysis of sequencing data. Thirty-five random urine samples from patients with suspected urinary tract infections were examined using conventional microbiology, WGS of isolated bacteria,

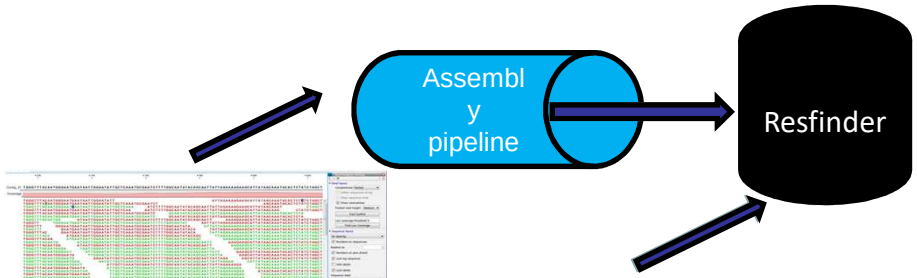
The study

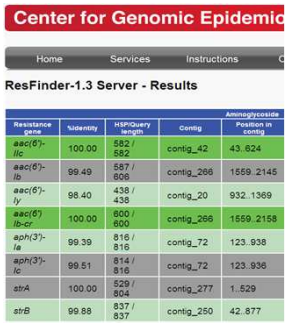
- 35 random urinary samples
- Routine culturing
  - All isolated bacteria WGS
- Direct sequencing
  - Reads mapped to databases

Results - ID

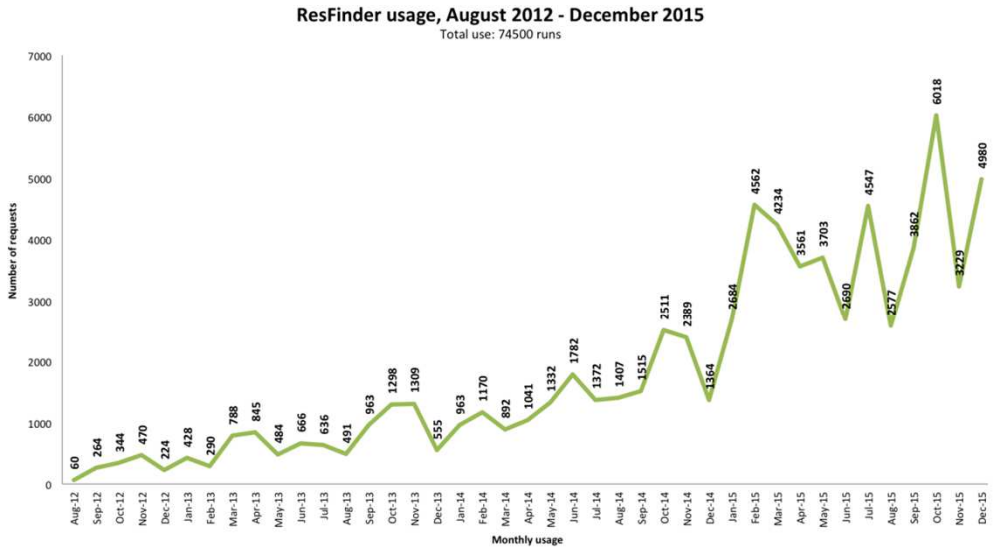
- Cultures from 19 (pure from 17)
  - WGS improved identification
- Metagenomics from 23
  - Four culture negative (G. vaginalis, L. iners, Prevotella, E. coli/E. faecalis)
  - Two mixed cultures (E. coli, E. coli/E. faecalis)

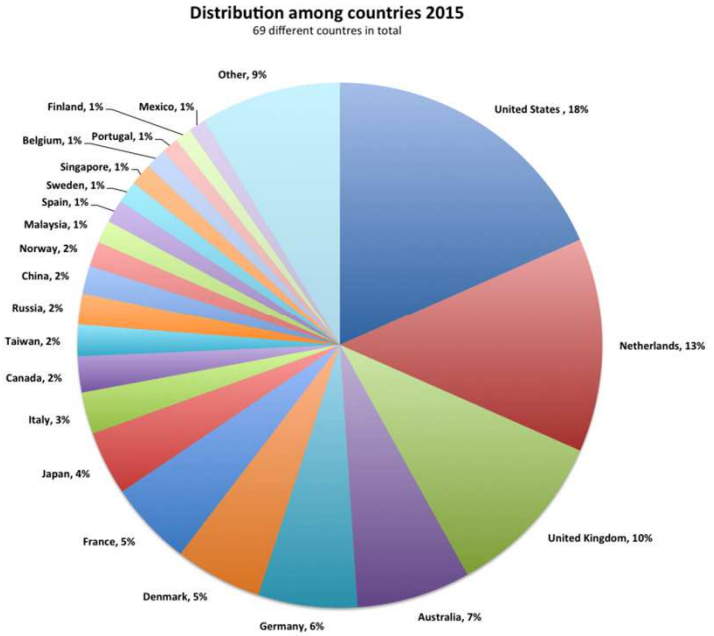
Online bioinformatic tool





Resistance gene	%Identity	HSP/Query length	Contig	Aminoglycoside Position in contig
aac(6')-Ilc	100.00	582 / 582	contig_42	43..624
aac(6')-Ib	99.49	587 / 606	contig_266	1559..2145
aac(6')-Iy	98.40	438 / 438	contig_20	932..1369
aac(6')-Ib-cr	100.00	600 / 600	contig_266	1559..2158
aph(3')-Ie	99.39	816 / 816	contig_72	123..938
aph(3')-Ic	99.51	814 / 816	contig_72	123..936
strA	100.00	529 / 804	contig_277	1..529
strB	99.88	837 / 837	contig_250	42..877





	Phenotypic	
	Resistant	Susceptible
Predicted resistant	475	7
Predicted susceptible	16	2553

99.2% concordance

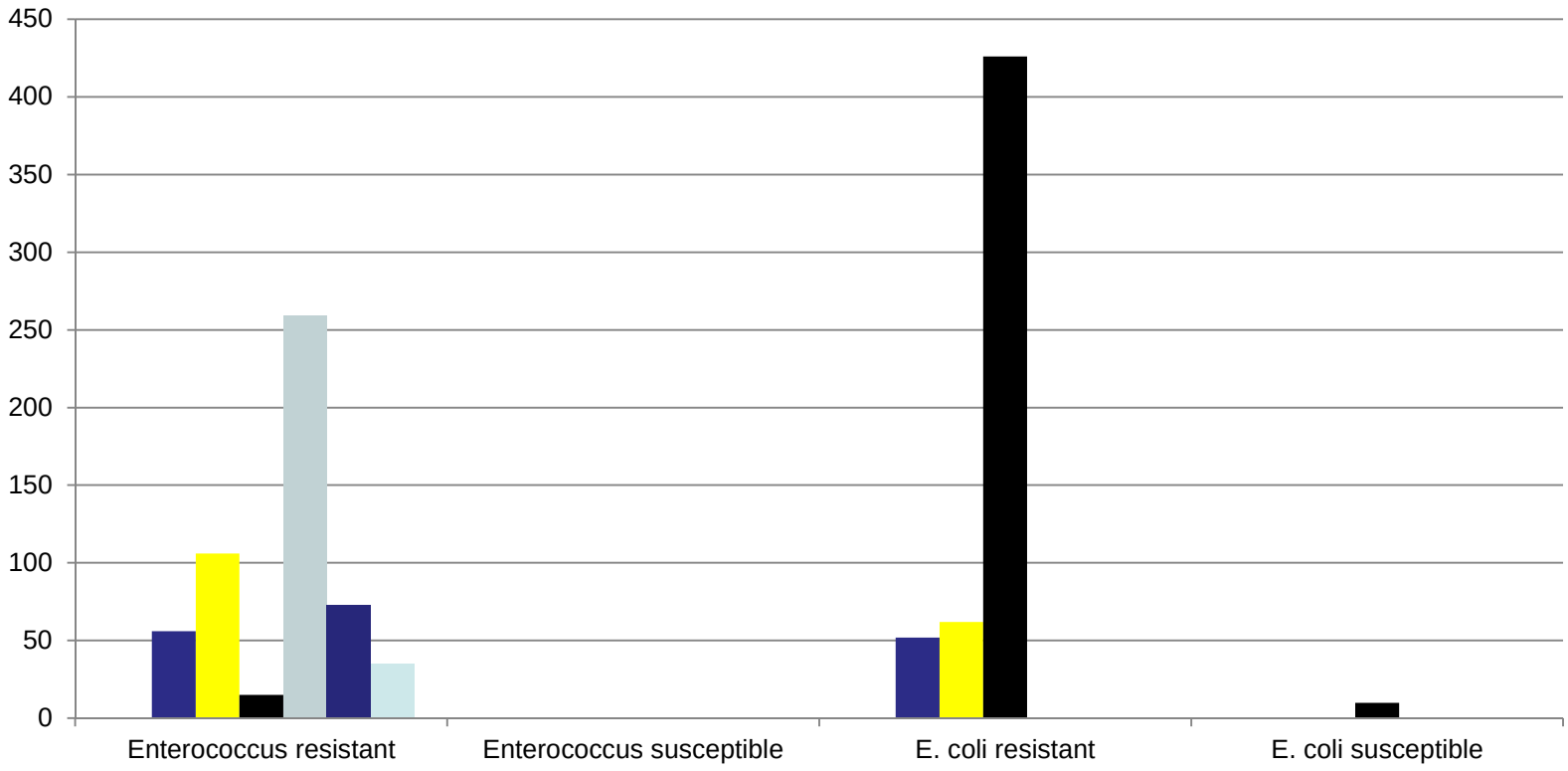
retest

	Phenotypic	
	Resistant	Susceptible
Resistant	475	7
Susceptible	0	2569

99.8% concordance

Spectinomycin in *E. coli*

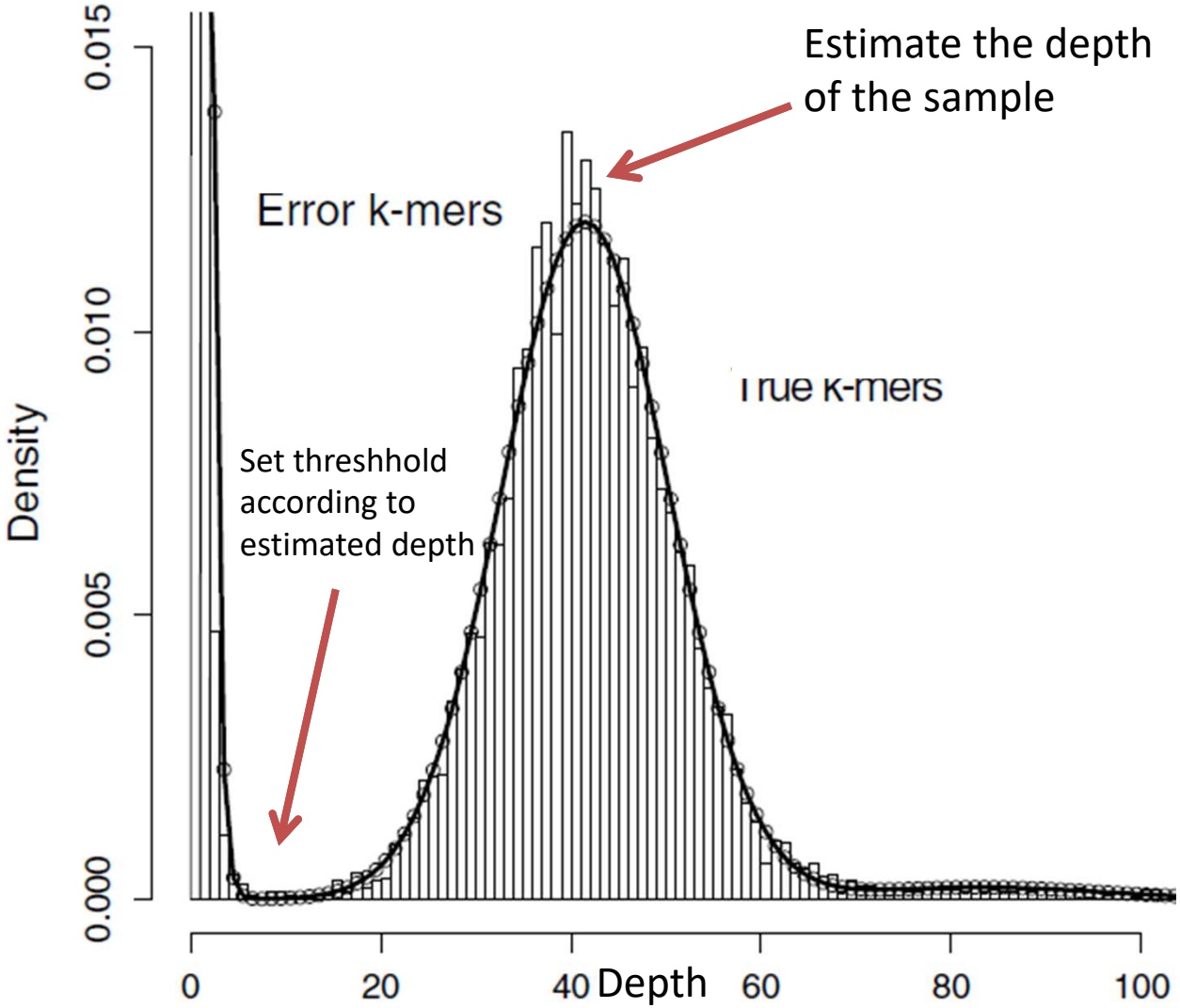
Number of reads mapping to
tetracycline resistant and tetracycline susceptible
Enterococci and E. coli



Agreement to resistance

Number	Species	Phenotypic resistance	WGS predicted	Metagenomic predicted
10	E. coli	S	S	ESBL
21	E. coli	AMP, CIP, GEN, NAL	AMP, GEN, TET	AMP, GEN, TET
27	E. coli	S	S	TET

Improving the methods
Bias the data



Test data

143 isolates from Oxford University Hospital, comprising 858 phenotypic susceptibility tests, most on beta-lactams.

193 isolates from Danish pig farms, comprising 2,547 phenotypic susceptibility tests, covering a broad spectrum of antibiotic classes.

Sequence quality and resistance determination

	SRST2	ResFinder	Kmer
SE	95	94	97
SP	96	96	97
	Down sampled		
SE	29	17	95
SP	98	98	97

Metagenomic assignment metamerfinder

$$P(T < t_i) = 1 - e^{-\lambda_i t_i} \quad , \quad \lambda = \frac{1}{\mu_i} \quad \wedge \quad i \in (Depth;\ Coverage)$$

Equation 2; The exponential survival function, μ: background expectation, t: measured quality

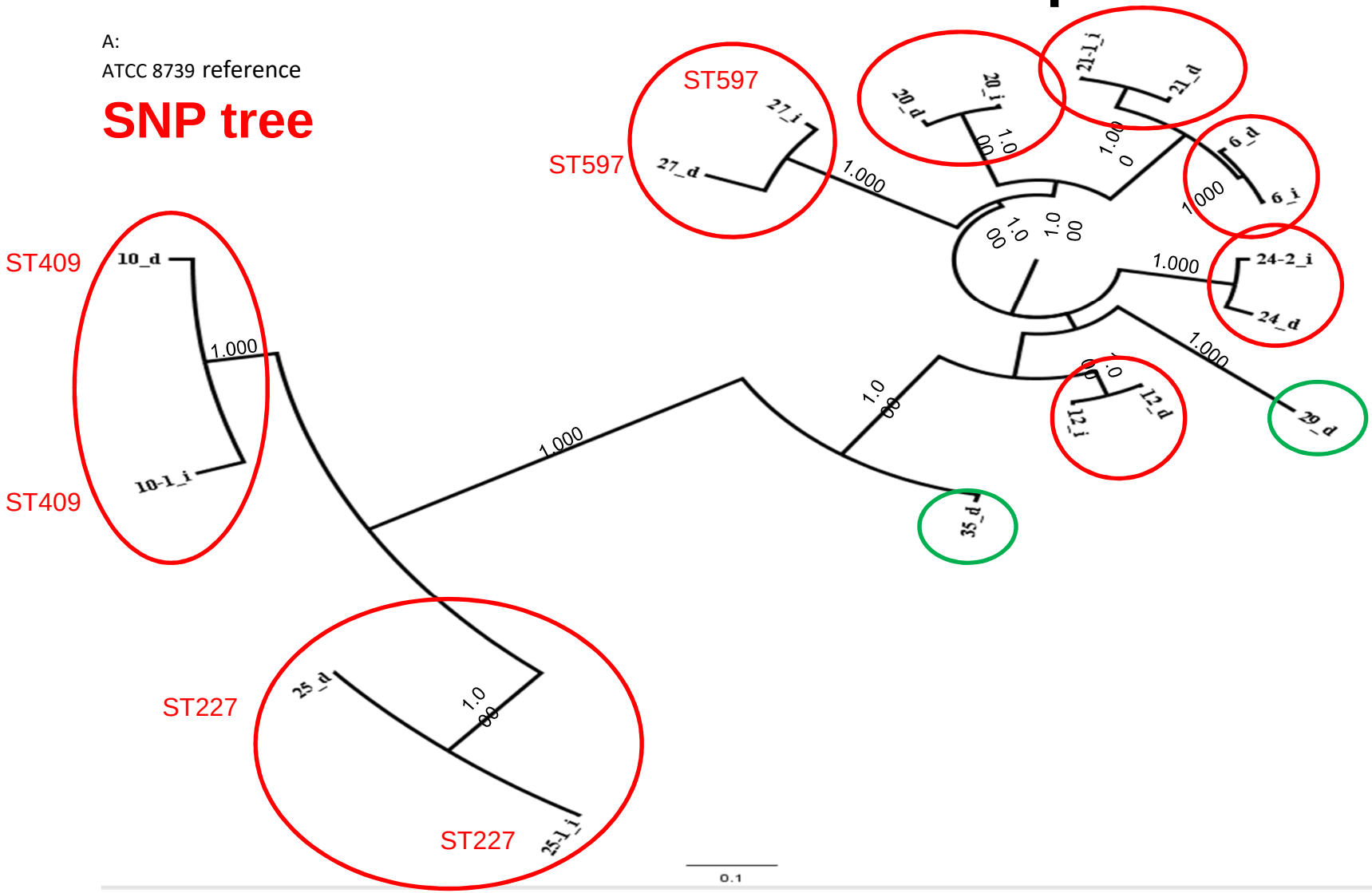
Agreement to resistance

No	Species	Phenotypic resistance	WGS predicted	Metagenomic predicted	Metamerfinder
3	E. faecalis	TET	TET	TET	E. faecalis (TET) E. coli (S) L. crispatus (ERY)
10	E. coli	S	S	ESBL	ESBL
21	E. coli	AMP, CIP, GEN, NAL	AMP, GEN, TET	AMP, GEN, TET	AMP, GEN, TET
27	E. coli	S	S	TET	TET, SUL, AMP, STR, TMP

E. coli in Urine samples

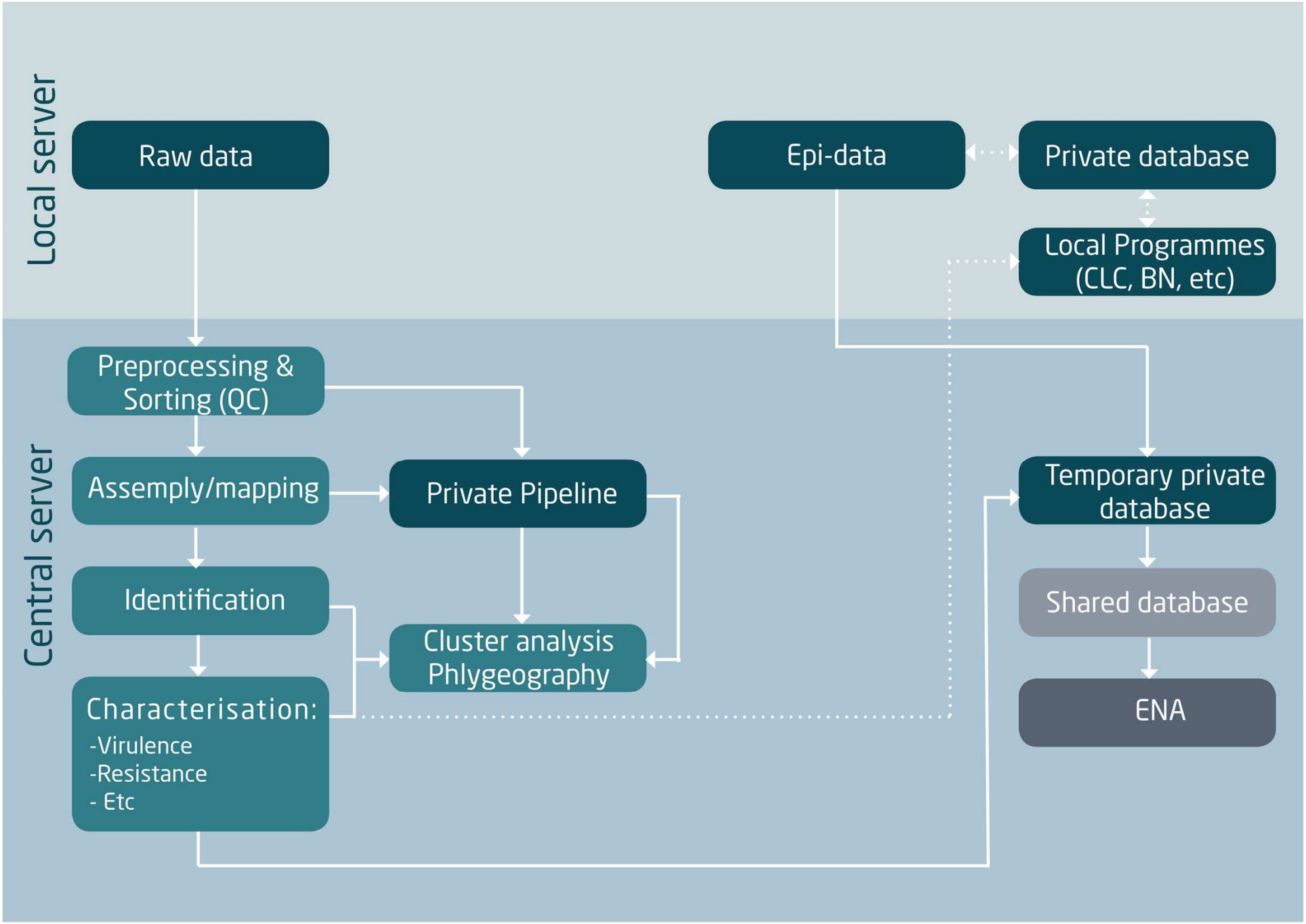
A:
ATCC 8739 reference

SNP tree



_d = Direct sequencing on urine

_i = sequencing of isolate from urine


Local server
Raw data
Epi-data
Private database
Local Programmes (CLC, BN, etc)
Central server
Preprocessing & Sorting (QC)
Assemply/mapping
Private Pipeline
Identification
Cluster analysis Phlygeography
Characterisation:
-Virulence
-Resistance
- Etc
Temporary private database
Shared database
ENA

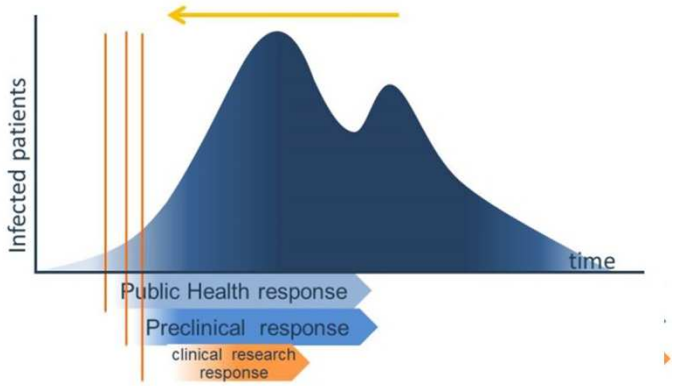
Why a central public repository?

Besides the language and altruistic issues

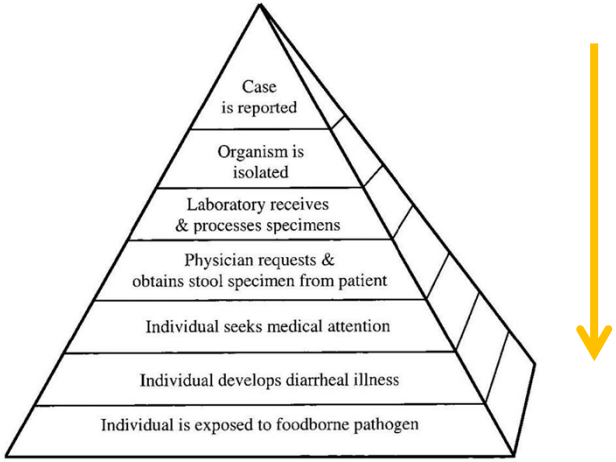
- The data comparison problem
- Allowing easy transfer between levels of access including public
- Allow access to bioinformatics for the frontline and frontline data for bioinformaticians
- Allowing for constantly improving the analytic pipelines

Establishing and improving surveillance

- Rapid sharing
  - Online bioinformatic tools
    - >1,000 jobs per day
  - Facilities for rapid sharing



- Natural reservoirs
  - Major issues with individual samples

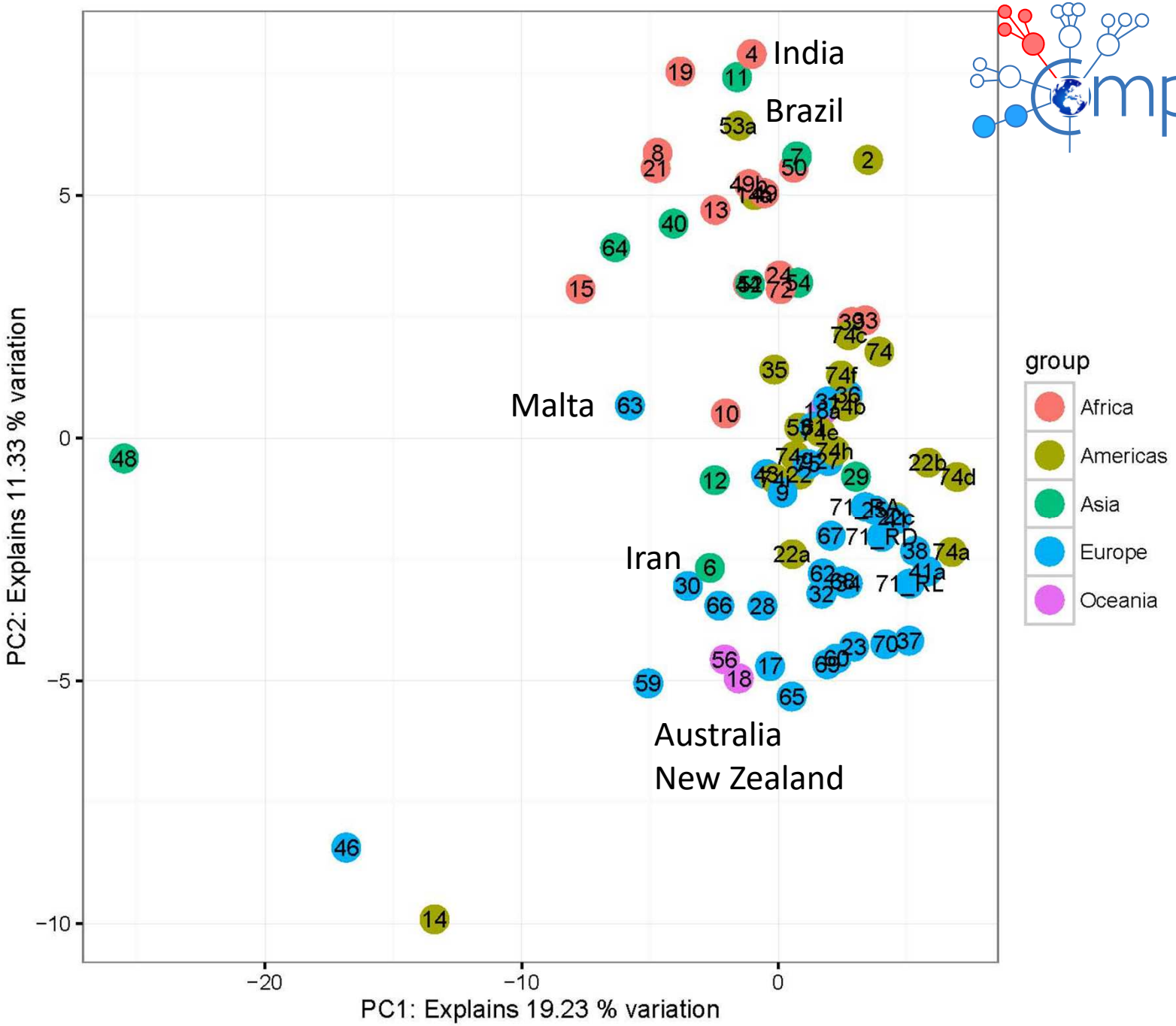


Convention on Biological Diversity

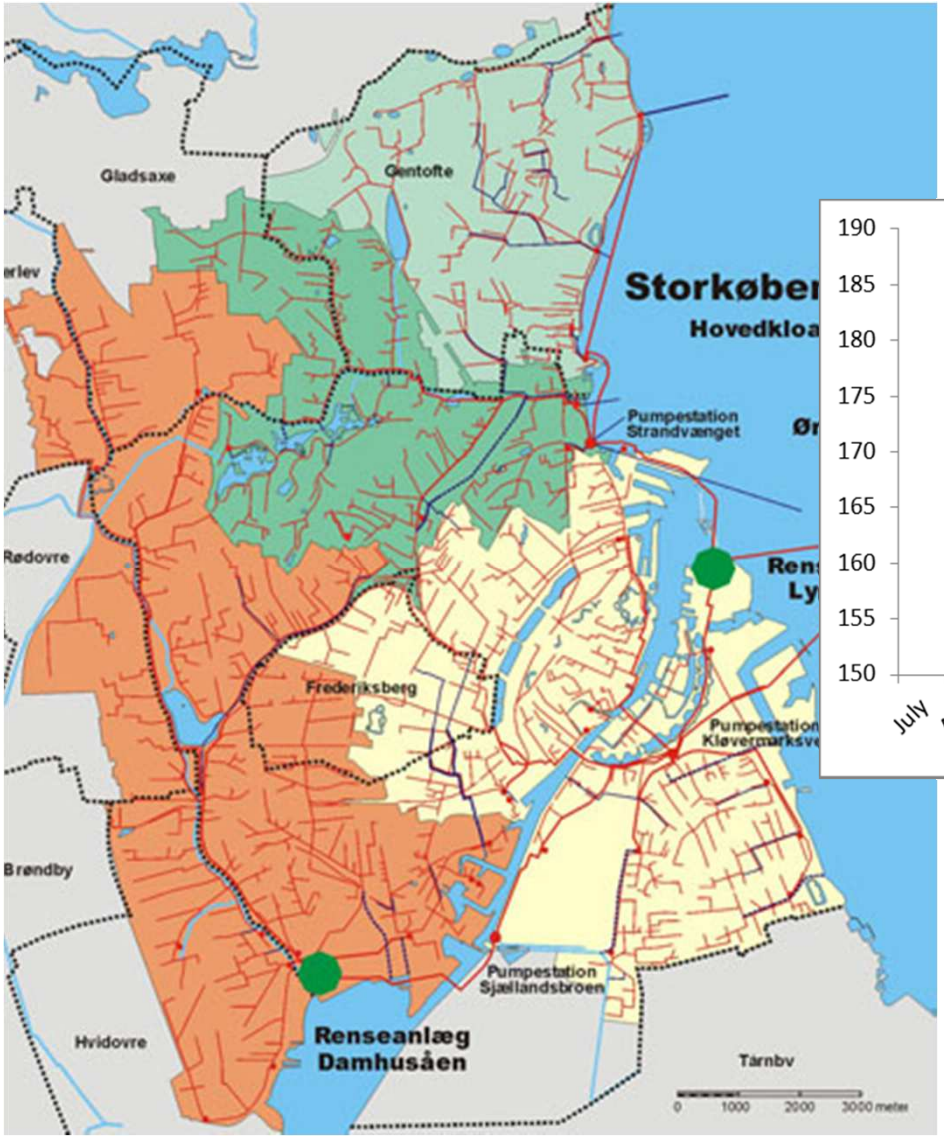
National ethical committees

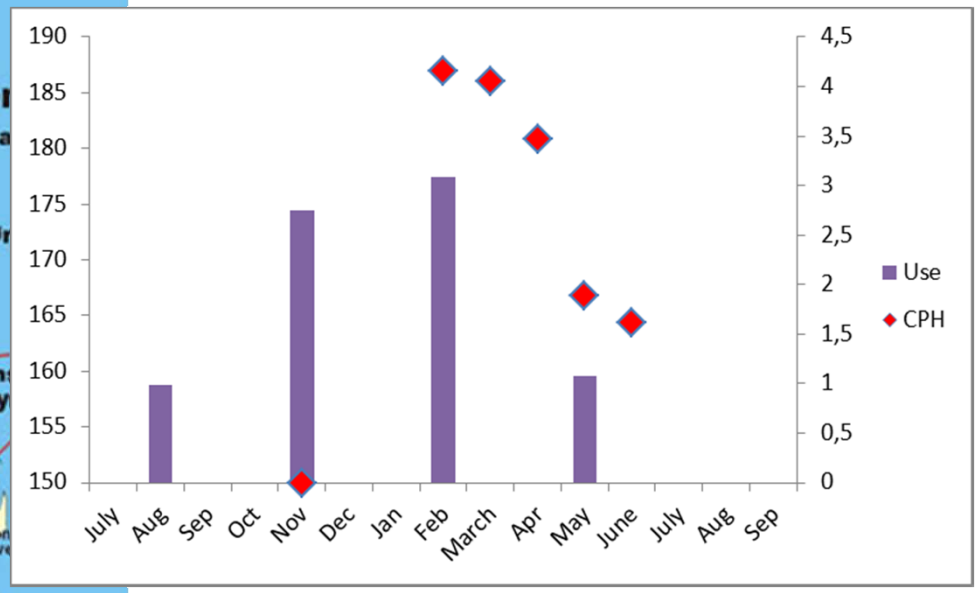
The Rio Convention
The Nagoya Protocol

Glo


India
Brazil
Malta
Iran
Australia
New Zealand
PC2: Explains 11.33 % variation
PC1: Explains 19.23 % variation
5
0
−5
−10
−20
−10
0
group
Africa
Americas
Asia
Europe
Oceania

Copenhagen according to sewage

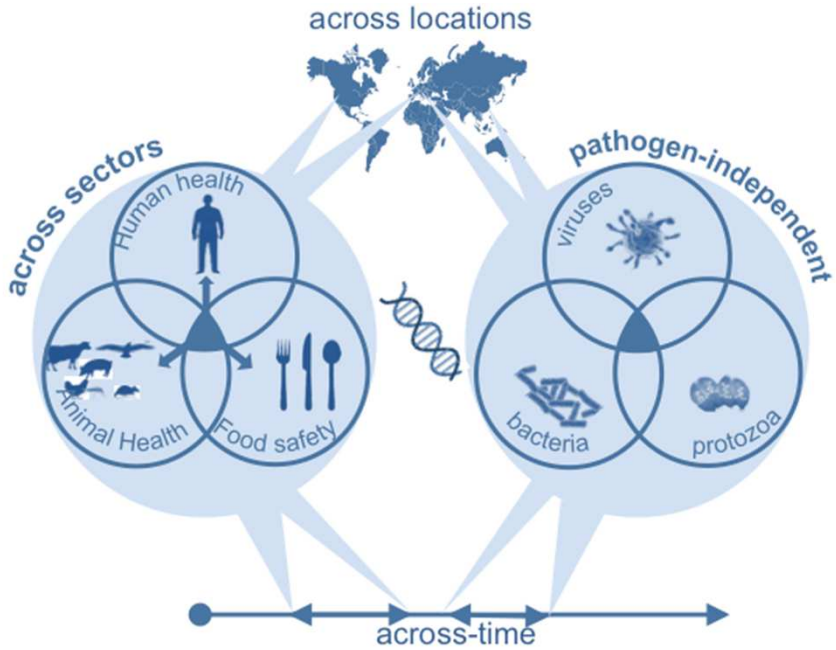




Very preliminary data

Drug use the previous year

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) (bring the tools to the data)

There can be no real-time detection & surveillance without real-time data sharing