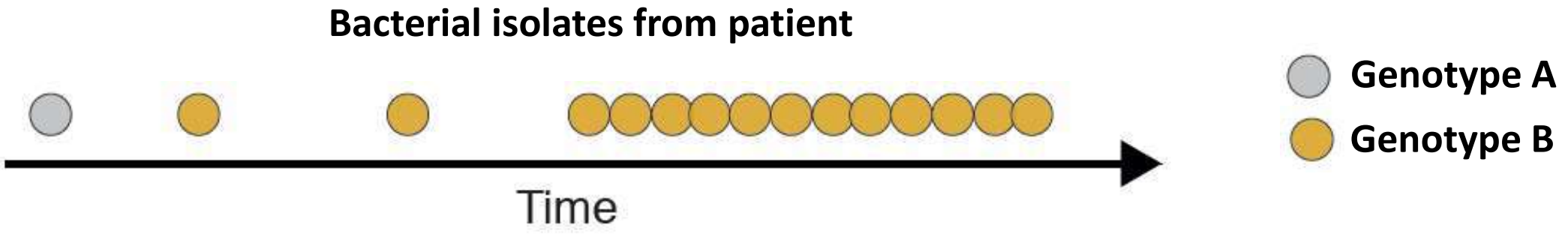
Genotyping offers increased resolution into strain dynamics and helps to determine if patient is chronically infected

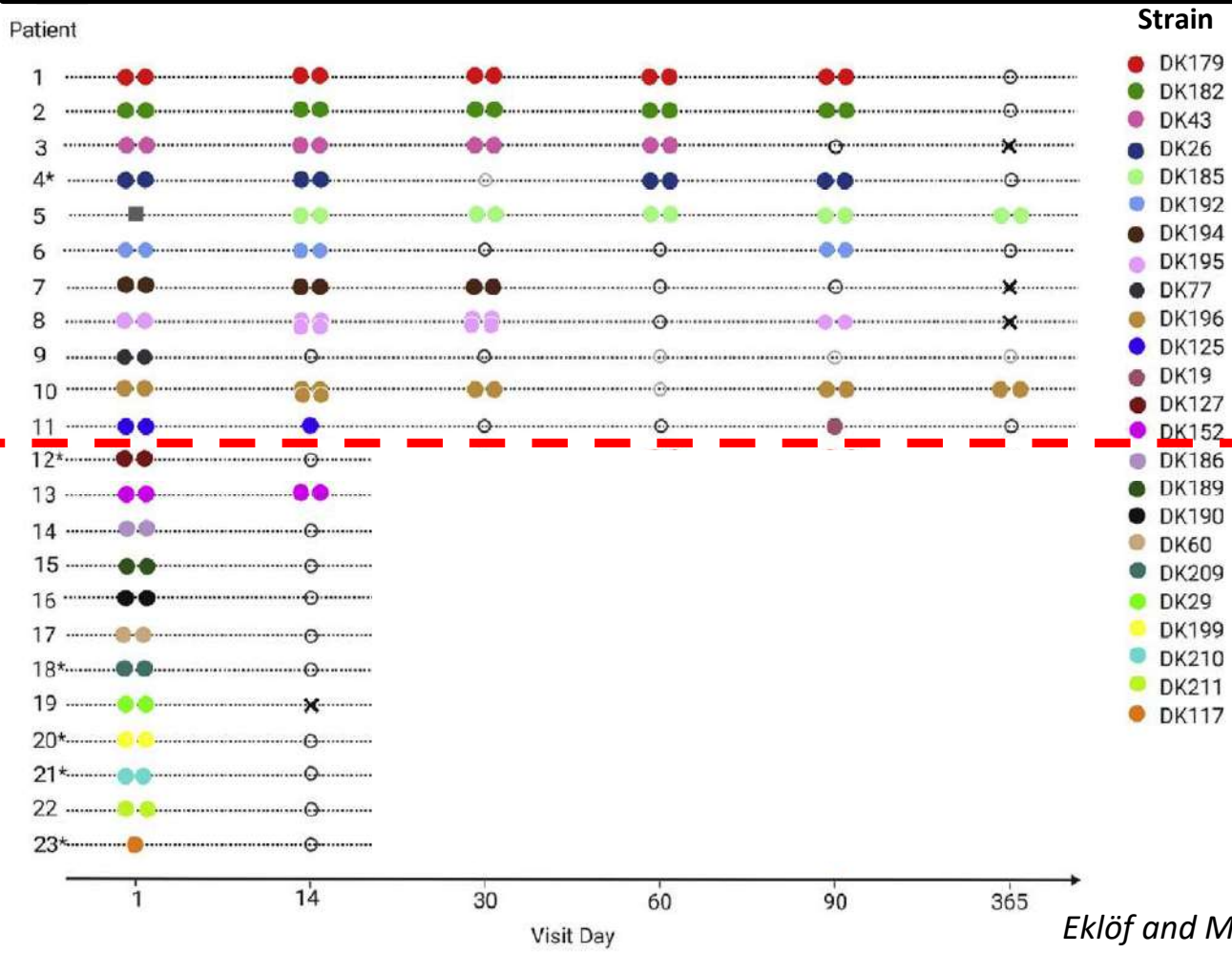


Intermittent infection — Chronic infection — Without genotype information

Intermittent infection — Chronic infection — With genotype information

Marvig, Nat Genet, 2015; Bartell, ERJ, 2021

Recurrence of *P. aeruginosa* in COPD patients is caused by persistence of the same strain – negative culture is false proxy for eradication

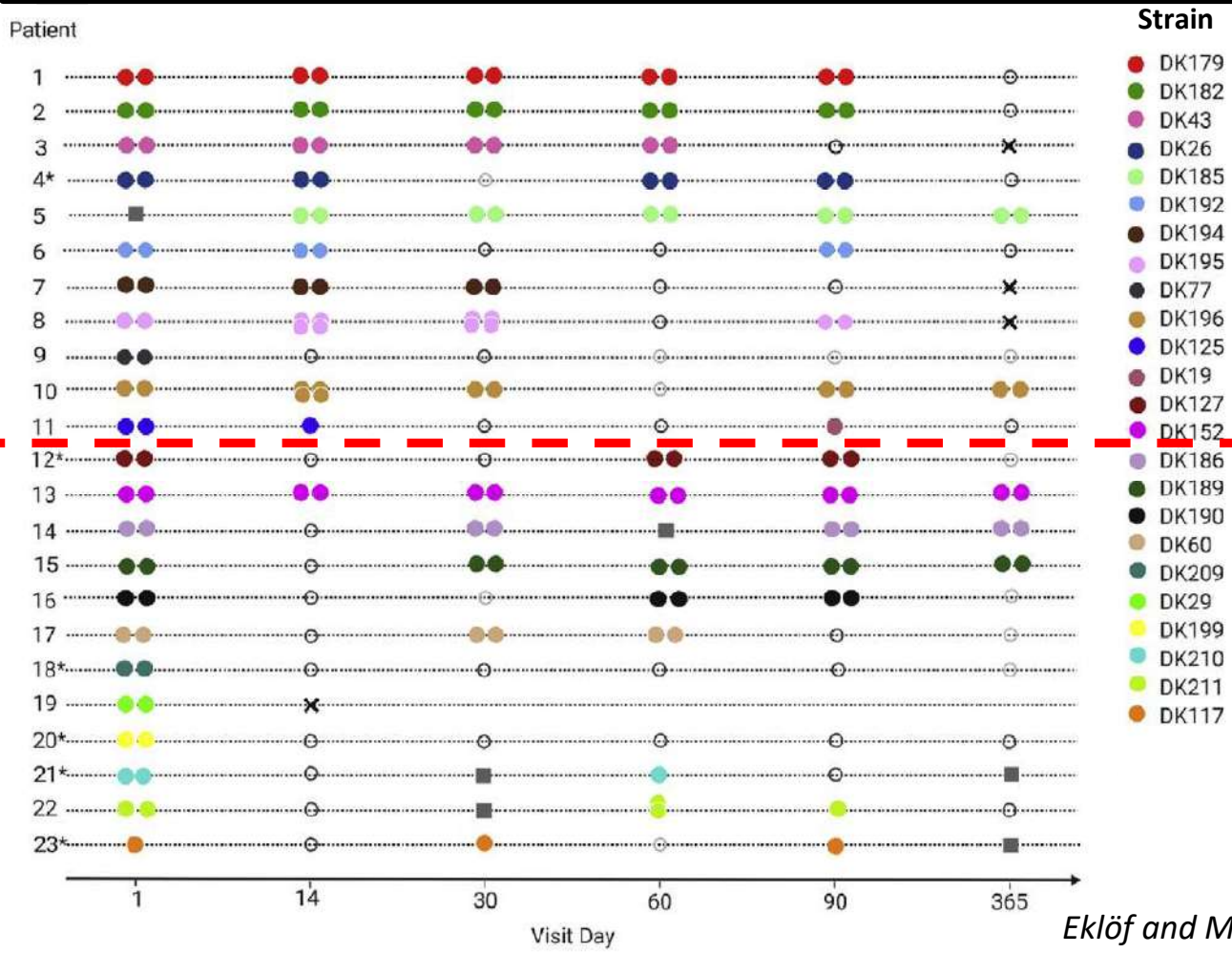


RCT control group:
No antipseudomonal treatment

RCT treatment group:
Antipseudomonal antibiotic treatment (intravenous b-lactam and oral ciprofloxacin) between day 1 and 14

Eklöf and Misiakou et al., Clinical Microbiology and Infection, 2022

Recurrence of *P. aeruginosa* in COPD patients is caused by persistence of the same strain – negative culture is false proxy for eradication



RCT control group:
No antipseudomonal treatment

RCT treatment group:
Antipseudomonal antibiotic treatment (intravenous b-lactam and oral ciprofloxacin) between day 1 and 14

Eklöf and Misiakou et al., Clinical Microbiology and Infection, 2022

Histogram of pairwise SNP distances between 446 P. aeruginosa isolates in the core genome: Red dotted line is chosen threshold for typing

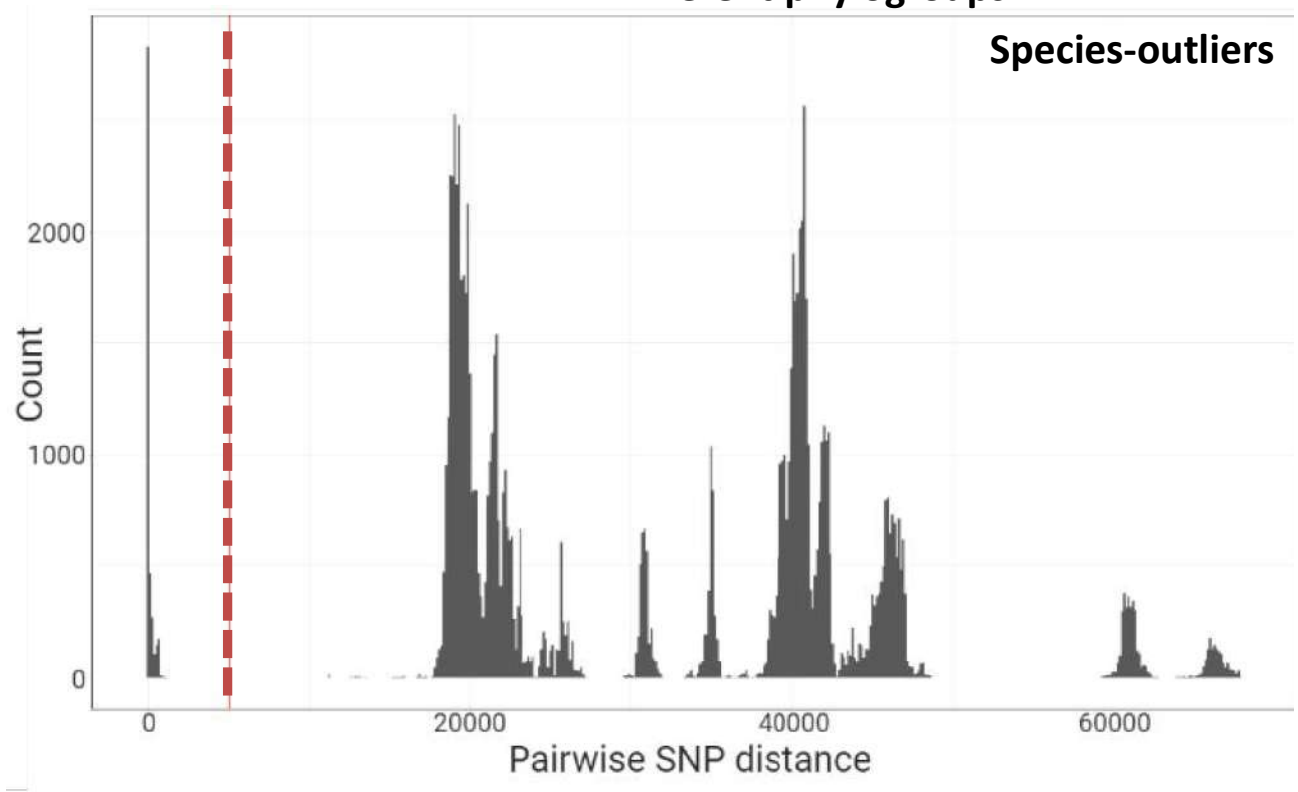
Sub-species typing

Same strain

Different type

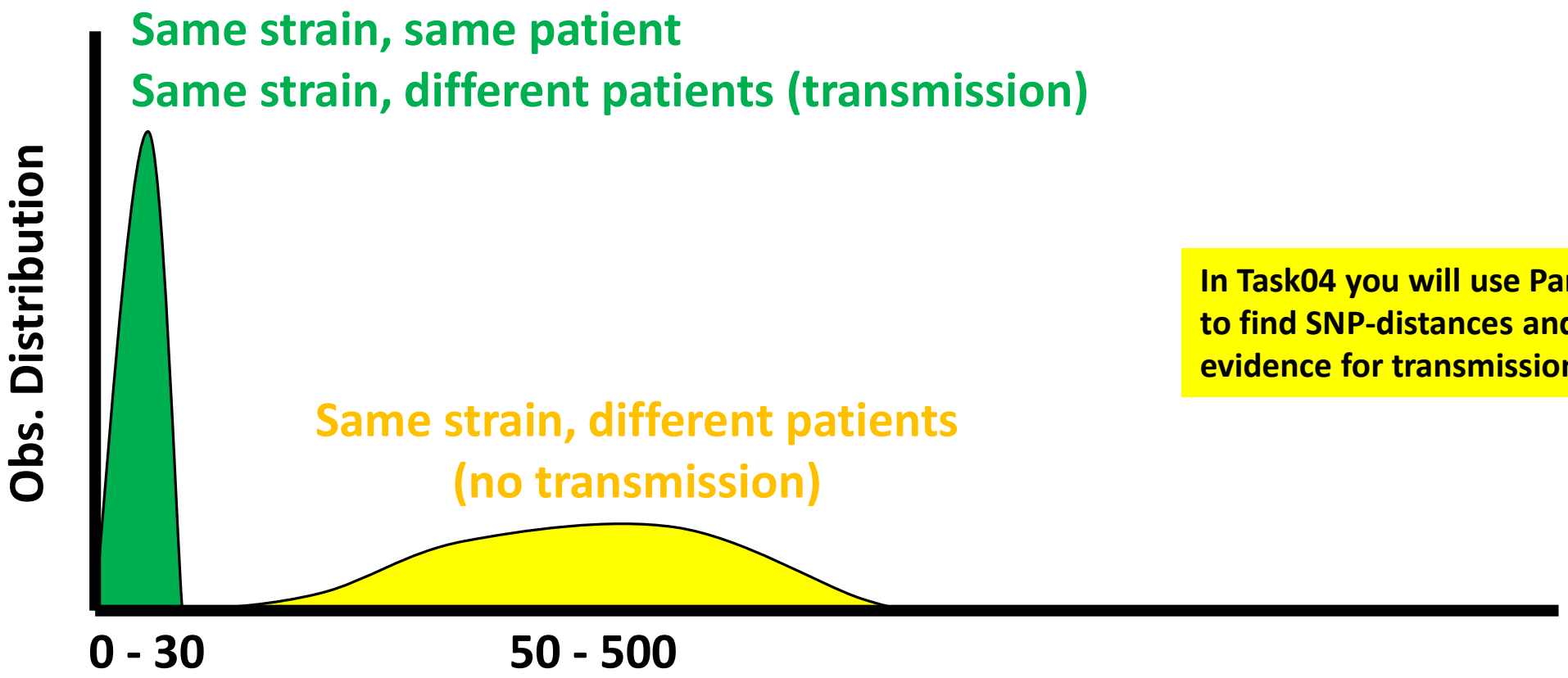
Different phylogroups

Species-outliers



Gabrielaite, mBio, 2020

Genetic distances (single nucleotide variants) between genomes of the same strain (clone type): Schematic example for *P. aeruginosa*



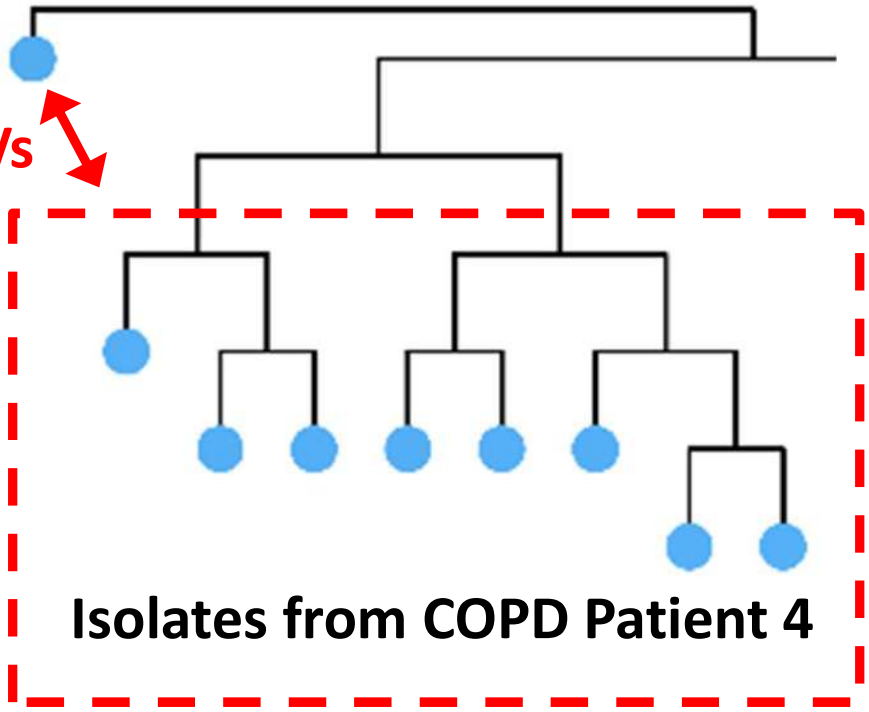
In Task04 you will use Parsnp to find SNP-distances and find evidence for transmission

Incidental finding: Close genetic relationship between isolates of COPD Patient 4 and CF Patient P2805 suggests within-hospital transmission

Phylogenetic tree

Isolate from CF Patient P2805

Genetic distance: 4-7 SNVs

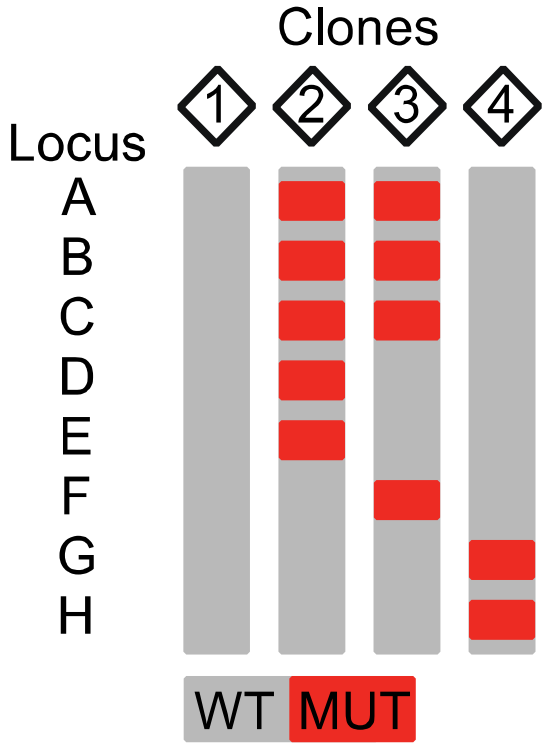


Both patients attended the same department at Rigshospitalet around the time of detection

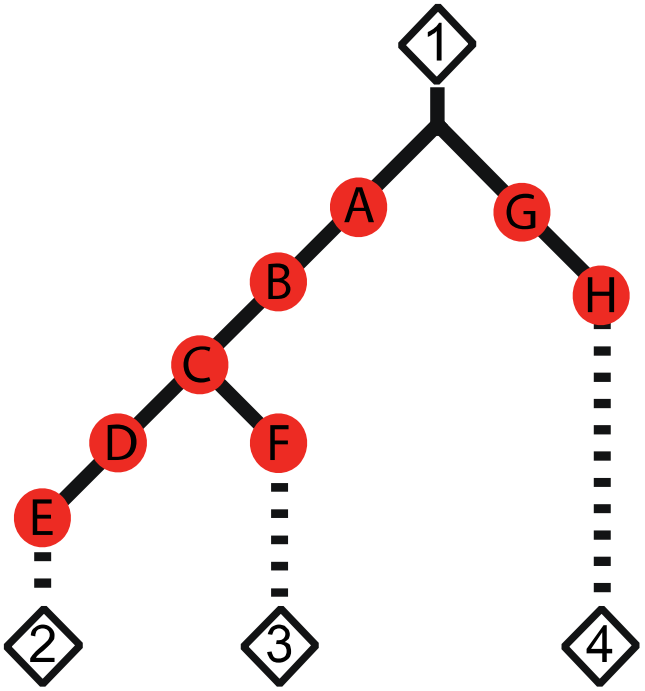
Eklöf and Misiakou et al., Clinical Microbiology and Infection, 2022

Phylogenomics: Inference of genetic relationship based on genomes

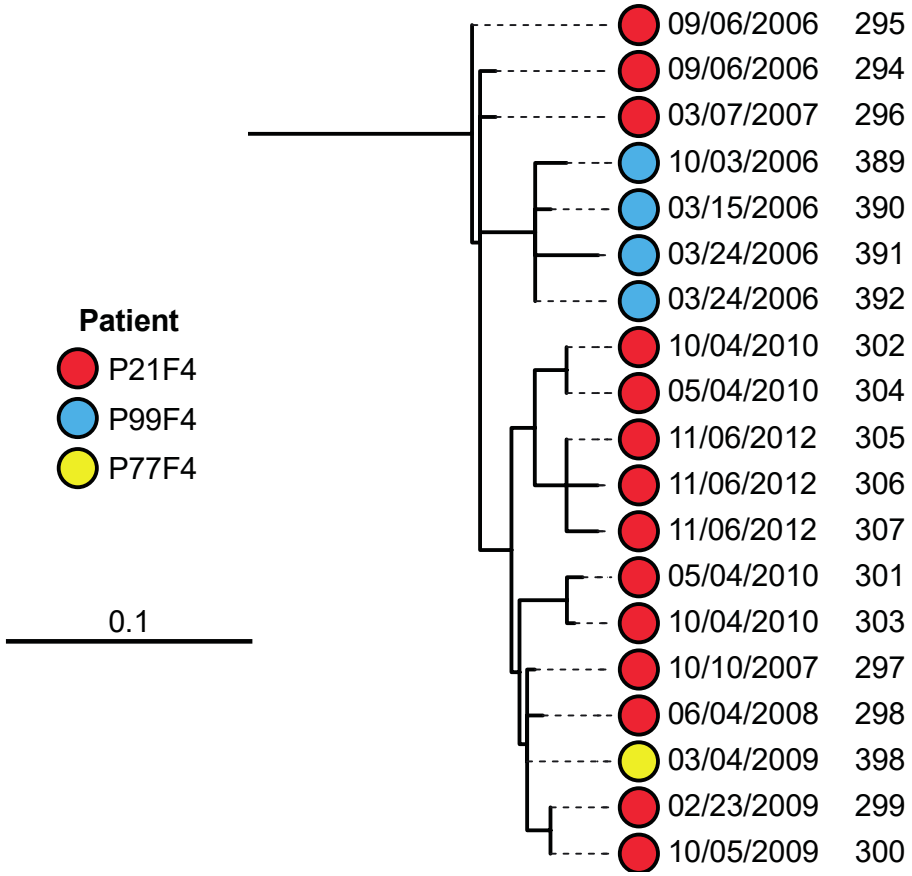
Whole genome sequencing



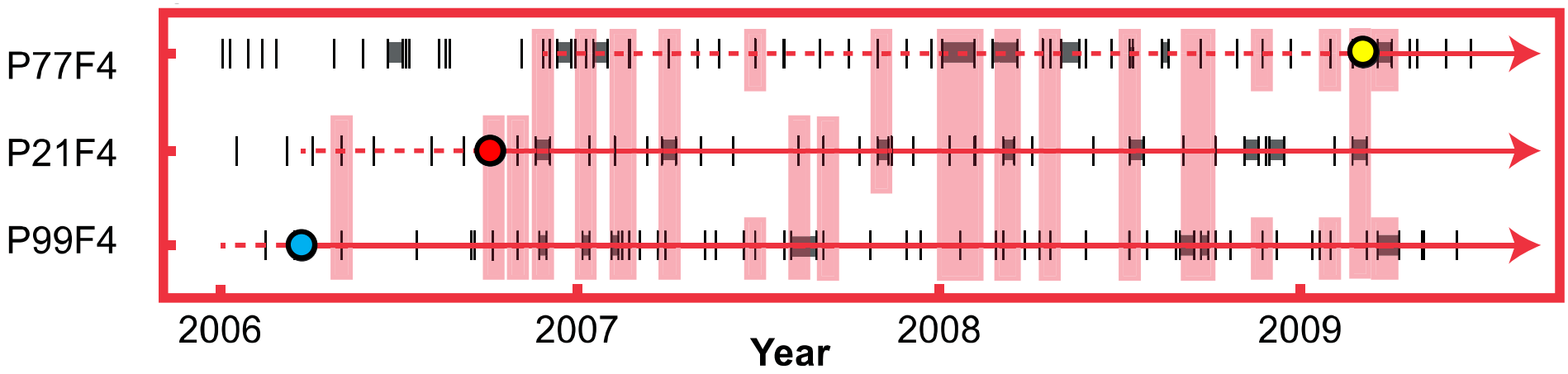
Genetic relationship



Phylogenomic analysis suggests transmission of *P. aeruginosa* between 7 of 34 cystic fibrosis patients: Example with <13 SNPs between patients



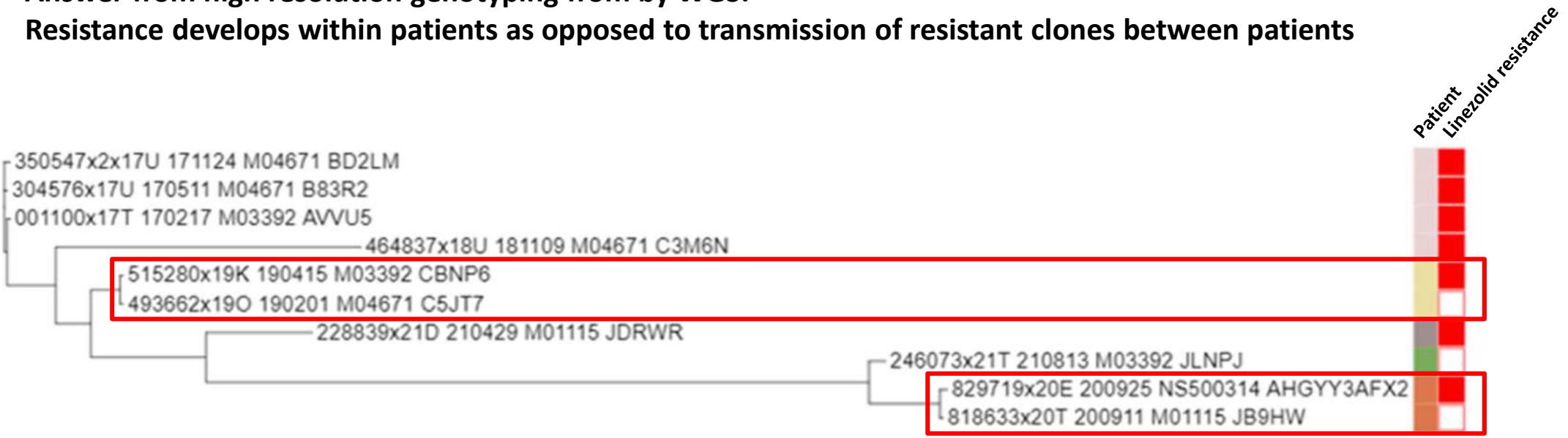
Temporal overlaps in the patient's hospital visits suggest that transmission took place at the hospital



Phylogenetic tree of clinical *E. faecium* isolates of type MLST ST-117

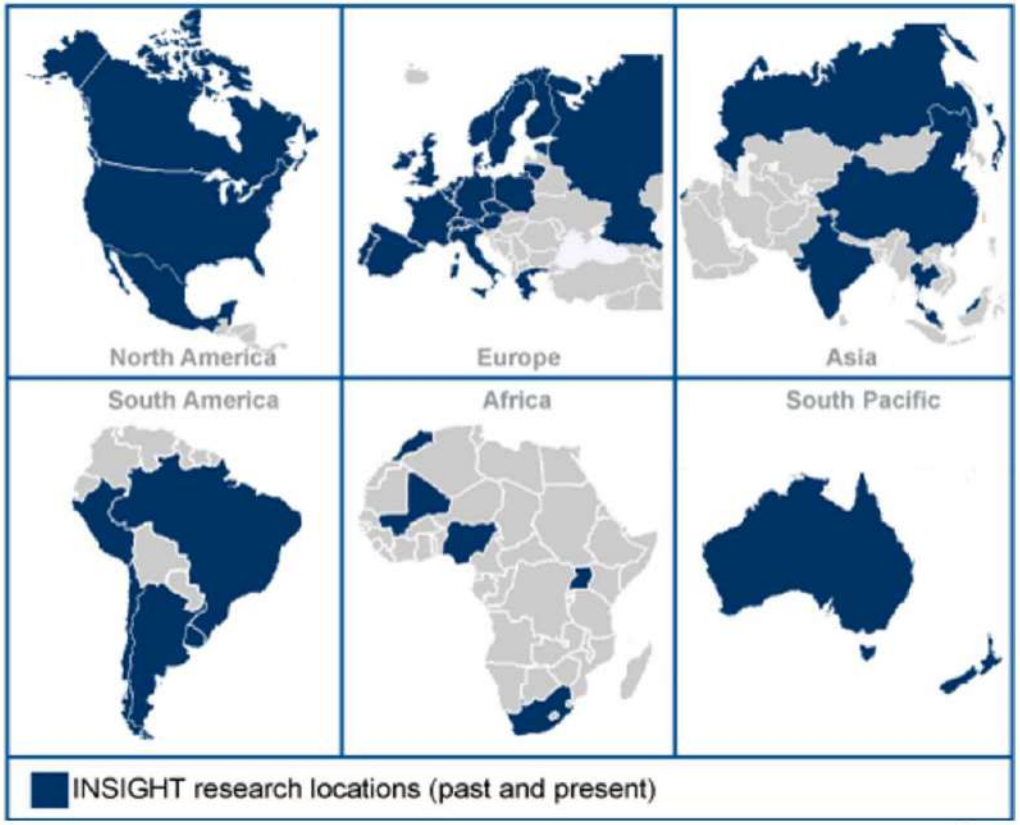
Clinical question: Linezolid resistant *E. faecium* – what it driving this ?

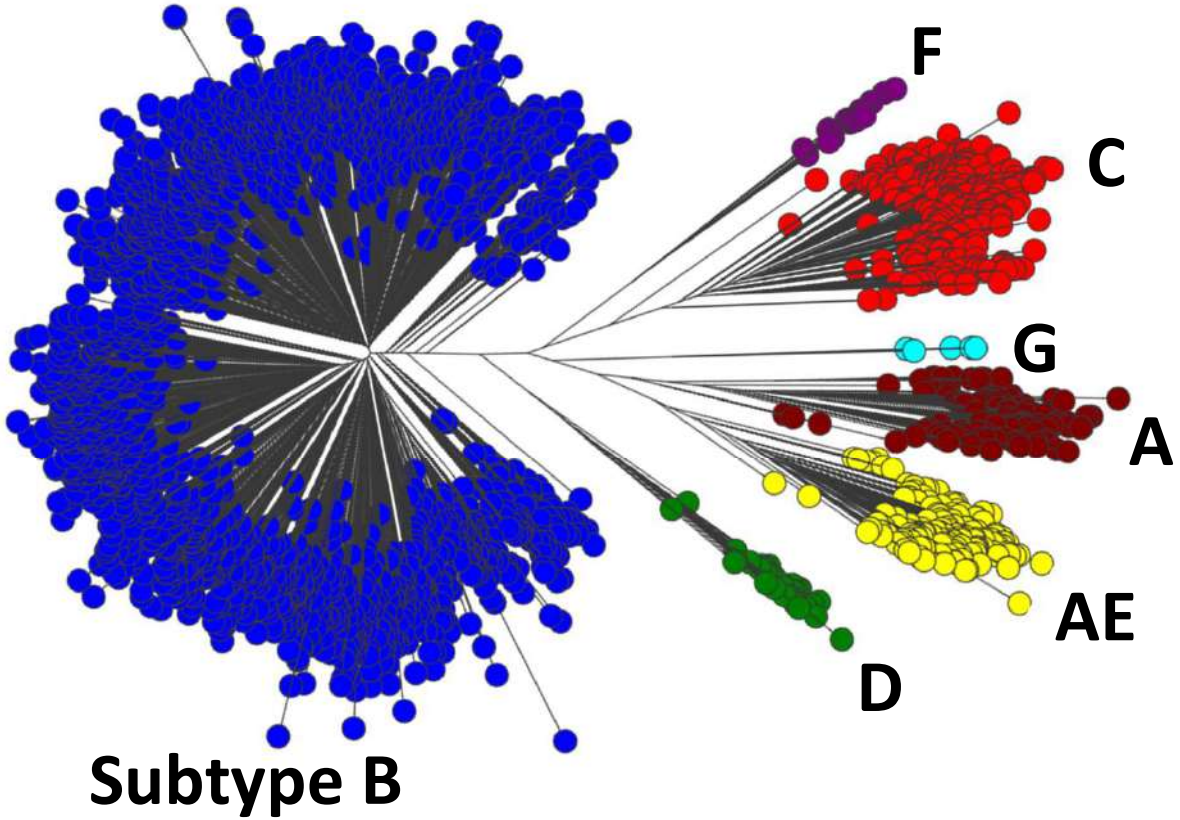
Answer from high resolution genotyping from by WGS:
Resistance develops within patients as opposed to transmission of resistant clones between patients



Unpublished data from Karen Leth Nielsen

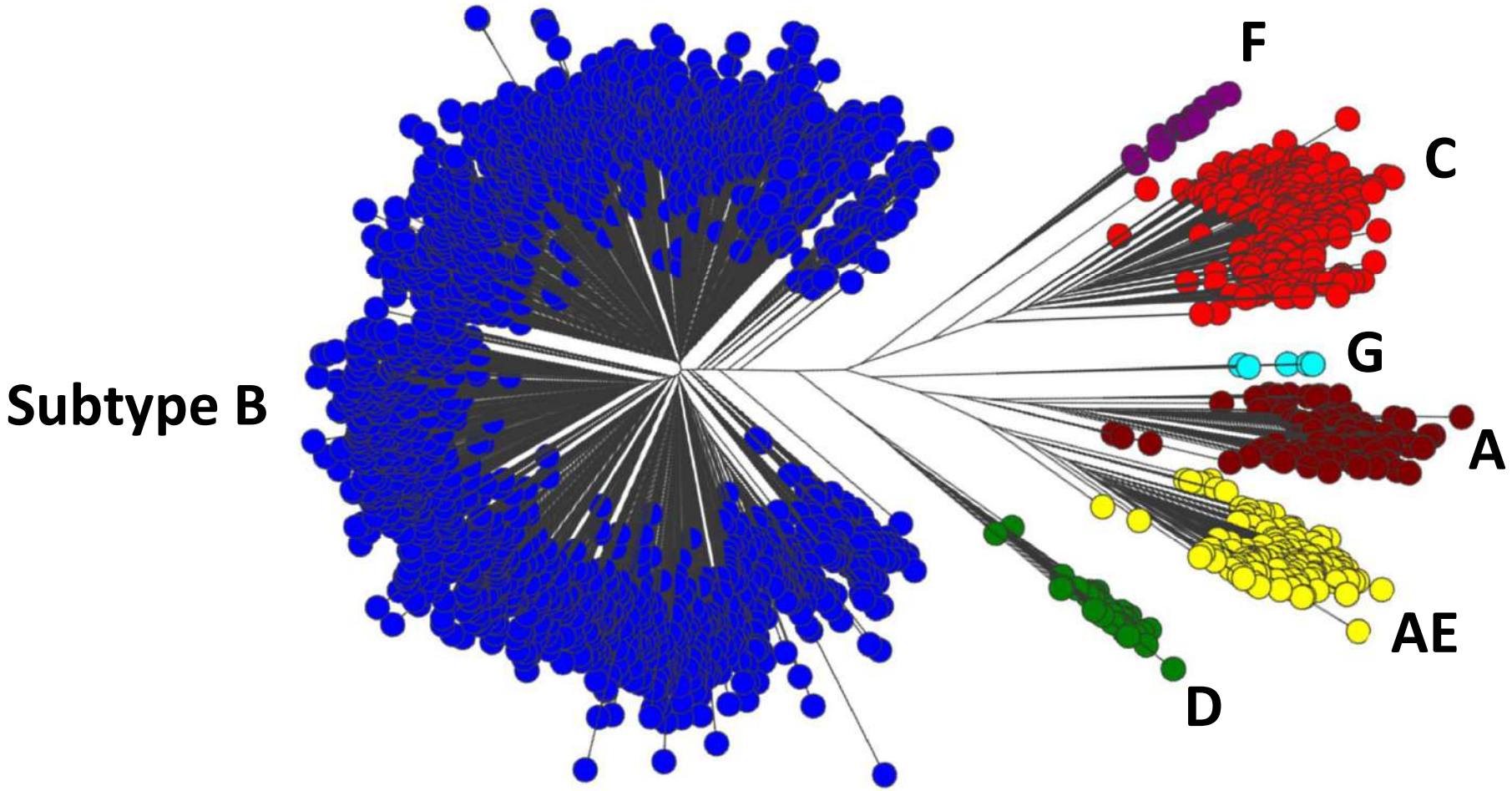
Phylogenetic tree of 2,501 HIV-1 genomes from START clinical trial
Samples have been collected in 2009-2013 from 35 countries



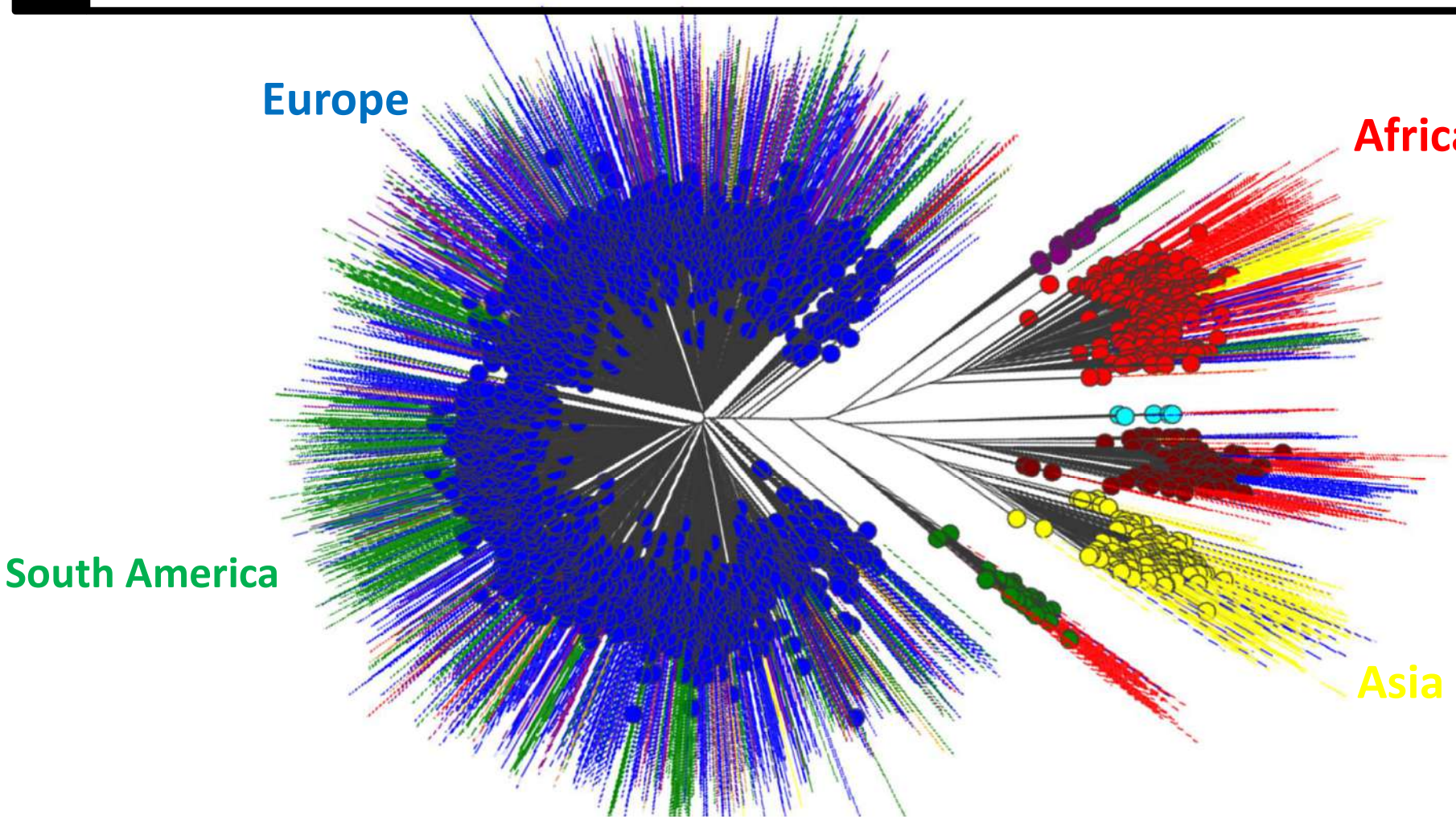


Bennedbæk, Virus Evolution, 2021

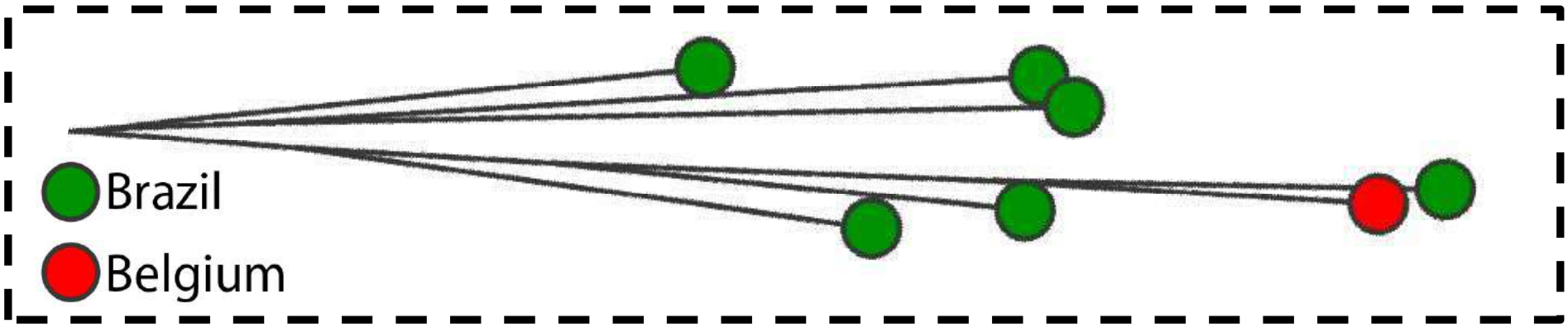
Phylogeny of 2,501 HIV genomes from START participants

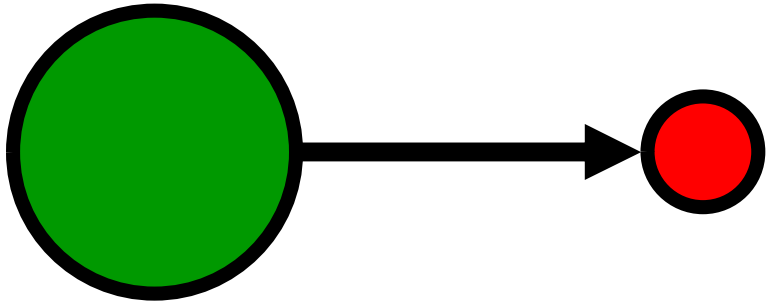


Demographic data (here region) can be added onto phylogeny
Ancestral state reconstruction can inform on origin and spread of virus



Ancestral state reconstruction can inform on origin and spread of virus: Infering clusters of transmission using PastML (pastml.pasteur.fr)

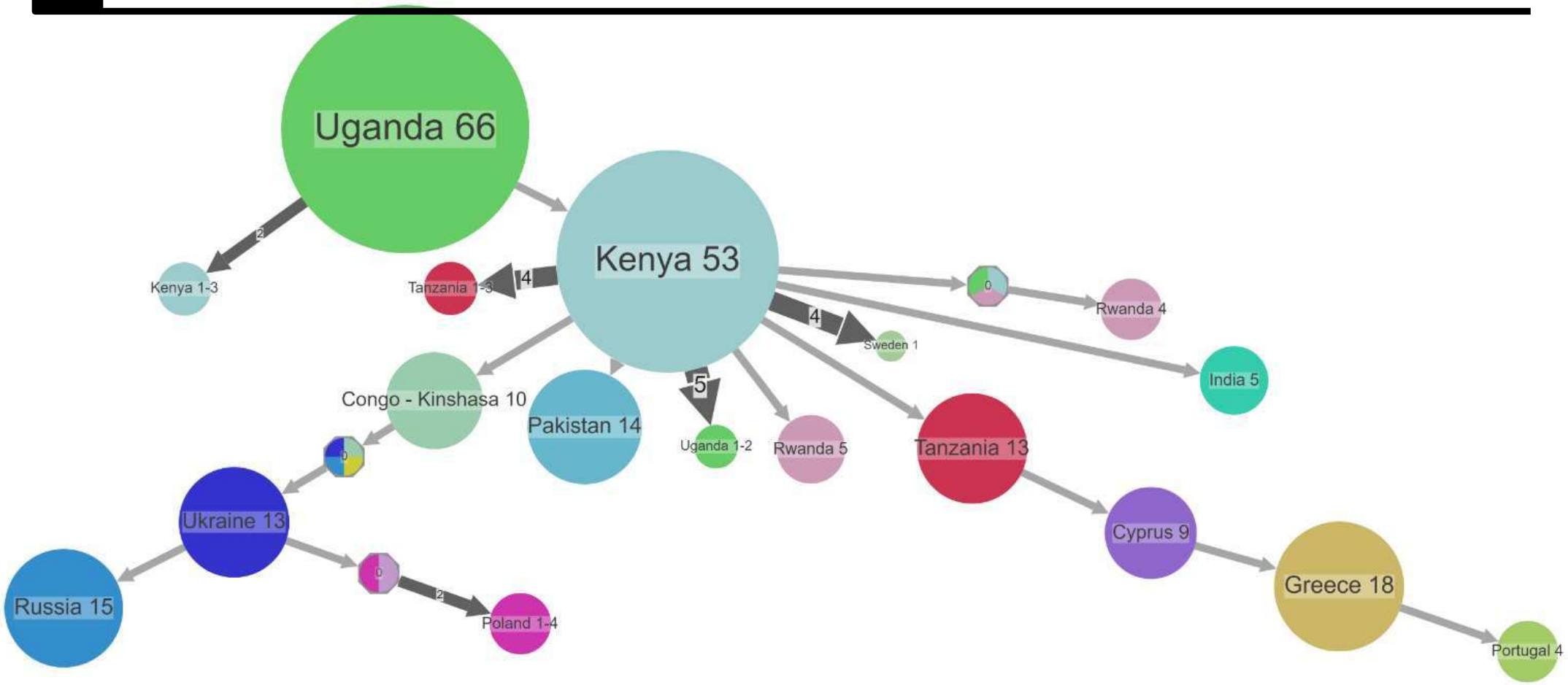




Brazil cluster
n=6

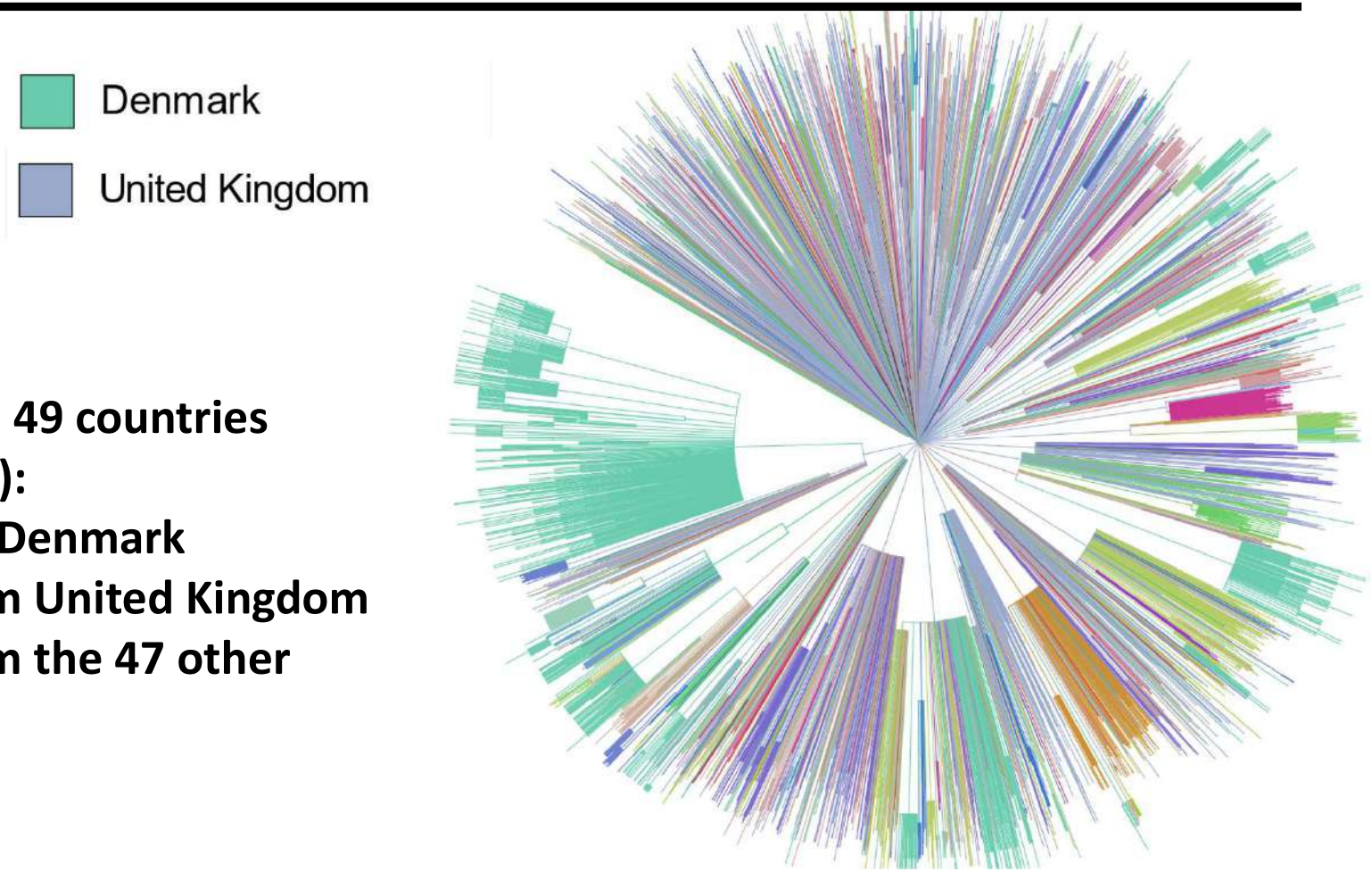
Belgium cluster
n=1

Subtype A samples (n=337) origin in Uganda and spread in Europe is via distinct country transmission chains with up to 6 links



Bennedbæk, Virus Evolution, 2021

Time-scaled maximum likelihood phylogenetic tree of B.1.1.7 (n=3,711) with tips colored by country

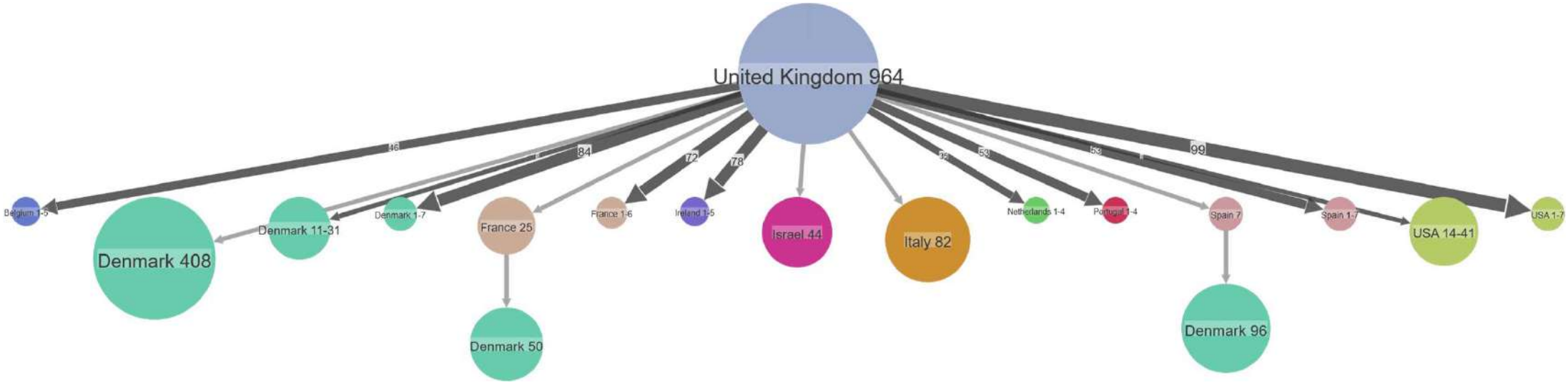


3,711 B.1.1.7 genomes from 49 countries (September 20 – January 25):

- **945 genomes from Denmark**
- **1,003 genomes from United Kingdom**
- **1,763 genomes from the 47 other countries**

Michaelsen, Genome Medicine, 2022

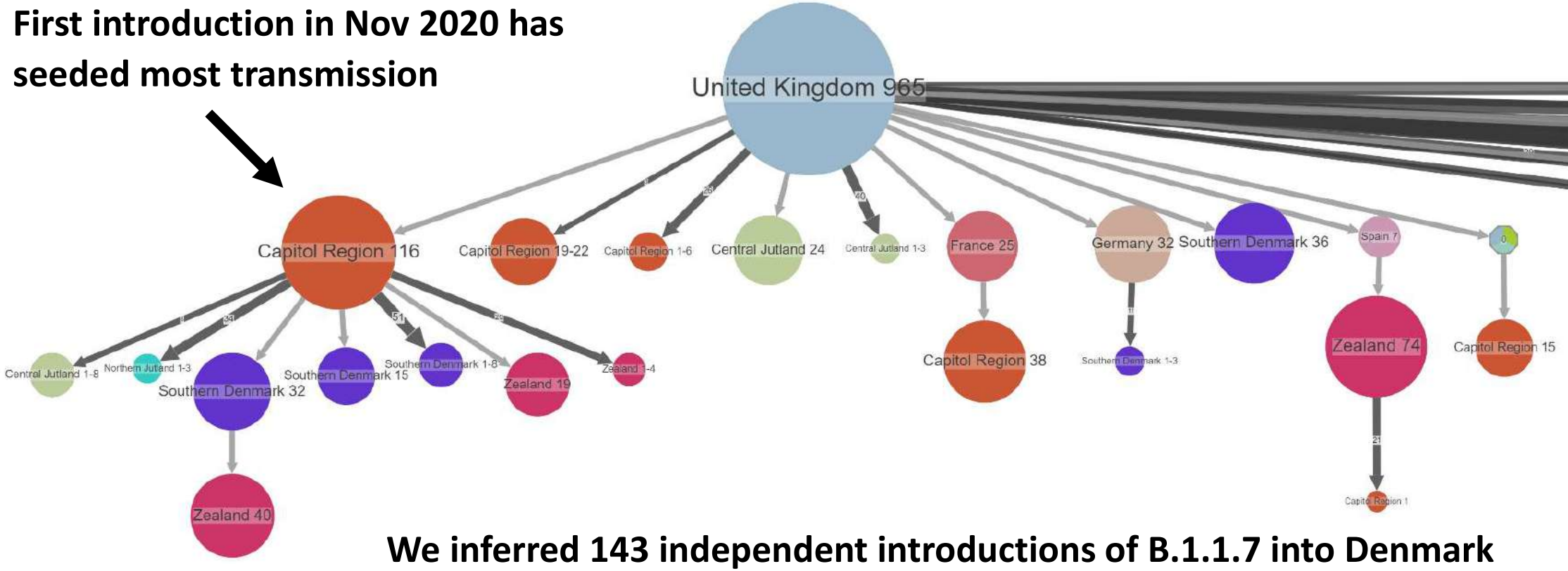
Visualisation of 18 largest country transmission clusters (compressed): Lineage B.1.1.7 origin in United Kingdom



Michaelsen, Genome Medicine, 2022

Same analysis but with Danish samples annotated according to Region (and only showing cluster involved in transmission to DK)

First introduction in Nov 2020 has seeded most transmission



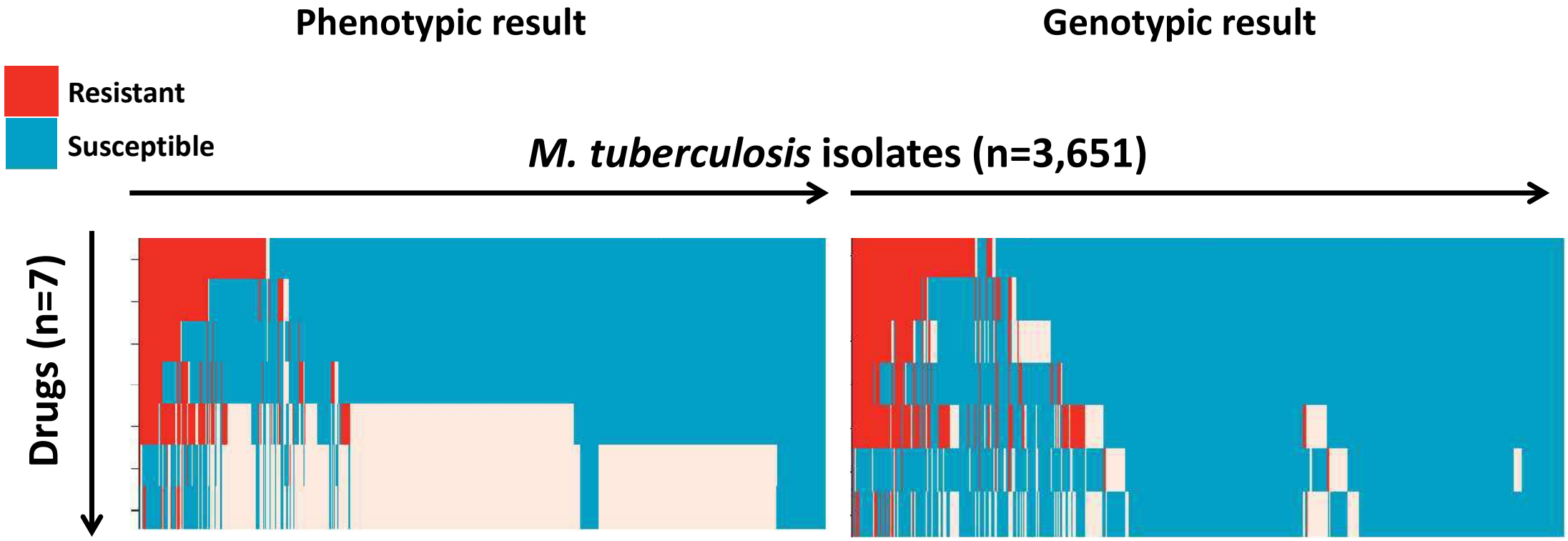
We inferred 143 independent introductions of B.1.1.7 into Denmark

Michaelsen, Genome Medicine, 2022

Fundamental diagnostic questions in clinical microbiology

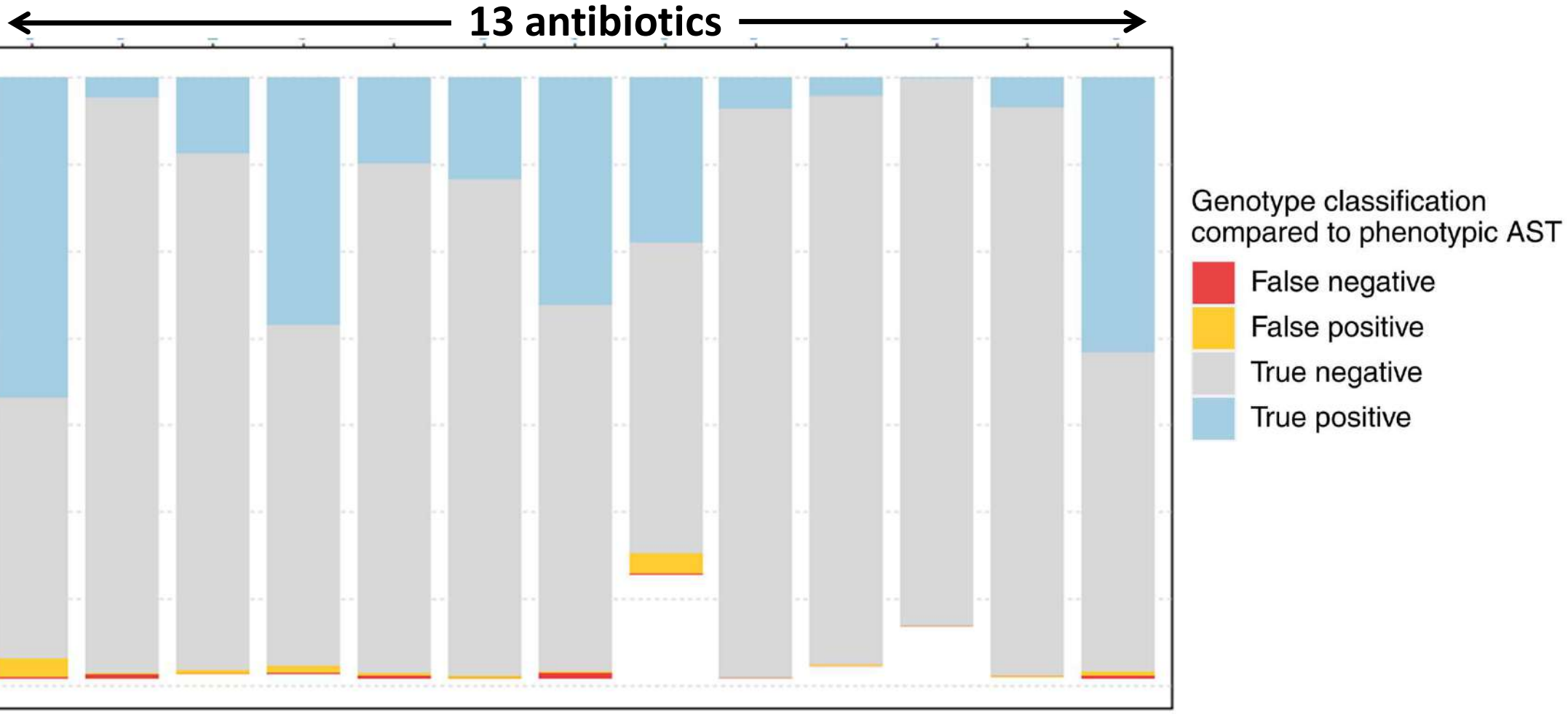
- Is there something ?
- What is it ?
- What can it do ?

89% concordance between phenotypic and genotypic antimicrobial susceptibility testing in *Mycobacterium tuberculosis*

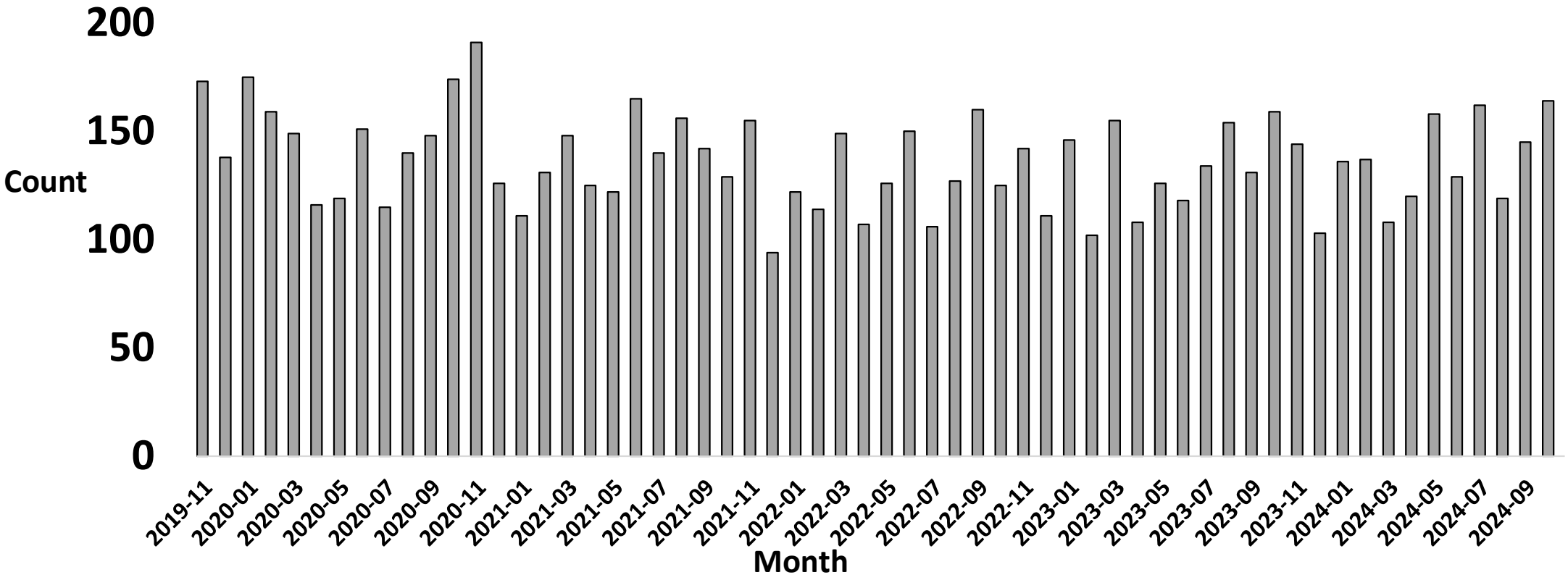


Walker et al. (2015) Lancet Infect Dis

98.9% accuracy of genotypic prediction of AST phenotype for 864 *Salmonella* spp. isolates (Sherry, Nature Communications, 2023)

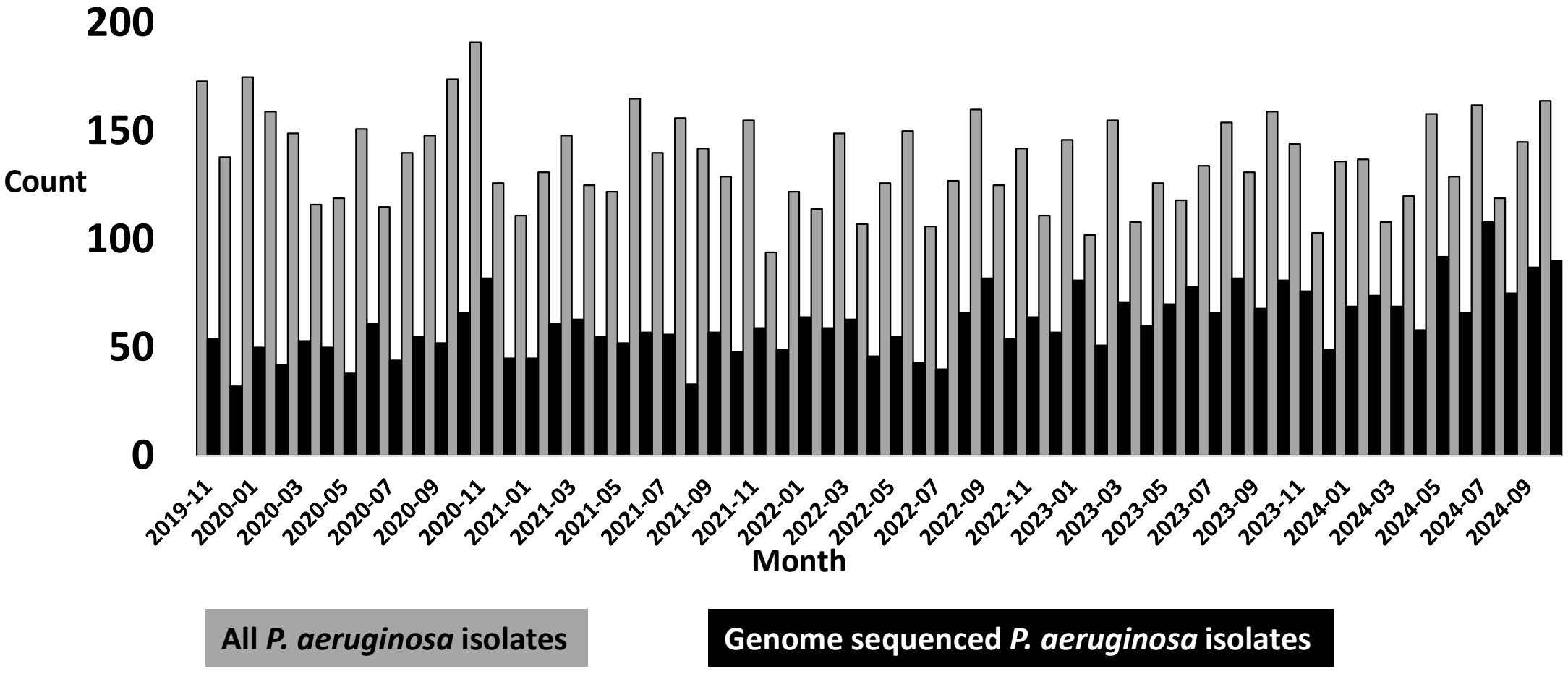


Number of samples with a *P. aeruginosa* isolate (n=8,189) during a 5-year period from November 2019 to October 2024

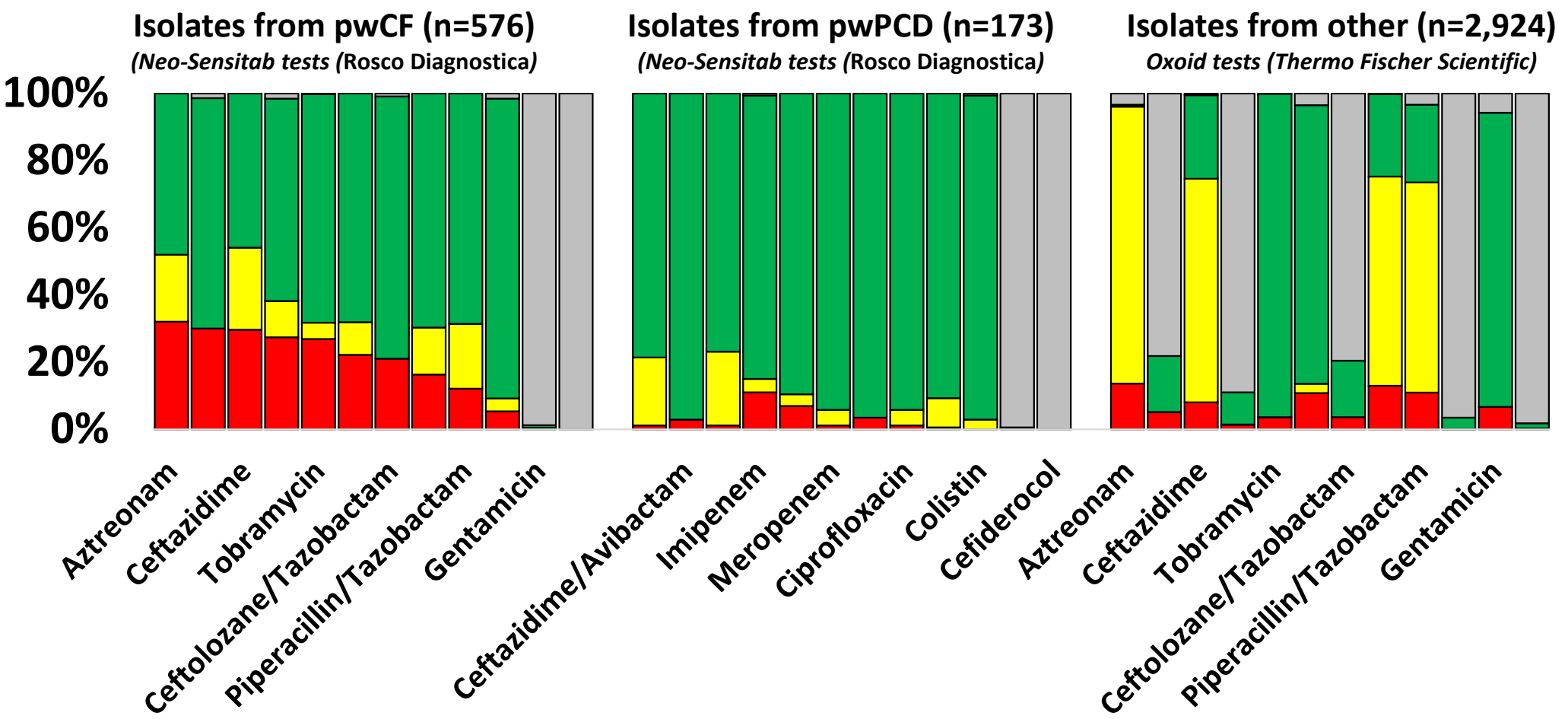


All *P. aeruginosa* isolates

Number of samples with a *P. aeruginosa* isolate (n=8,189)
Number of genome sequenced P. aeruginosa isolates (n=3,673, 45%)



In vitro antibiotic susceptibility test result distribution: Susceptible (S), susceptible at increased exposure (I), resistant (R) , not determined



Antibiotic determinants observed in a collection of genomes including only one *P. aeruginosa* genome per person (2,089 genomes)

AMRFinderPlus mutations

Observations (count)	Gene	Mutations	Drug class for resistance
<=1	Not shown		
2	*mexZ*	G195E,Q134STOP	BETA-LACTAM
5	*ampR*	D135G,D135N	BETA-LACTAM
5	*ampD*	H157Y,Q155STOP,Q44STOP,H77Y	BETA-LACTAM
6	*phoQ*	V260G,LG364del	COLISTIN
22	*fusA1*	A555E,E100G,R371C,T456A,Y552C,P618L, R680C,T671A,P554L	AMINOGLYCOSIDE
30	*parC*	S87W,S87L	QUINOLONE
31	*pmrB*	A54V,H340R,A248T	COLISTIN
33	*gyrB*	E468D,S466Y,S466F	QUINOLONE
41	*ftsI*	A454V,A539T,F507L,Q458R,V471G,F533L, N427S,H394R,V523M,G63S,P527S,R504C	BETA-LACTAM
46	*gyrA*	Q106L,D87G,T83A,D87Y,D87N	QUINOLONE
49	*oprD*	L11P,S278P, 13 different STOP mutations	BETA-LACTAM

Mutations *gyrA*(T83I), *oprD*(V359L), *parE*(A473V), and *pmrB*(V15I) were ignored in mutation analysis as they were observed in 48 to 983 of the genomes:.
blaOXA- and *blaPDC*-genes were ignored in gene presence analysis.

AMRFinderPlus genes

Observations (count)	Gene	Drug class for resistance
<=4	Not shown	
5	*aadA7*	AMINOGLYCOSIDE
5	*blaNDM-1*	BETA-LACTAM
5	*dfrA1*	TRIMETHOPRIM
5	*rmtB4*	AMINOGLYCOSIDE
6	*aac(6')-Ib3*	AMINOGLYCOSIDE
6	*aadA11*	AMINOGLYCOSIDE
6	*ant(2'')-Ia*	AMINOGLYCOSIDE
6	*blaIMP-1*	BETA-LACTAM
6	*tbtB*	EFFLUX
6	*tbtM*	EFFLUX
8	*aadA6*	AMINOGLYCOSIDE
10	*floR2*	PHENICOL
10	*qnrVC1*	QUINOLONE
10	*tet(G)*	TETRACYCLINE
21	*aph(6)-Id*	AMINOGLYCOSIDE
27	*aph(3'')-Ib*	AMINOGLYCOSIDE
32	*sul1*	SULFONAMIDE
>=1375	Not shown	

Positive predictive values (PPVs) for resistant phenotype in *in vitro* antibiotic susceptibility test

AMRFinderPlus mutations

Observations (count)	Gene	Mutations	Drug class for resistance
<=1	Not shown		
2	*mexZ*	G195E,Q134STOP	BETA-LACTAM
5	*ampR*	D135G,D135N	BETA-LACTAM
5	*ampD*	H157Y,Q155STOP,Q44STOP,H77Y	BETA-LACTAM
6	*phoQ*	V260G,LG364del	COLISTIN
22	*fusA1*	A555E,E100G,R371C,T456A,Y552C,P618L, R680C,T671A,P554L	AMINOGLYCOSIDE
30	*parC*	S87W,S87L	QUINOLONE
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46	*gyrA*	Q106L,D87G,T83A,D87Y,D87N	QUINOLONE
49	*oprD*	L11P,S278P, 13 different STOP mutations	BETA-LACTAM

In Task05 you will use Abricate to find antibiotic resistance genes in genomes

PPV 20% for Colistin resistant (40%)

PPV 100%/55% for Gentamicin/Tobramycin resistant (100%/64%)

PPV 97% for Ciprofloxacin resistant (100%)

PPV 19% for Colistin resistant (43%)

PPV 33% for Ciprofloxacin resistant (64%)

PPV 76% for Ceftazidime resistant (83%)

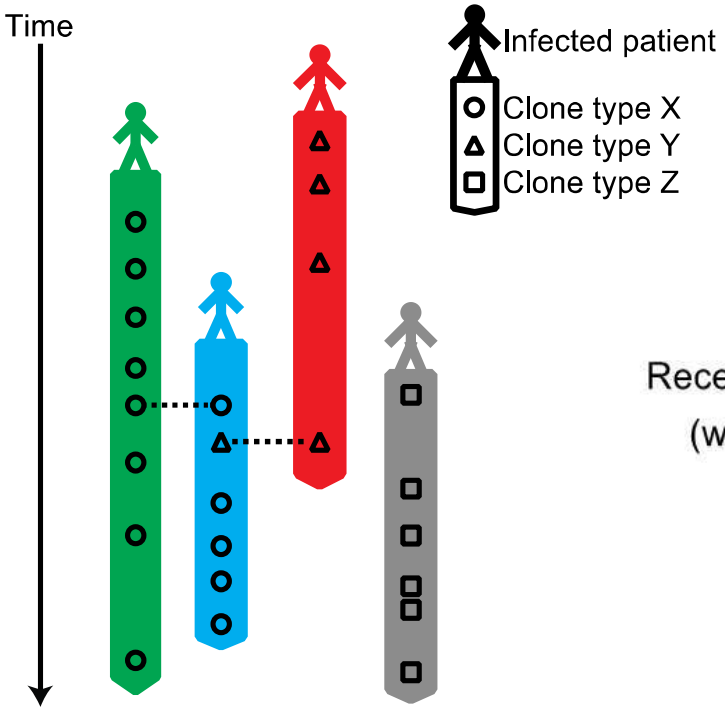
PPV 37% for Ciprofloxacin resistant (65%)

PPV 56% for Meropenem resistant (75%)

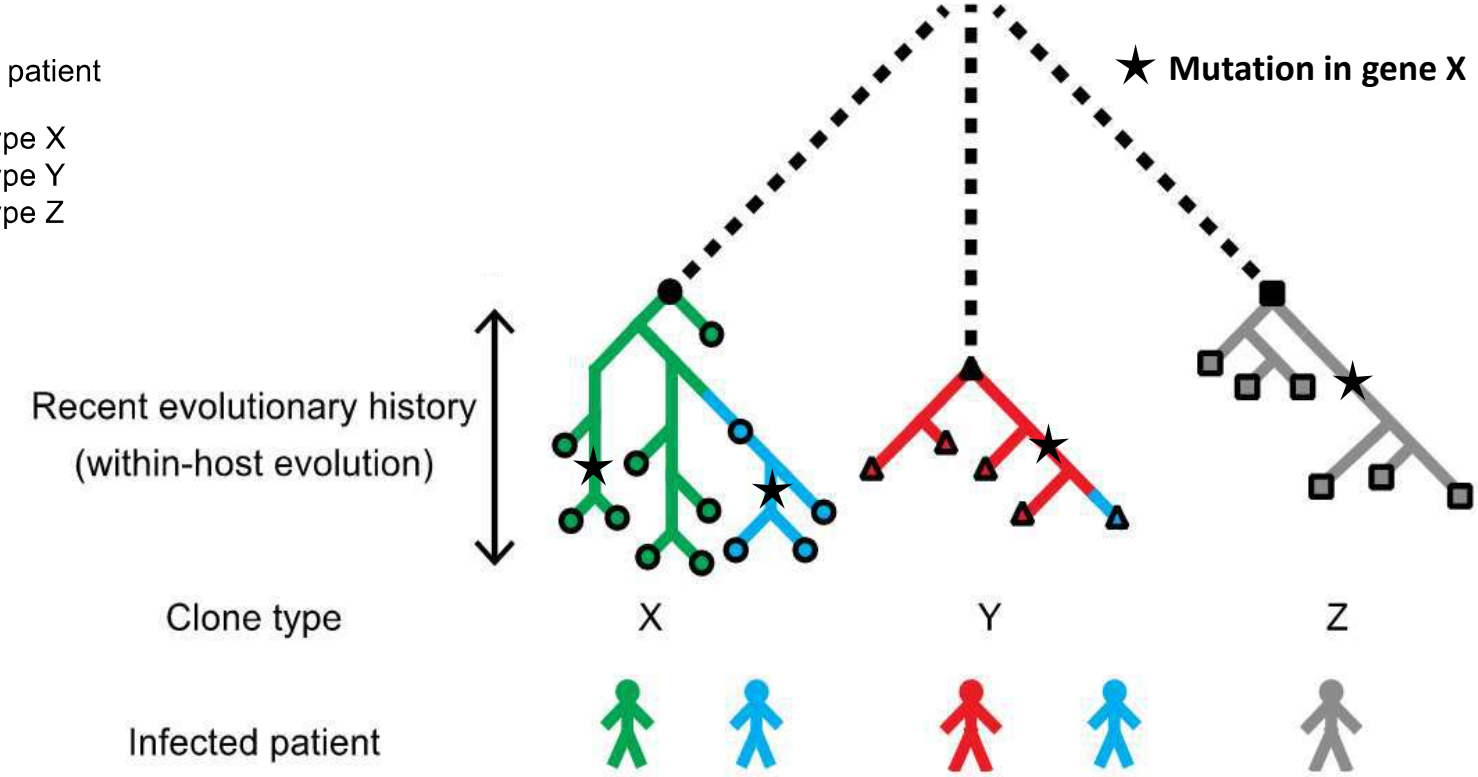
Parenthesis shows PPV for either resistant (R) or susceptible at increased exposure (I)

Genetic adaptation evidenced by independent mutation of the same bacterial gene across patients (convergent and/or parallel evolution)

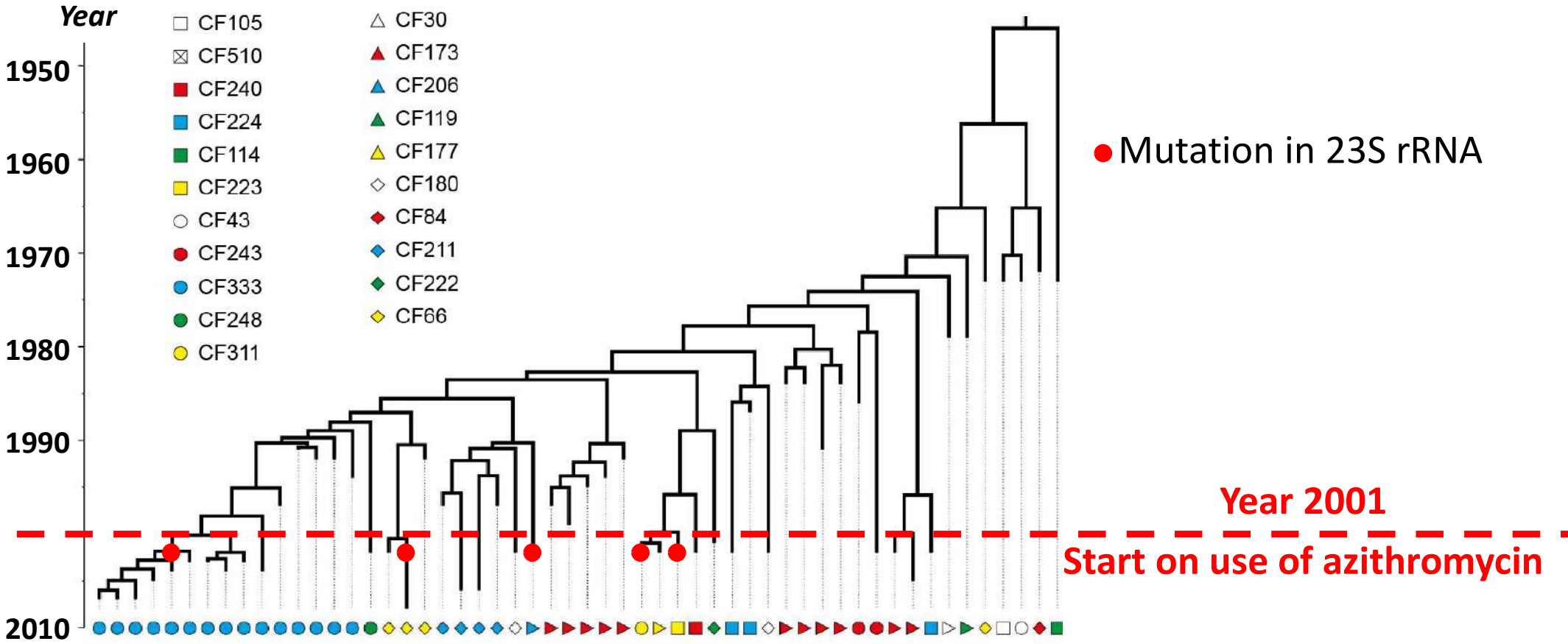
Collection of bacterial isolates from patients



Genetic relationship of bacterial isolates

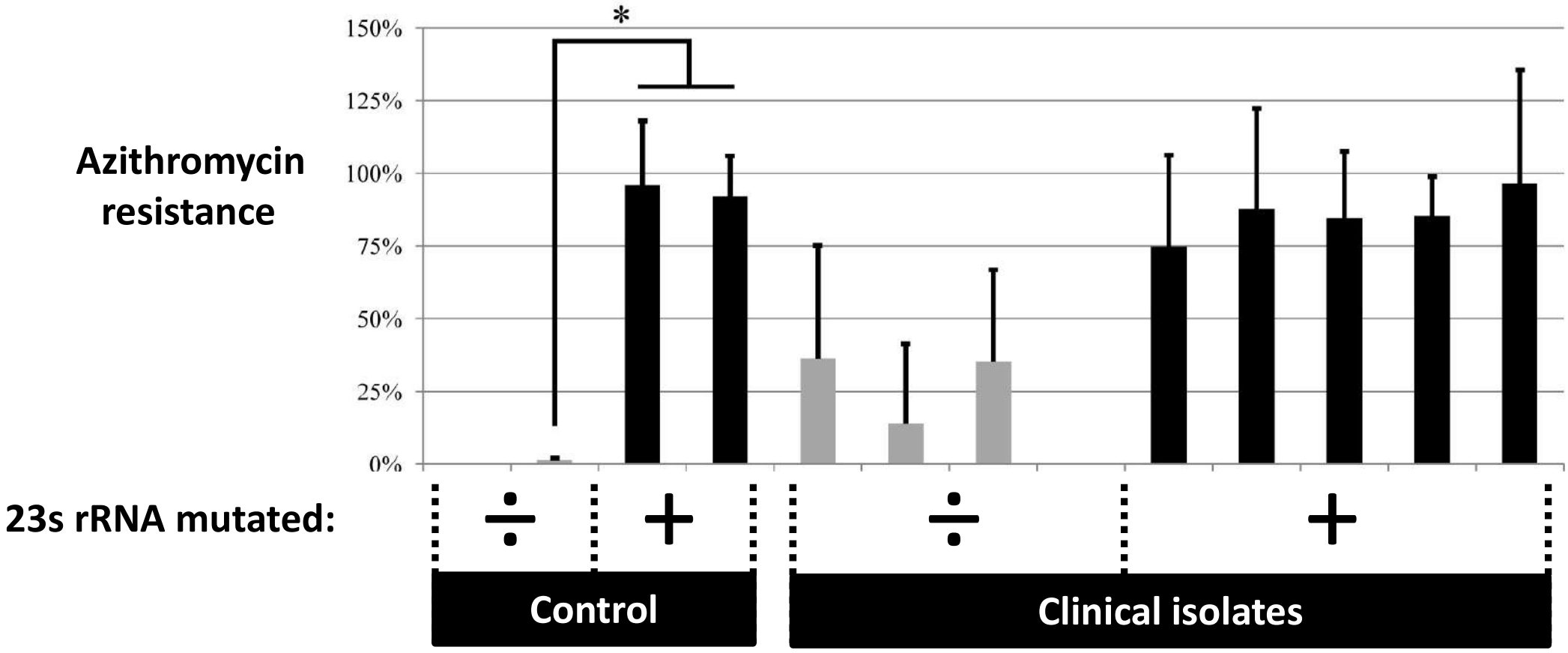


Parallel adaptive evolution: Identification of a new antibiotic resistance mutation not captured by standard antibiotic susceptibility tests



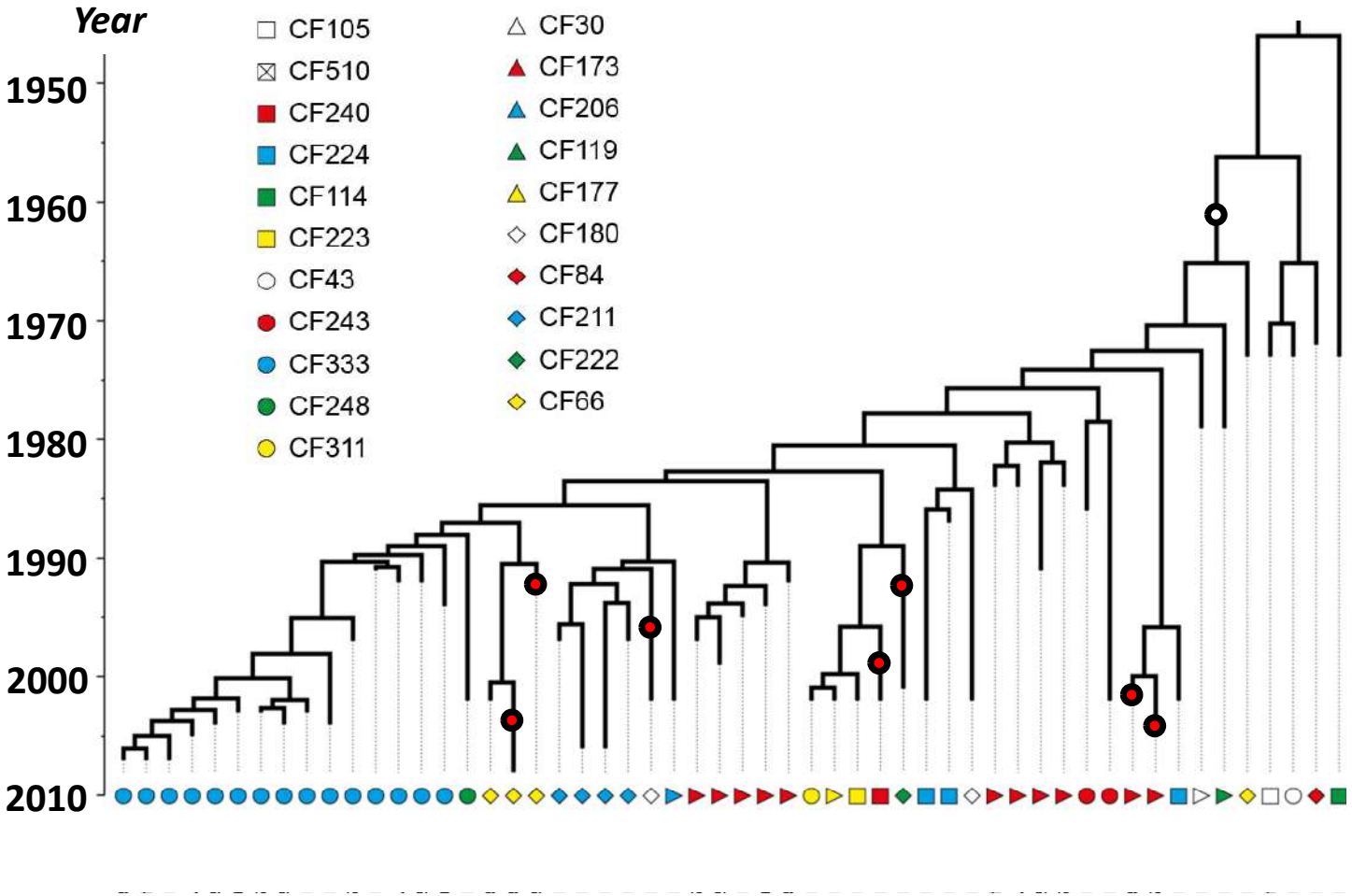
Marvig, Søndergaard et al. (2012) Antimicrob Agents Chemother

Parallel adaptive evolution: Parallel adaptive evolution: Identification of a new antibiotic resistance mutation



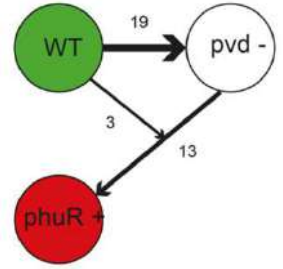
Marvig, AAC, 2012

Taste of blood: Adaptation Towards Iron Acquisition from Hemoglobin by mutations in *Pseudomonas* heme uptake system (*phu*)



- Loss of function mutation in *pvdS* (cooperative iron system)
- Gain of function mutations in *phu* (private iron system)

Always same mutation order



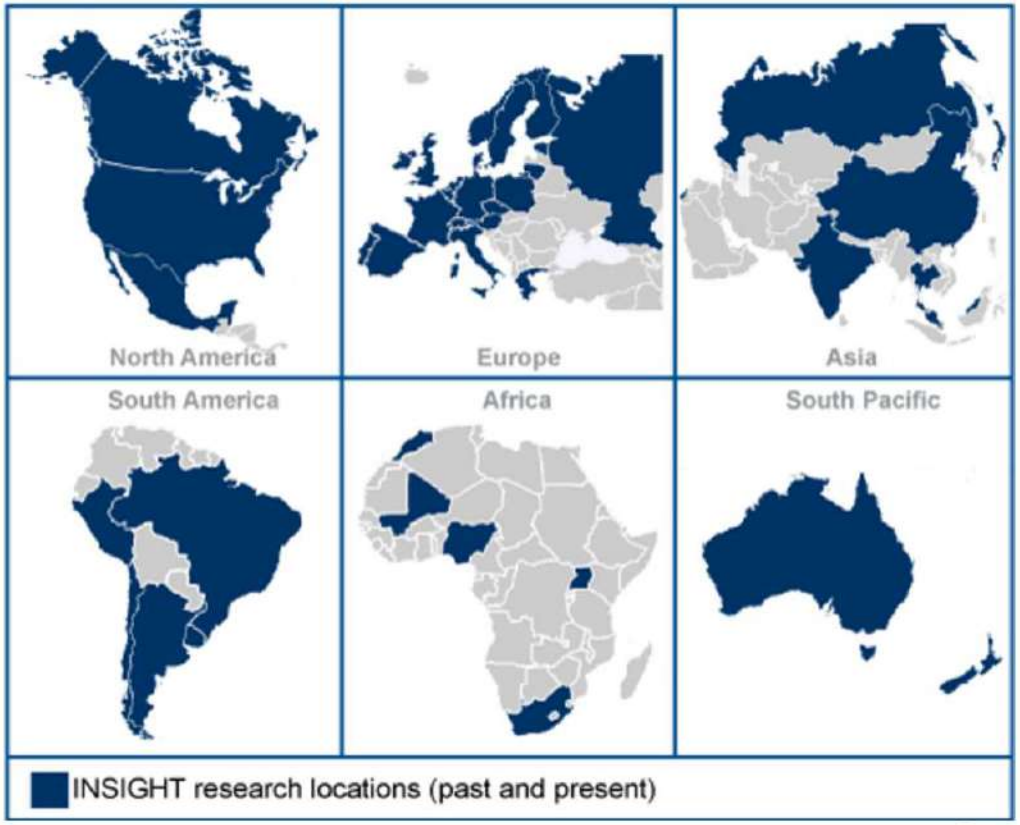
Marvig et al. (2013) PLoS Genet

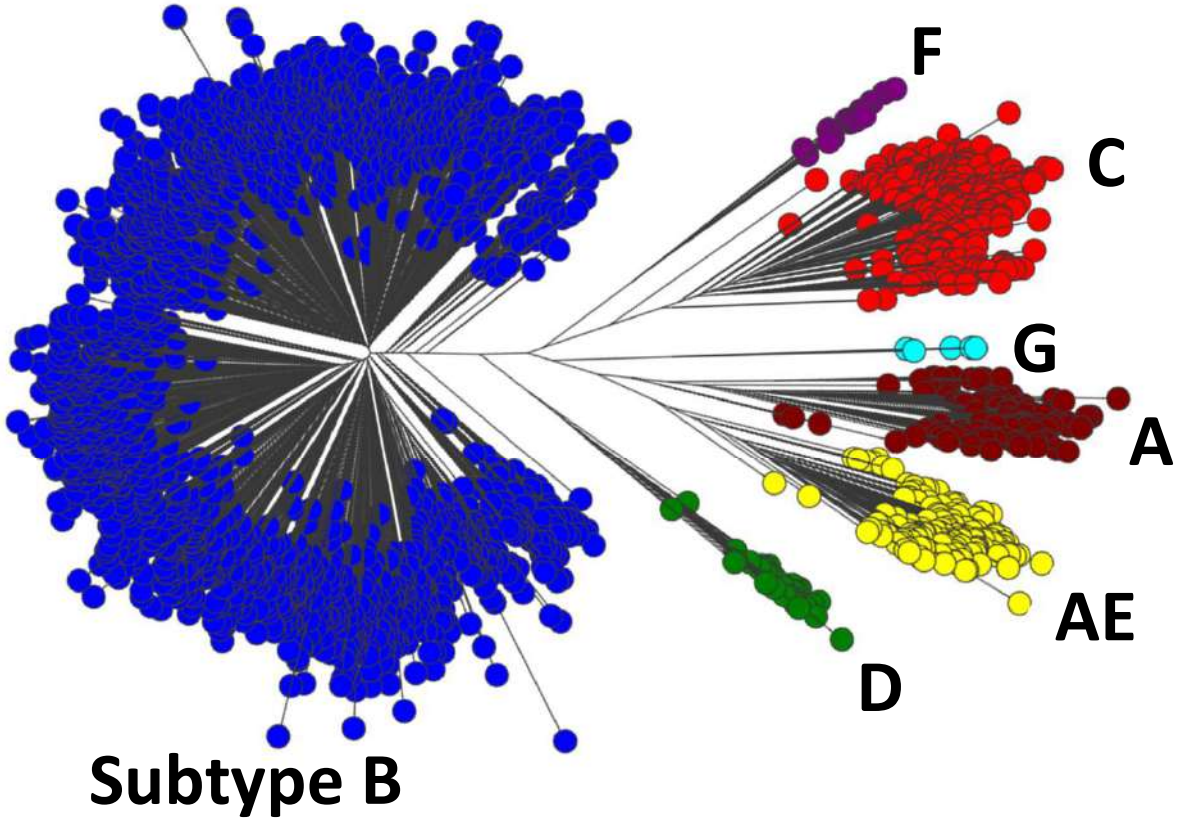
Marvig et al. (2014) mBio

Andersen et al. (2015) PNAS

Andersen et al. (2018) eLife

Phylogenetic tree of 2,501 HIV-1 genomes from START clinical trial
Samples have been collected in 2009-2013 from 35 countries





Bennedbæk, Virus Evolution, 2021

2,122 persons: Data available for association analysis using generalized linear models

Virus genetics
3,010 AA variants (observed min >=5% of virus genomes)

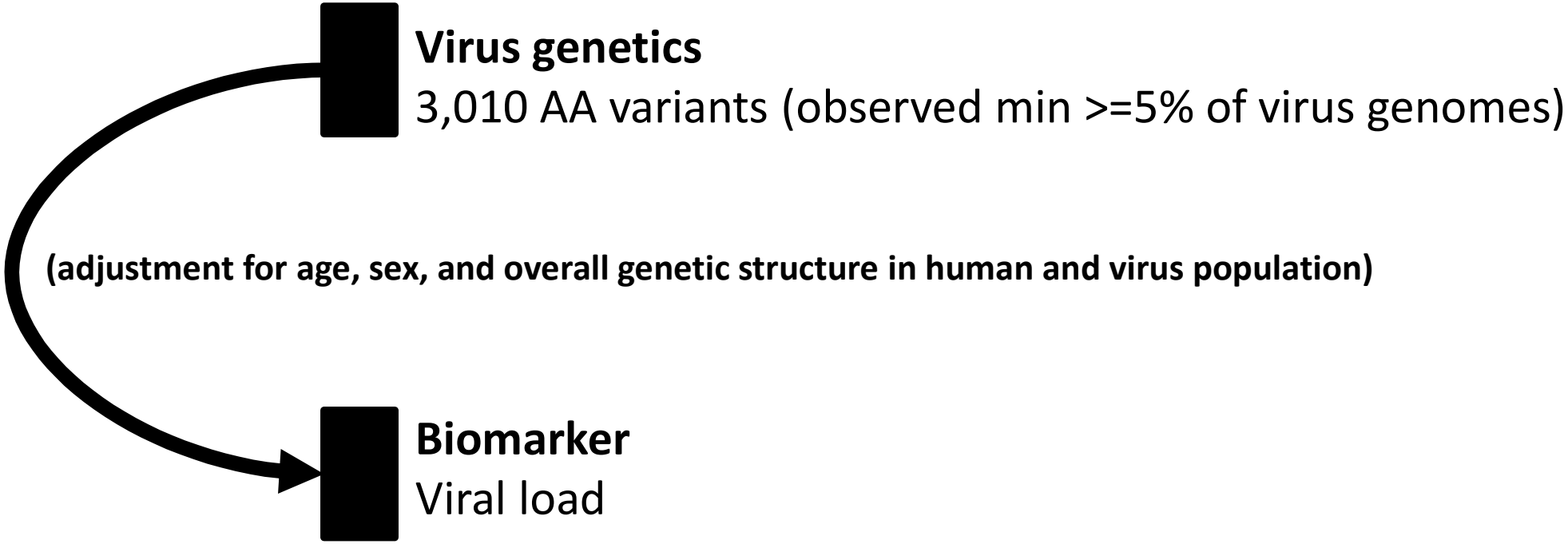
(adjustment for age, sex, and overall genetic structure in human and virus population)

Biomarkers
Viral load, CD4+, CD8+, hs CRP, IL-6, D-dimer

HIV GWAS studies:
Gabrielaite, Bennedbæk et al., J Infect Dis, 2021
Gabrielaite, PloS Comput Biol, 2023

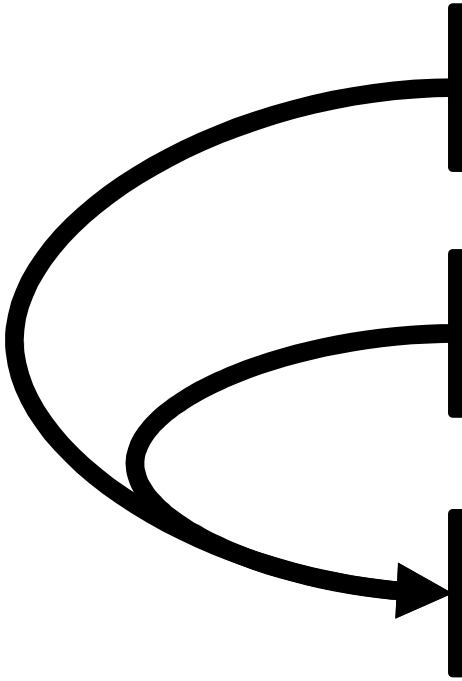
No significant associations btw virus genetics and biomarkers …

2,122 persons: Data available for association analysis using generalized linear models



Gabrielaite, Bennedbæk et al., J Infect Dis, 2021
Gabrielaite, PloS Comput Biol, 2023

2,122 persons: Data available for association analysis using generalized linear models



Virus genetics
3,010 AA variants (observed min >=5% of virus genomes)

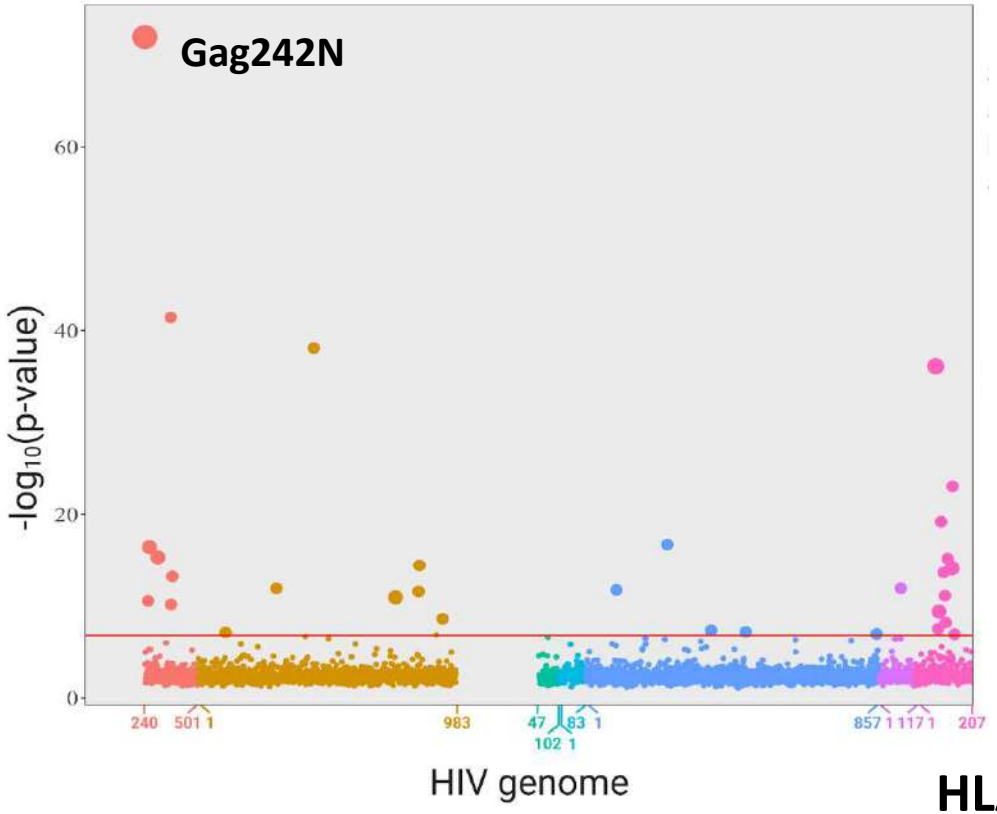
Adjustment for host immune type
388,501 SNPs → 130 HLA alleles

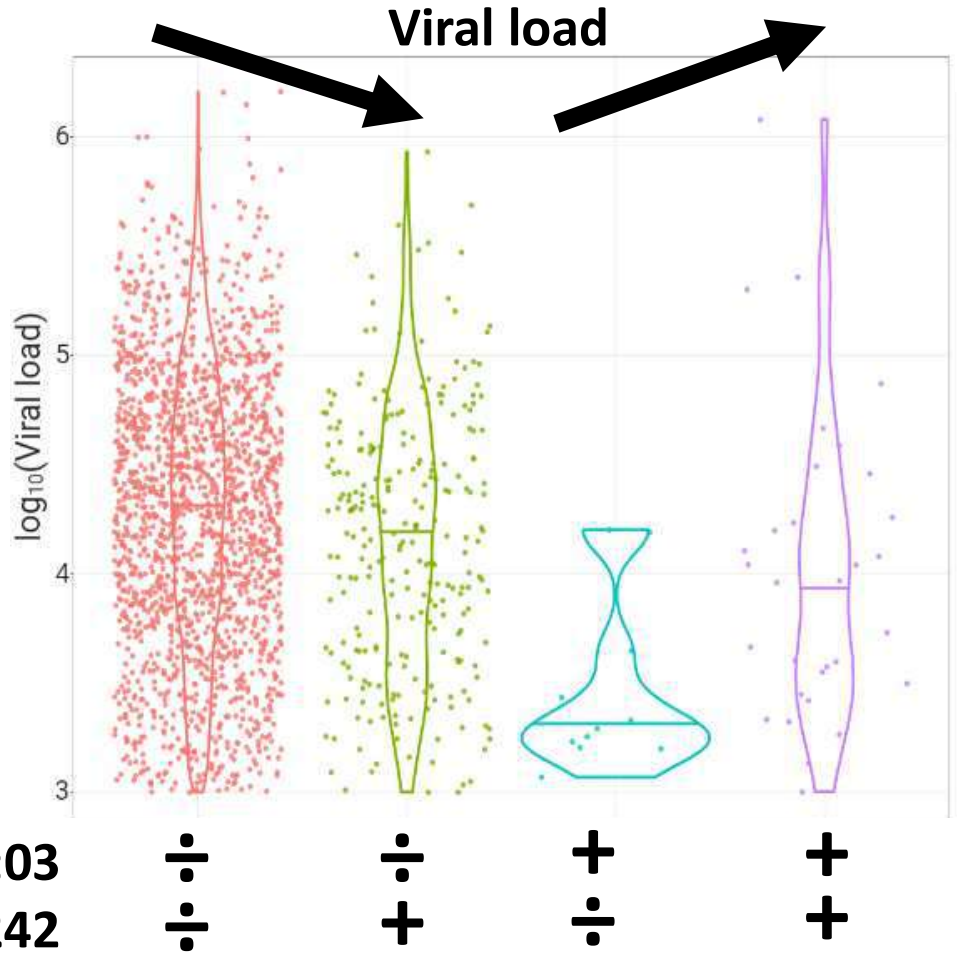
Biomarker
Viral load

Gabrielaite, Bennedbæk et al., J Infect Dis, 2021
Gabrielaite, PloS Comput Biol, 2023

Interaction between HIV genetic variant and HLA allele (immune type) explains viral load variance

Virus genome to human HLA association





Gabrielaite, Bennedbæk et al., J Infect Dis, 2021
Gabrielaite, PloS Comput Biol, 2023

Microbial genomics in a clinical setting can be used for

… for high resolution genetic typing

… detection of transmission

… identification of genetic predictive markers of clinical phenotypes

… a lot, but not all and still difficult to translate into clinical action

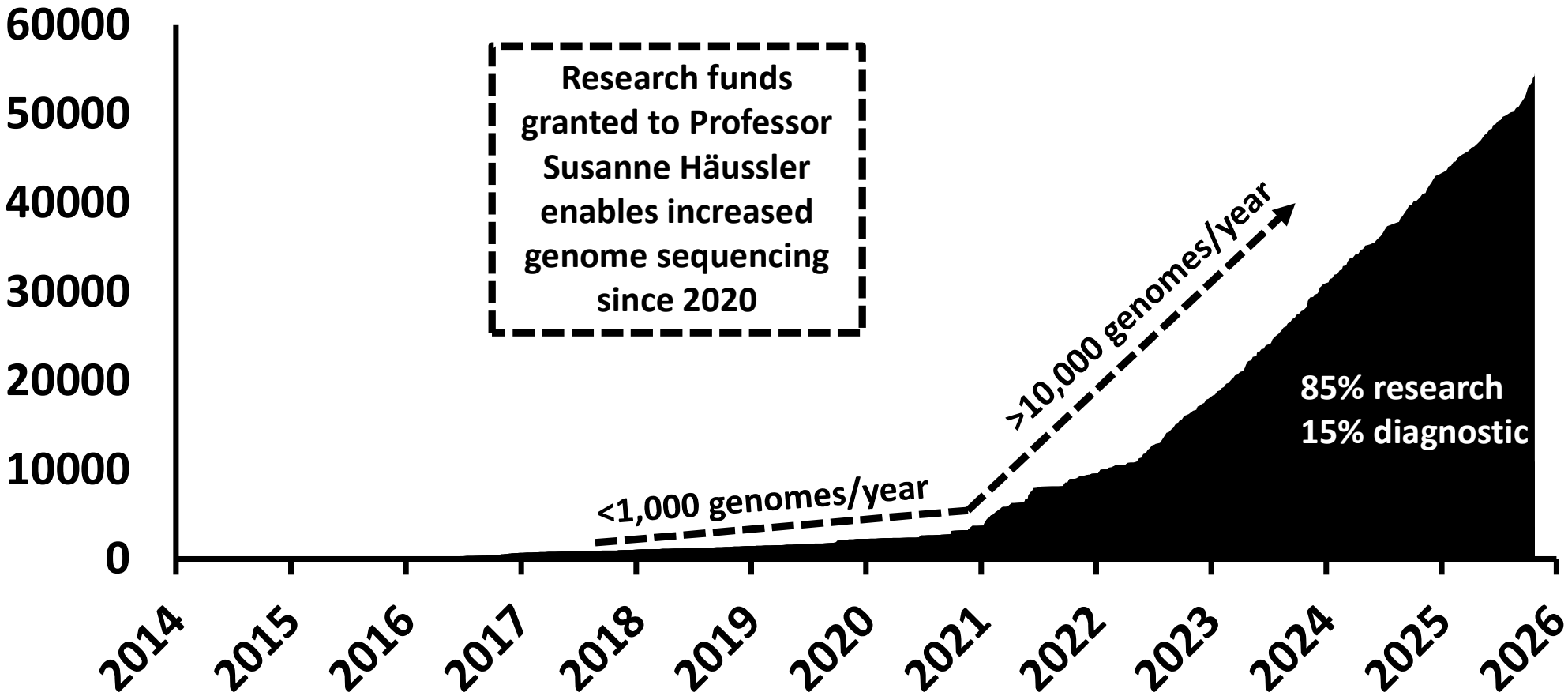
Hospital directorship: Are NGS-based tests cost-effective ?

Fundamental diagnostic questions in clinical microbiology

- Is there something ?
- What is it ?
- What can it do ?

- Hospital directorship: Are NGS-based tests cost-effective ?

Bacterial WGS at Rigshospitalet (accumulated count): 54,599 genomes sequenced per 2025-10-31



Key persons that drive scale-up of bacterial genome sequencing at Rigshospitalet, Copenhagen, Denmark

Karen Leth Nielsen, Senior Scientist (now at Roche Diagnostics)
Driver of laboratory workflow for genomics

Helle Krogh Johansen, Professor
Driver of bacterial genomics in pwCF and pwPCD

Susanne Häussler, Professor
Driver of bacterial genomics in other patients

What is the potential if we sequence the genome of all bacterial isolates ?

ELSEVIER

Clinical Microbiology and Infection

Available online 7 October 2025

In Press, Journal Pre-proof ⓘ What's this?

Original Article

Estimating the potential economic and health impact of integrated genomic surveillance in a hospital setting

Frederik Boetius Hertz [1 2 ¶], Karen Leth Nielsen [1 ¶], Dmytro Strunin [1 ¶], Jelena Erdmann [4 5 ¶], Martin Lucas Jørgensen [3], Theiss Bendixen [3], Roshkan Srinathan [3], Rasmus L. Marvig [6], Steen Christian Rasmussen [1], Asger Nellemann Rasmussen [1], Christian Salgaard Jensen [1], Jenny Dahl Knudsen [1], Susanne Häussler [1 4 5]

Abstract

Objectives

Integrated genomic surveillance, combining whole genome sequencing (WGS) of bacterial isolates with patient movement data, promises improved detection and prevention of pathogen transmission. However, evidence on its cost-effectiveness and clinical utility remains limited, not least because the full extent of transmission in hospital settings is difficult to capture.

Methods

We conducted a 28-month observational study at Rigshospitalet, Copenhagen, collecting patient movement data and sequencing 18,438 bacterial isolates from 7,398 patients across diverse species, infection sites, and resistance profiles. We estimated the hypothetical benefits of implementing integrative WGS surveillance, assuming that continued transmission could be prevented when WGS information was acted upon immediately.

Results

We found that 1,975 of 7,398 of culture-positive hospitalized patients (26.7 %) harboured a pathogen genetically related to another patient´s isolate. 1359 of those (68.8 %) had an epidemiological link in the hospital, with *Enterococcus faecium* by far being the most prevalent involved in transmissions. WGS-informed prevention could hypothetically generate net savings of €1.35 million annually if transmission was stopped once a clonal isolate was detected in a second patient. Furthermore, this approach could potentially have prevented an estimated 1,284 hospital-acquired infections over the 28-month study period, including 94 cases characterized by the recovery of bloodstream isolates.

Conclusions

Our integrated genomic surveillance approach reveals previously unexplored transmission landscapes. We discovered that transmission is widespread, varies between species, and is not limited to resistant isolates. Our results emphasise the potential of integrated genomic surveillance, the identification of local transmission hotspots, potential greater savings by including susceptible isolates, and an incremental cost-effectiveness ratio classification by pathogen species.

PROMISING*: An investigator initiated randomized clinical trial to test if WGS all bacterial isolates improves infection control

**Prevention of nosocomial infections through prospective surveillance of bacterial pathogens by whole genome sequencing – a cluster randomised clinical trial*

Rigshospitalet (RH)

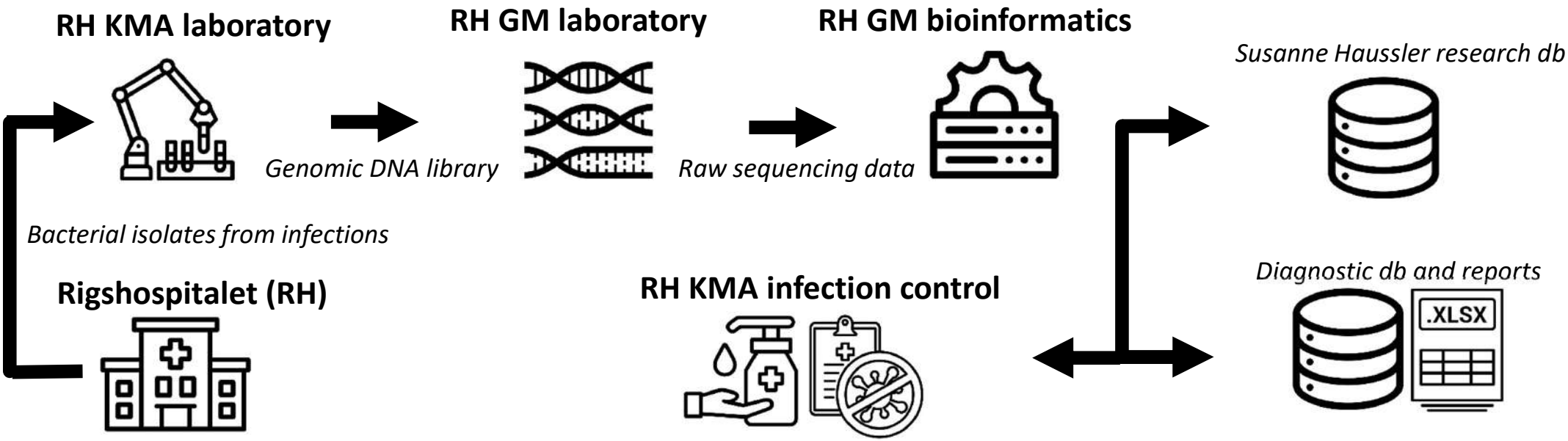
Hvidovre Hospital

RH Dept A **RH Dept B**	***Active arm:*** ***WGS of all isolates***	**HH Dept A** **HH Dept B**
RH Dept C **RH Dept D**	***Control arm:*** ***WGS upon indication***	**HH Dept C** **HH Dept D**

Investigator: Susanne Haussler
Trial contact: Melissa Hornbæk Øvre

Primary trial endpoint:
Does the intervention (WGS-informed infection control) reduce hospital-acquired infections ?

Genome sequencing of bacterial isolates at Rigshospitalet

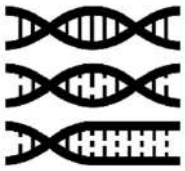


Genome sequencing of bacterial isolates at Rigshospitalet

RH KMA laboratory

Genomic DNA library

RH GM laboratory



Raw sequencing data

RH GM bioinformatics

Susanne Haussler research db

WGS twice a week

Bacterial isolates from infections

Rigshospitalet (RH)

RH KMA infection control

Diagnostic db and reports

Bacterial isolates from infections

Hvidovre Hospital (HH)

HH KMA infection control

Clinical trial db and reports

Retrieval of metadata from HH

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