Whole-genome sequencing for syphilis, gonorrhoea and shigella epidemiology and control

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NHS Foundation Trust

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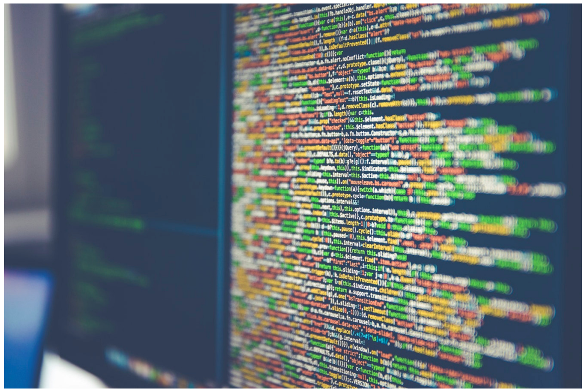
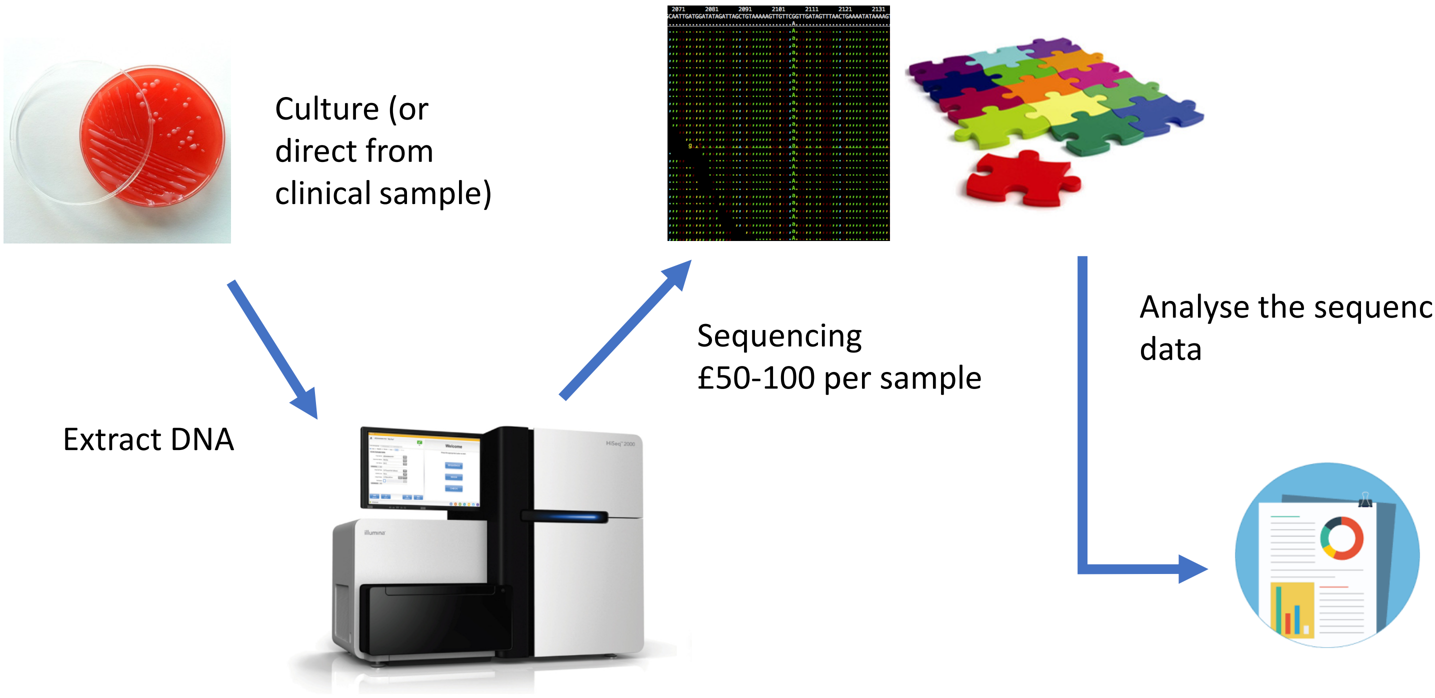
What can whole-genome sequencing tell us?

- Reconstruct historical transmission events at a population scale
  - When did **Syphilis** come to Europe?
- Track the spread of infections in particular populations
  - **Shigella** as a cause of colitis in MSM
- Track the spread of antibiotic resistance
  - Identifying the likely origin of cases of MDR/XDR **gonorrhoea**
- Individual transmission tracking
  - Understand local transmission networks, e.g. evidence for serosorting or associative selectivity
  - Enhanced partner notification
- Better diagnostics?



Decoding pathogen DNA: Sequencing



Culture (or
direct from
clinical sample)

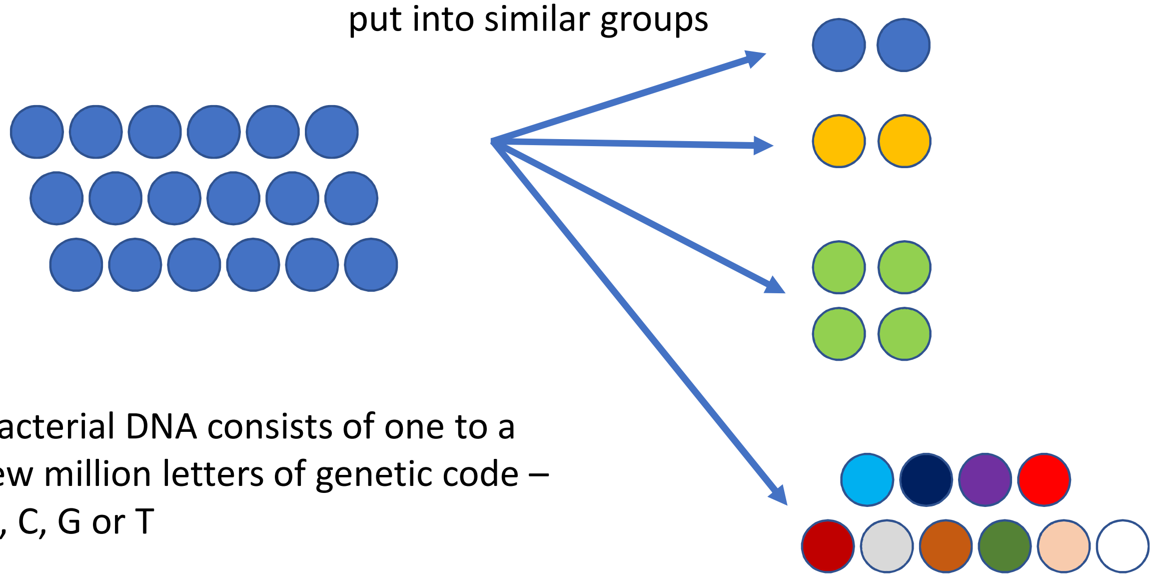
Extract DNA

Sequencing
£50-100 per sample

Analyse the sequence
data

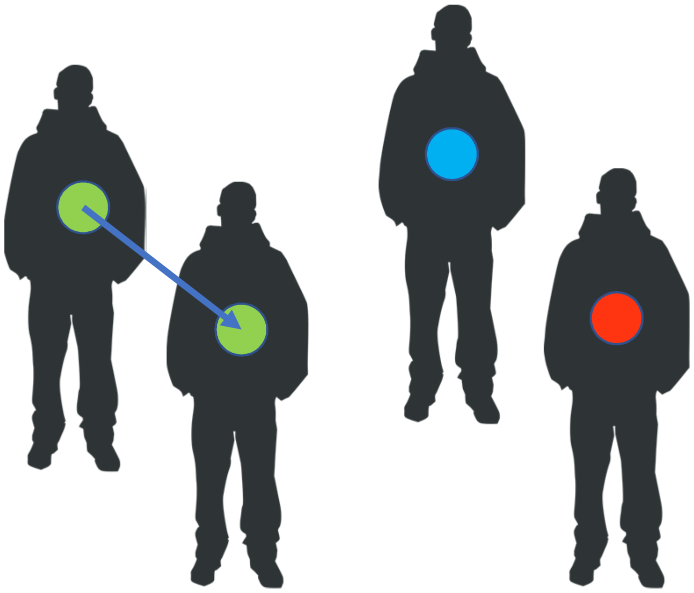
Pathogen DNA

This DNA allows pathogens to be put into similar groups

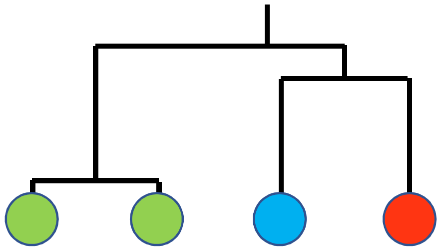


Bacterial DNA consists of one to a few million letters of genetic code – A, C, G or T

If infection spreads between two people the DNA of the bacteria is likely to be identical or very similar



Phylogenetic tree:



Two theories of how Syphilis came to Europe

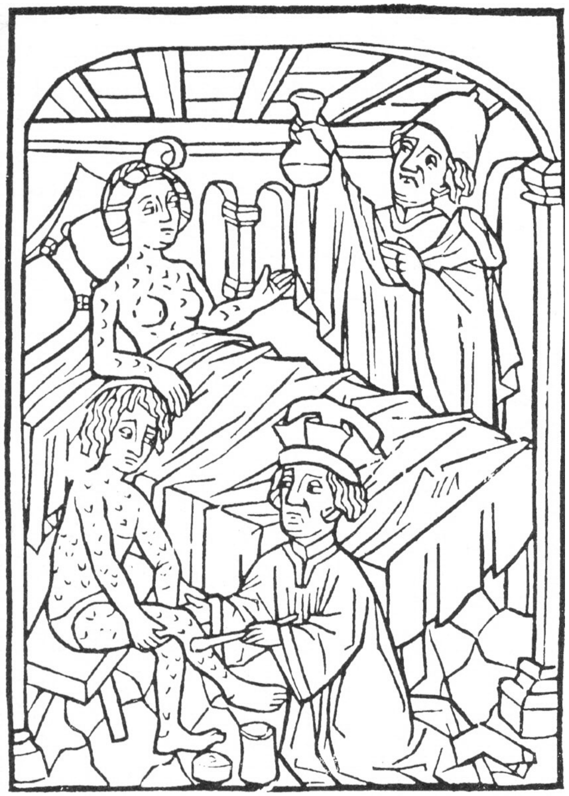
Columbian theory

- Brought back to Europe by Christopher Columbus' crew
- Spread to Italy by the French during the siege of Naples in 1495 (via Spanish mercenaries)

Pre-Columbian

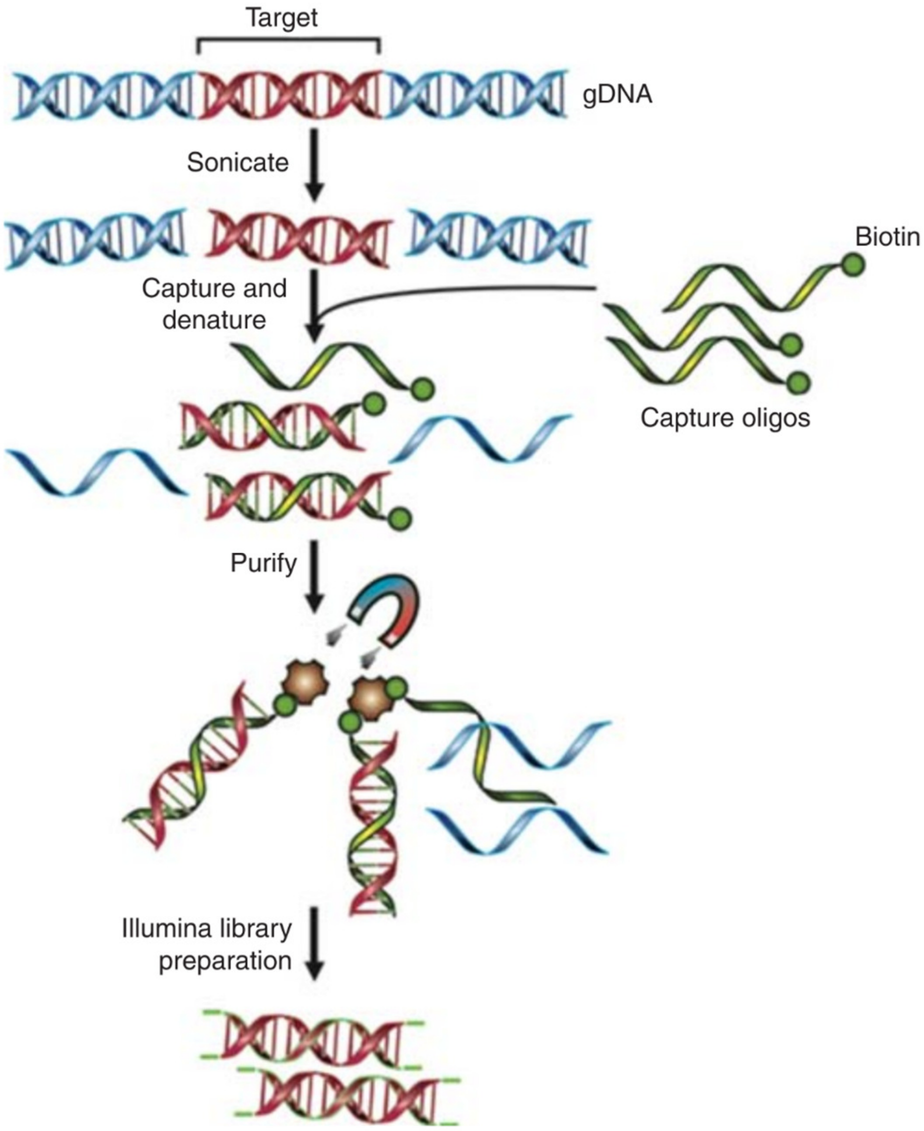
- Existed before - descriptions in keeping with tertiary syphilis in ancient Greece
- Skeletons consistent with congenital syphilis from Pompeii

Can whole-genome sequencing tell us the answer?



Treponema pallidum: DNA capture

- First genome sequenced in 1998, but only a handful of genomes until 2016…
- Sequencing possible direct from clinical samples in primary and secondary syphilis using RNA ‘baits’ → allows selective capture of low concentrations of pathogen DNA

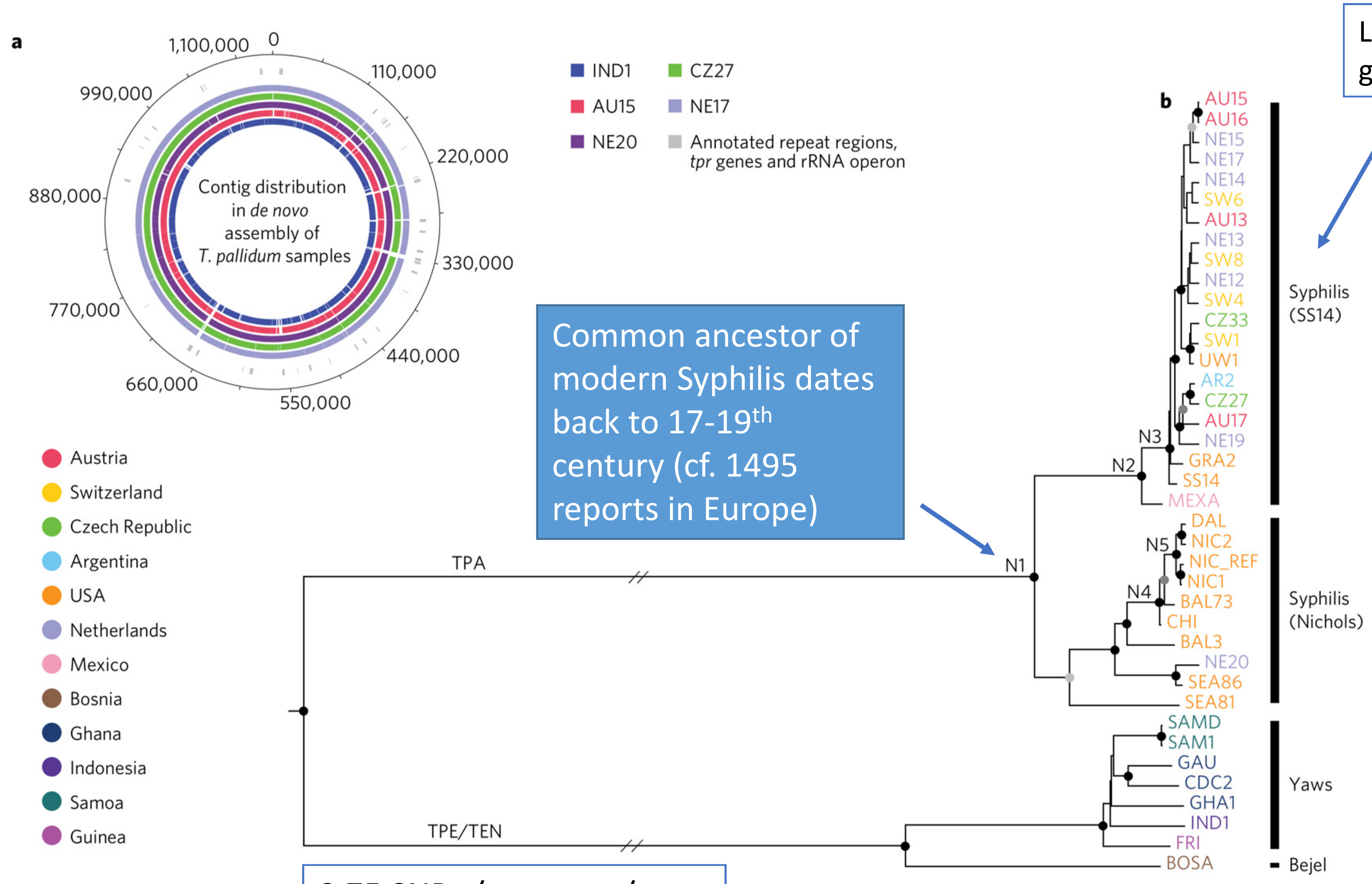


Cold Spring Harbor Protocols 2015.7 (2015): pdb-

Historical perspective

- 70 clinical samples 2012-13 and 18 historic samples from 1912 onwards

NATURE MICROBIOLOGY 2, 16245 (2016)



Lineages named after reference genomes

Common ancestor of modern Syphilis dates back to 17-19th century (cf. 1495 reports in Europe)

0.75 SNPs / genome / year

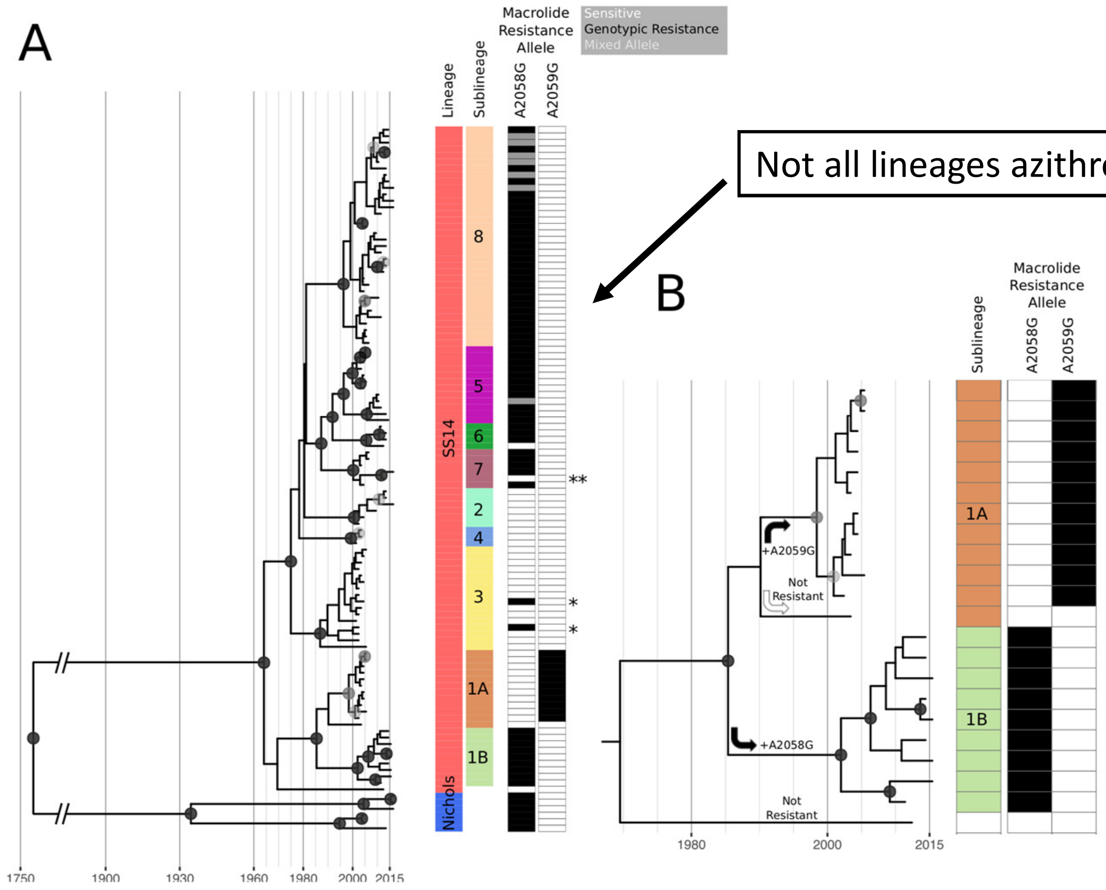
Most circulating strains worldwide are from the SS14 lineage

- Emerged 1950s to 1970s
- Most azithromycin resistant

Nichols limited mostly to N America ≤ 1980s

Success of the SS14 lineage likely multifactorial

- WGS of 73 samples from UK and USA + 49 previous sequences



Not all lineages azithromycin resistant

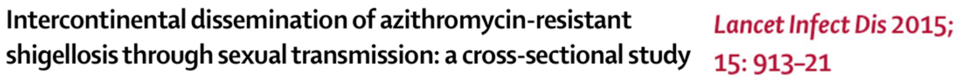
Multiple mutations have emerged that confer macrolide resistance

bioRxiv
THE PREPRINT SERVER FOR BIOLOGY

Genomic epidemiology of syphilis reveals independent emergence of macrolide resistance across multiple circulating lineages

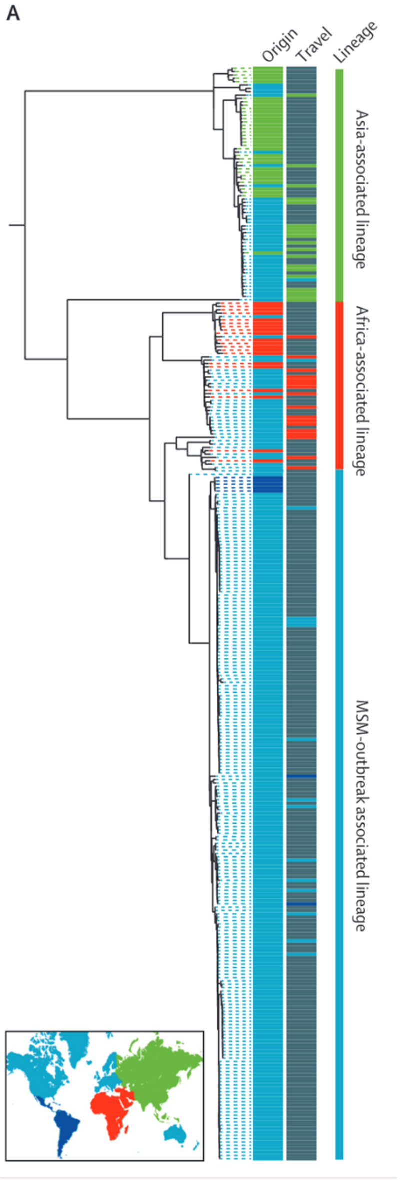
Shigellosis as a sexually transmitted infection

- Outbreaks of colitis due to *Shigella flexneri* serotype 3a reported from 1970s, large UK outbreak in 2009 onwards in MSM
- *Shigella flexneri* serotype 3a from UK 2004-2013 sequenced together with isolates from 29 countries



Intercontinental dissemination of azithromycin-resistant shigellosis through sexual transmission: a cross-sectional study

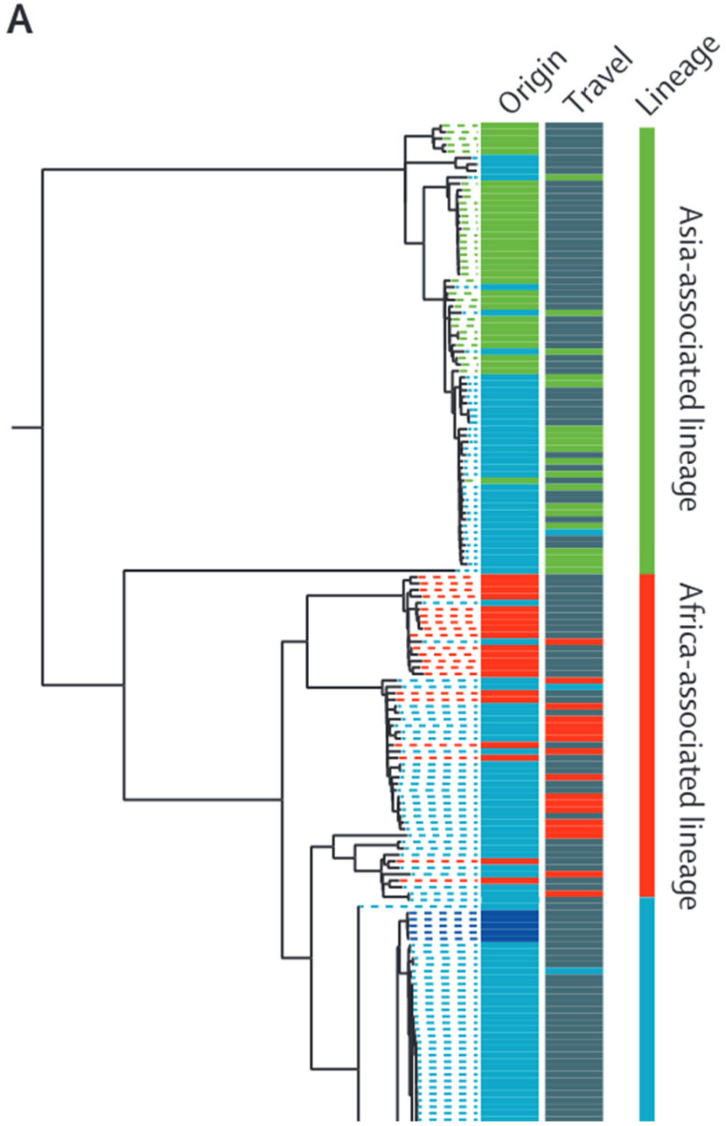
Lancet Infect Dis 2015; 15: 913–21



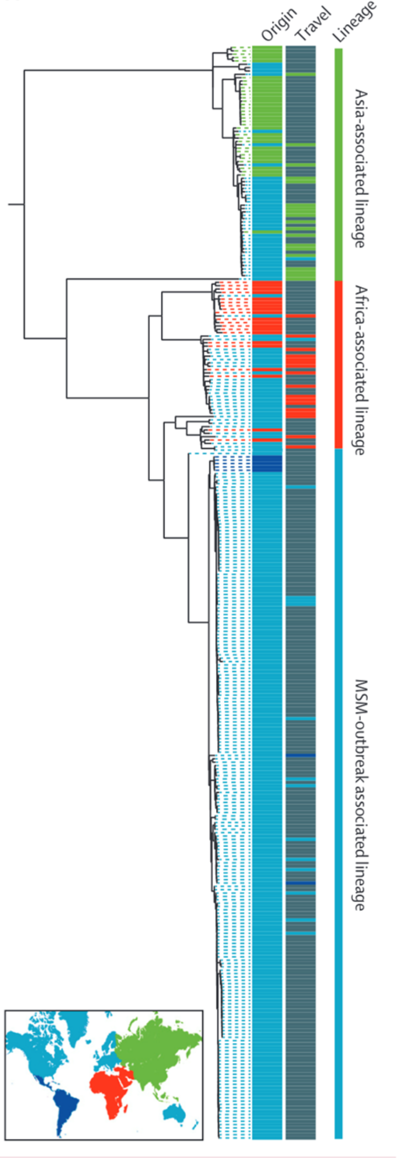
Cases associated with isolates from Asia or travel to Asia

Cases associated with isolates from Africa or travel to Africa

No travel to high risk areas for foodborne acquisition

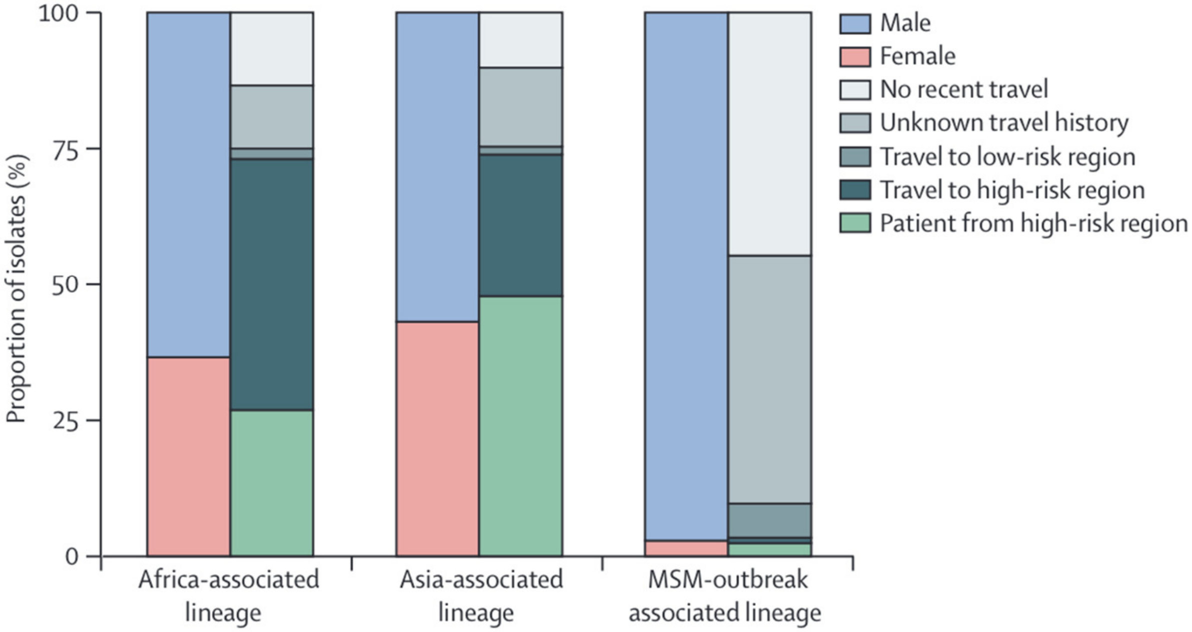


A

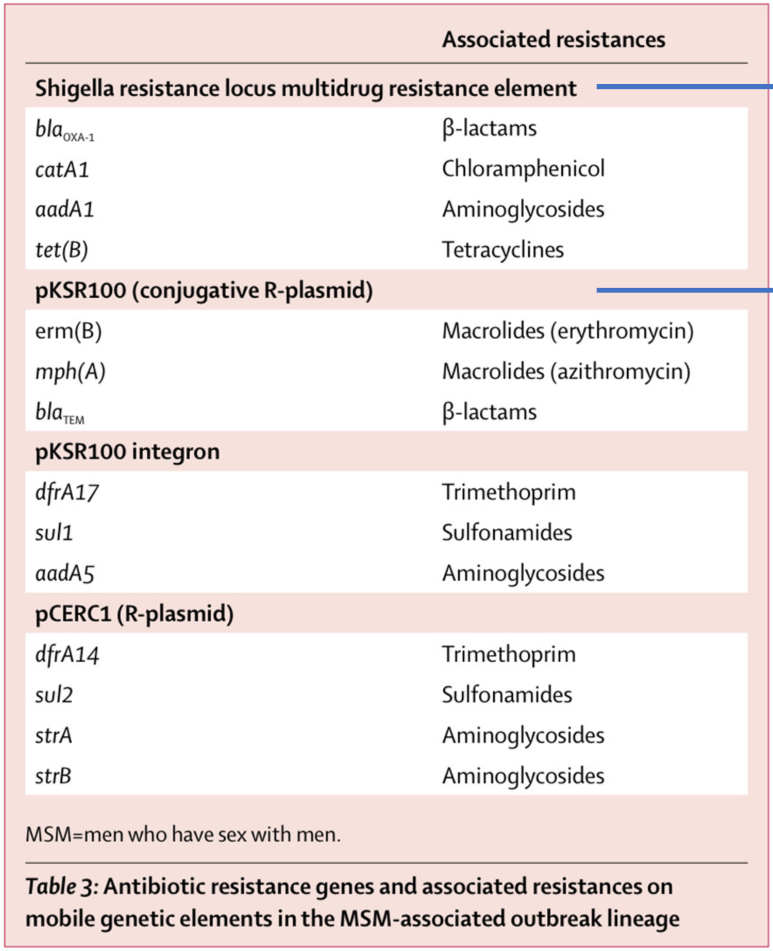


MSM-outbreak lineage found predominant in men without a history of recent travel; of those with data on sexuality 40/41 MSM had MSM-outbreak lineage

C



Antibiotic resistance



	Associated resistances
Shigella resistance locus multidrug resistance element	
$bla_{\text{OXA-1}}$	β-lactams
catA1	Chloramphenicol
aadA1	Aminoglycosides
tet(B)	Tetracyclines
pKSR100 (conjugative R-plasmid)	
erm(B)	Macrolides (erythromycin)
mph(A)	Macrolides (azithromycin)
bla_{TEM}	β-lactams
pKSR100 integron	
dfrA17	Trimethoprim
sul1	Sulfonamides
aadA5	Aminoglycosides
pCERC1 (R-plasmid)	
dfrA14	Trimethoprim
sul2	Sulfonamides
strA	Aminoglycosides
strB	Aminoglycosides

MSM=men who have sex with men.

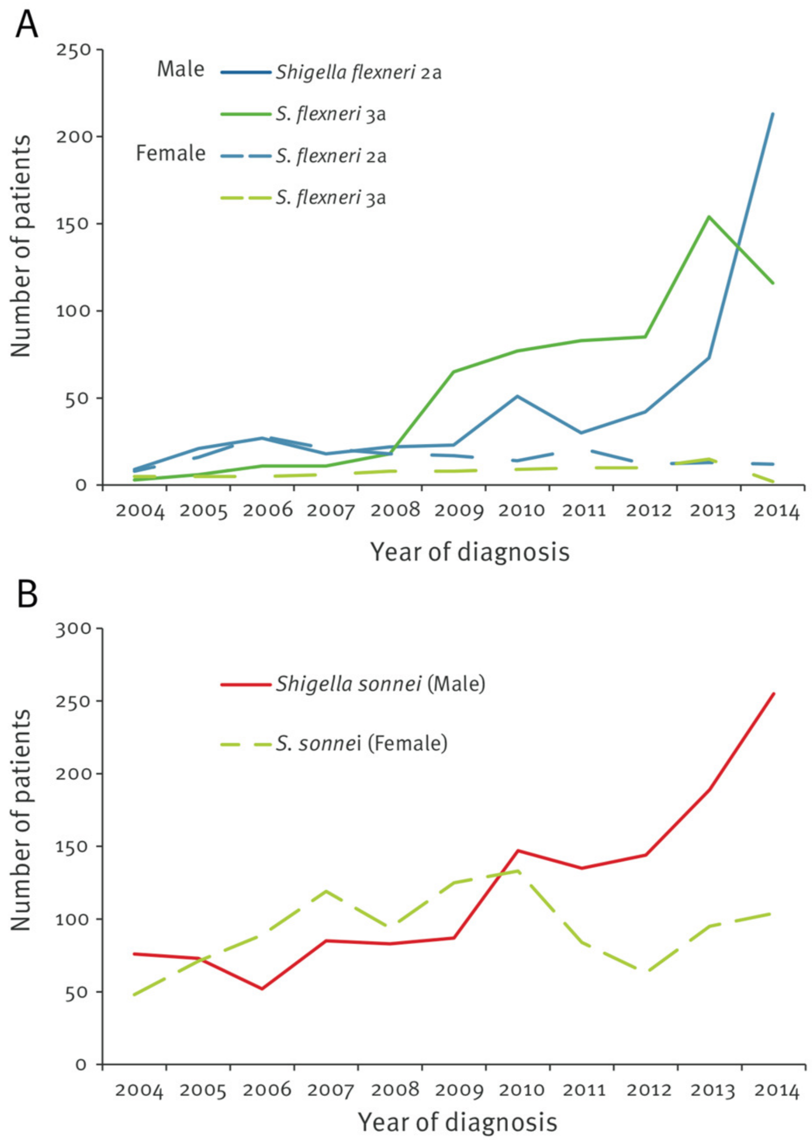
Table 3: **Antibiotic resistance genes and associated resistances on mobile genetic elements in the MSM-associated outbreak lineage**

In all isolates

In common isolates from 2009 onward
Macrolide resistance important in success of MSM-outbreak lineage

Also associated with other serotypes, e.g. 2a

and *S. sonnei*



Eurosurveillance

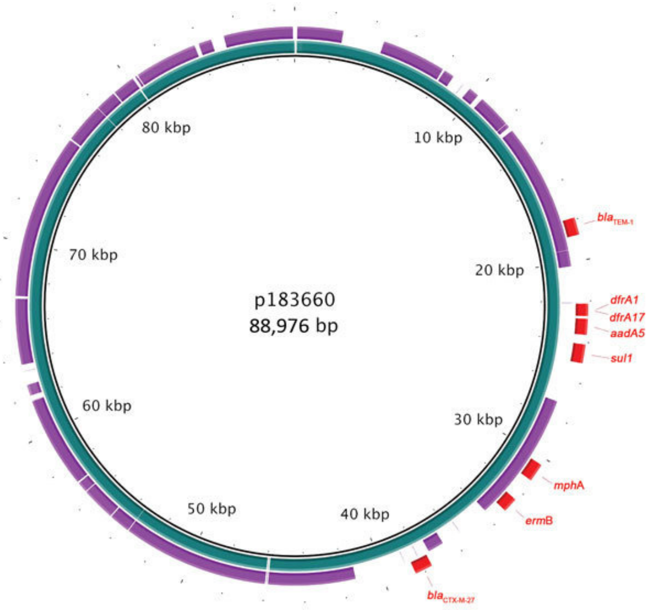
Intensified shigellosis epidemic associated with sexual transmission in men who have sex with men - *Shigella flexneri* and *S. sonnei* in England, 2004 to end of February 2015

ESBL-Producing and Macrolide-Resistant *Shigella sonnei* Infections among Men Who Have Sex with Men, England, 2015

EMERGING INFECTIOUS DISEASES

Shigella sonnei

- In 2015, *Shigella sonnei*, isolated in 4 men in London
  - Macrolide resistant, but also ESBL
- pKRS100-like plasmid found (same as *S. flexneri* 3a outbreak), with additional $\mathit{bla}_{\text{CTX-M-27}}$ gene
- Look back over WGS of all *S. sonnei* sent to PHE identified 9 patients within 5 SNPs, 7 identified as MSM, but no epidemiological links found between them



Tracking XDR gonorrhoea

- March 2018 – **XDR gonorrhoea** diagnosed
  - Ceftriaxone resistant and high-level azithromycin resistance
  - UK resident heterosexual male
  - Recent sexual contact with female in Thailand
- Key **public health questions**
  - **Where did this strain come from**? → is it circulating elsewhere?
  - **How has it become resistant**?
  - What has this strain evolved from? What is its **potential to spread**?

Man has 'world's worst' super-gonorrhoea

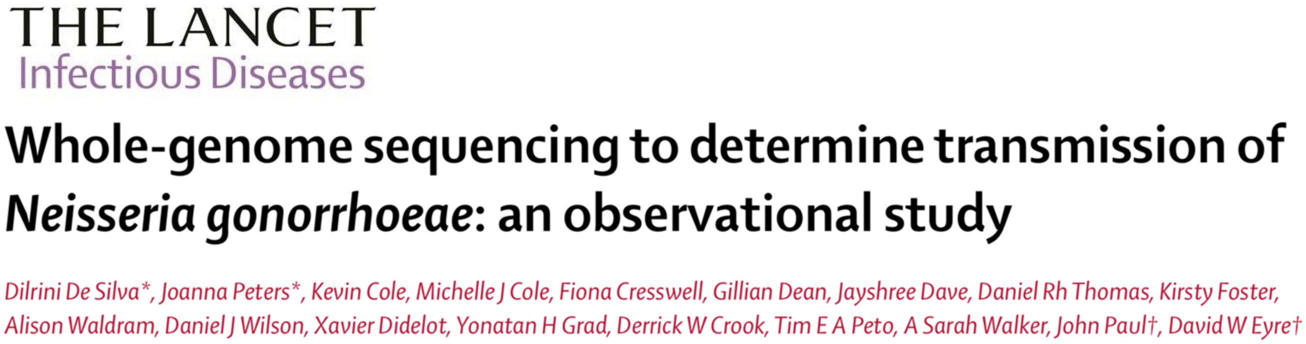
By James Gallagher
Health and science correspondent, BBC News

28 March 2018

GETTY IMAGES

How do we use WGS to identify person-to-person transmission?

- Context from previous studies: 1407 sequences obtained from Brighton from 1061 genetically distinct infections
- **How many genetic differences do we expect between recently transmitted genomes?**
  - **SNPs, single nucleotide polymorphisms**



THE LANCET
Infectious Diseases

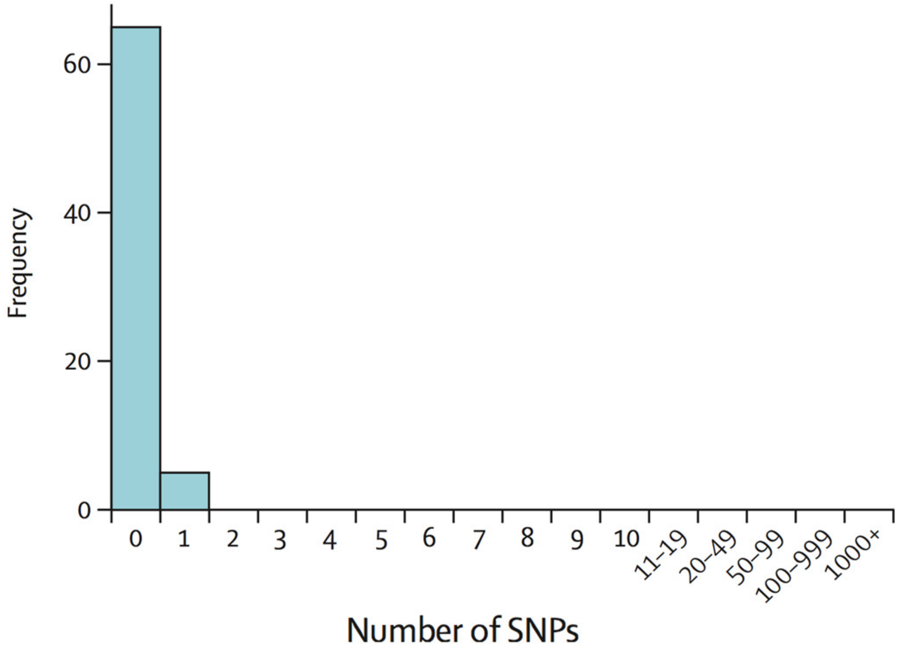
Whole-genome sequencing to determine transmission of *Neisseria gonorrhoeae*: an observational study

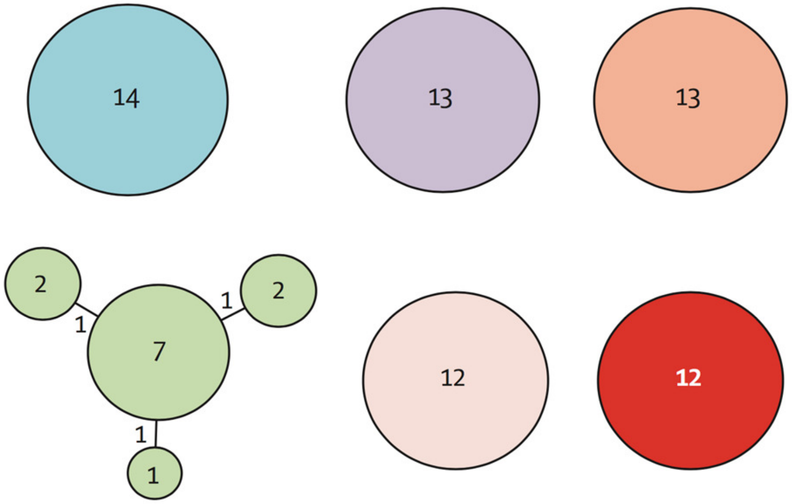
Dilrini De Silva, Joanna Peters*, Kevin Cole, Michelle J Cole, Fiona Cresswell, Gillian Dean, Jayshree Dave, Daniel Rh Thomas, Kirsty Foster, Alison Waldram, Daniel J Wilson, Xavier Didelot, Yonatan H Grad, Derrick W Crook, Tim E A Peto, A Sarah Walker, John Paul†, David W Eyre†*

Lancet Infect Dis 2016; 16: 1295–303

Within-sample diversity:

12-14 colonies picked from 6 randomly chosen samples

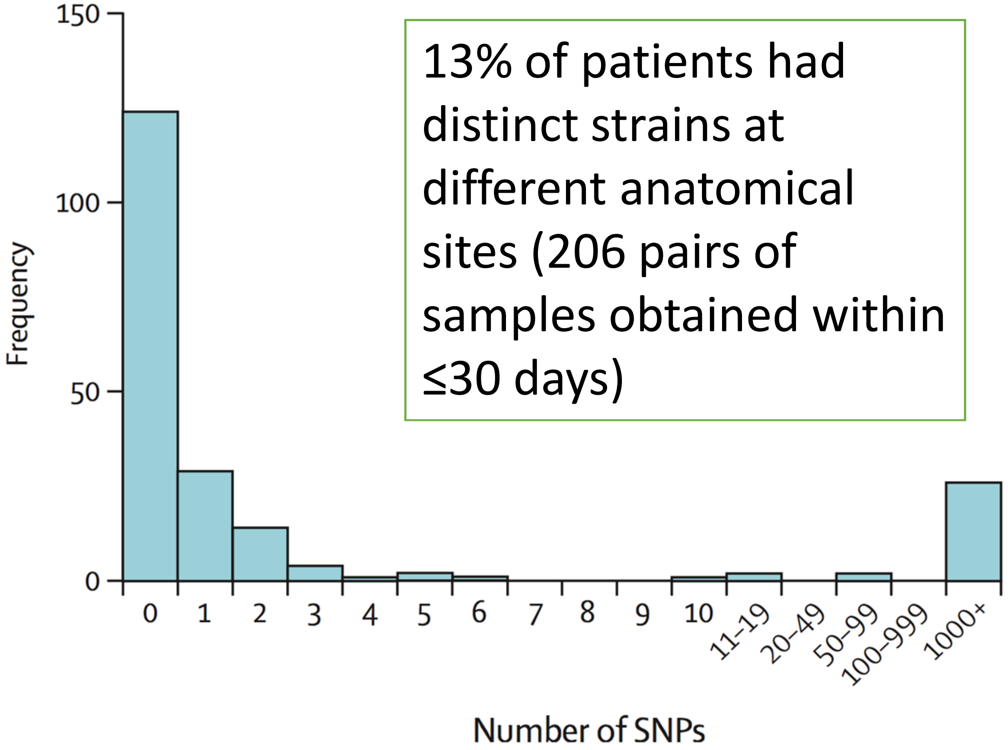




Minimal within sample diversity, a sweep of growth sequenced for remaining samples

Anatomical site diversity:

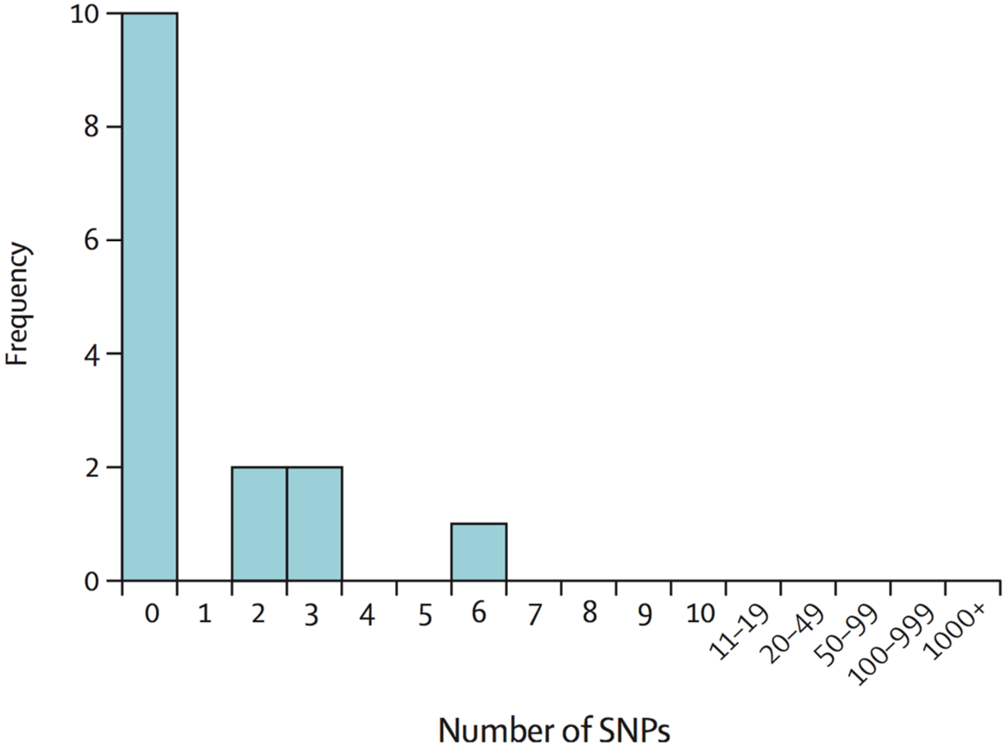
Opportunistic sequencing of all patients
with samples from more than one site



13% of patients had distinct strains at different anatomical sites (206 pairs of samples obtained within ≤30 days)

Transmission pairs

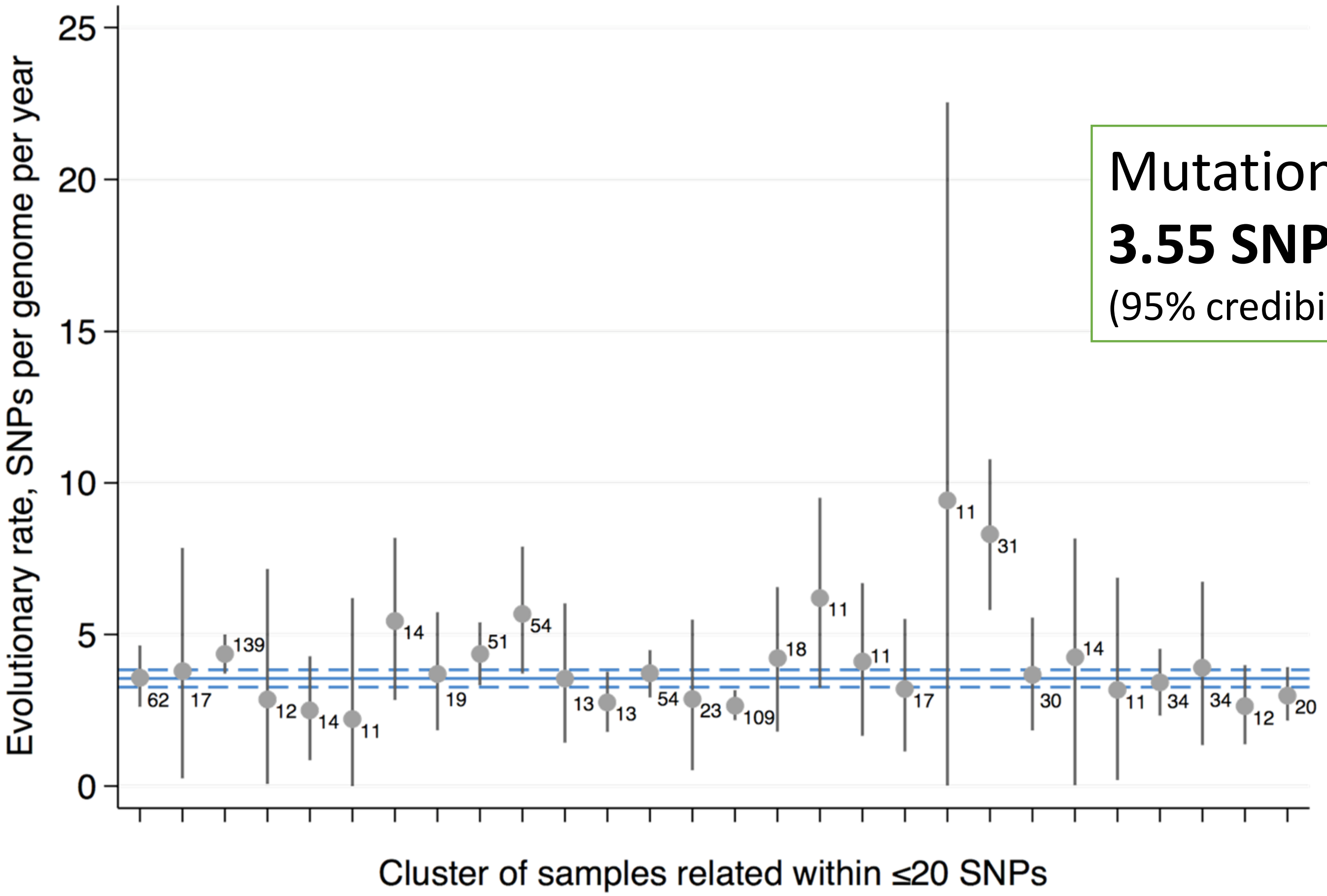
30 samples from 15 contact pairs



Both used together to **estimate within-host diversity**

Evolutionary rates:

Very little chronic infection, so estimated from time-scaled phylogenies



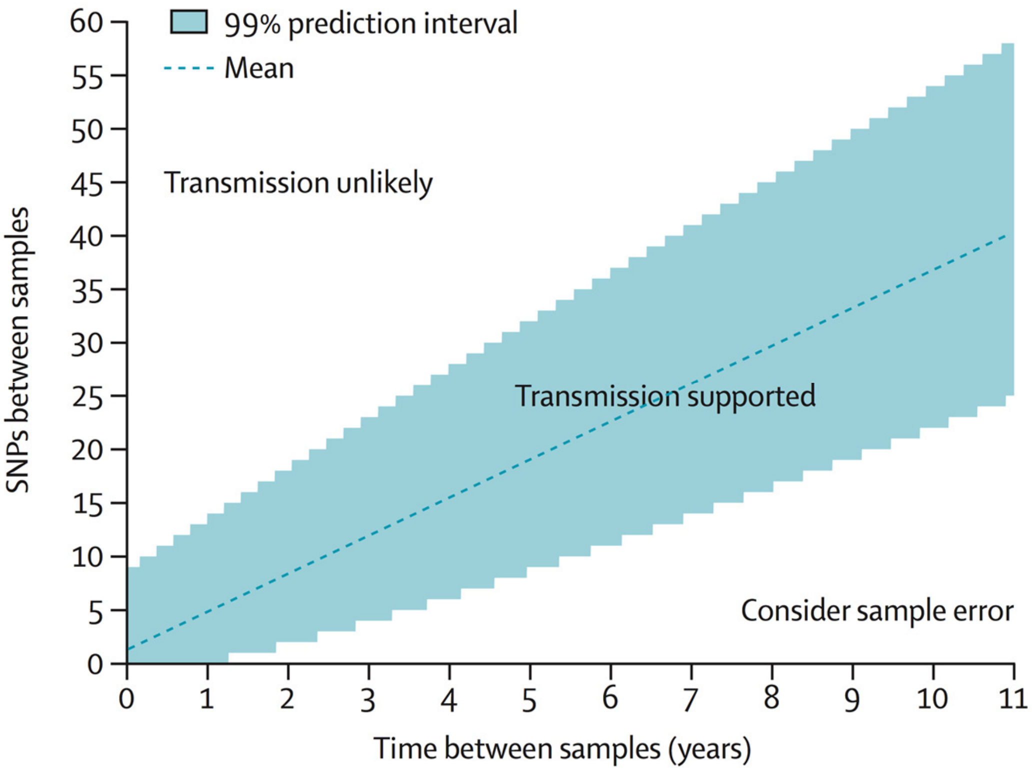
Mutation rate:

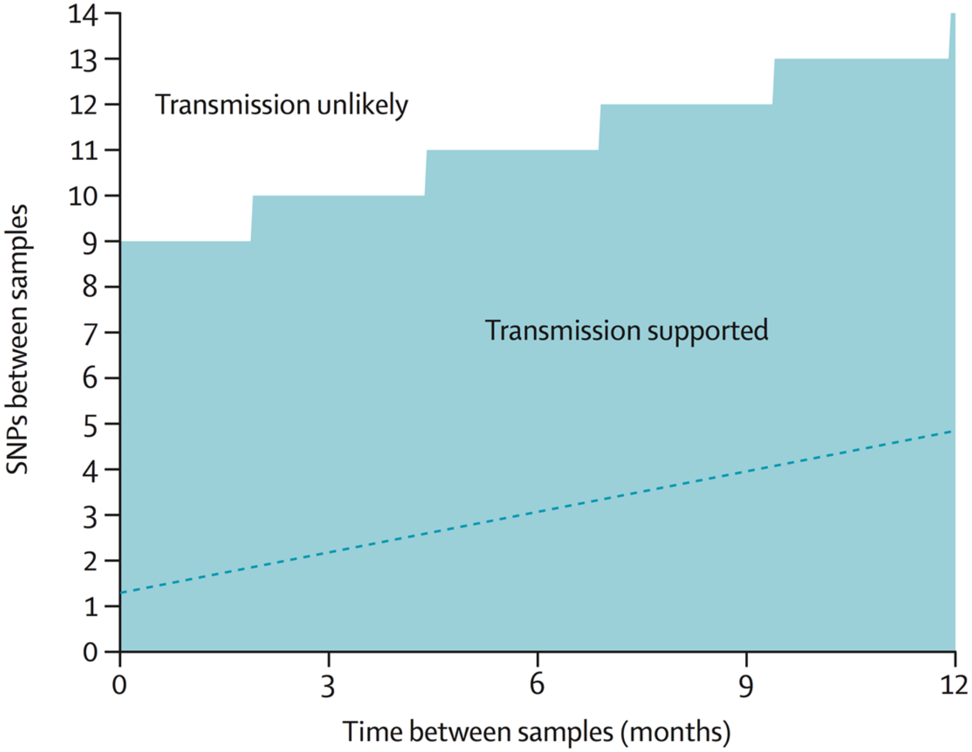
3.55 SNPs / genome / year

(95% credibility interval 3.27–3.83)

Transmission nomogram

Number of SNPs expected between isolates linked by direct or indirect transmission





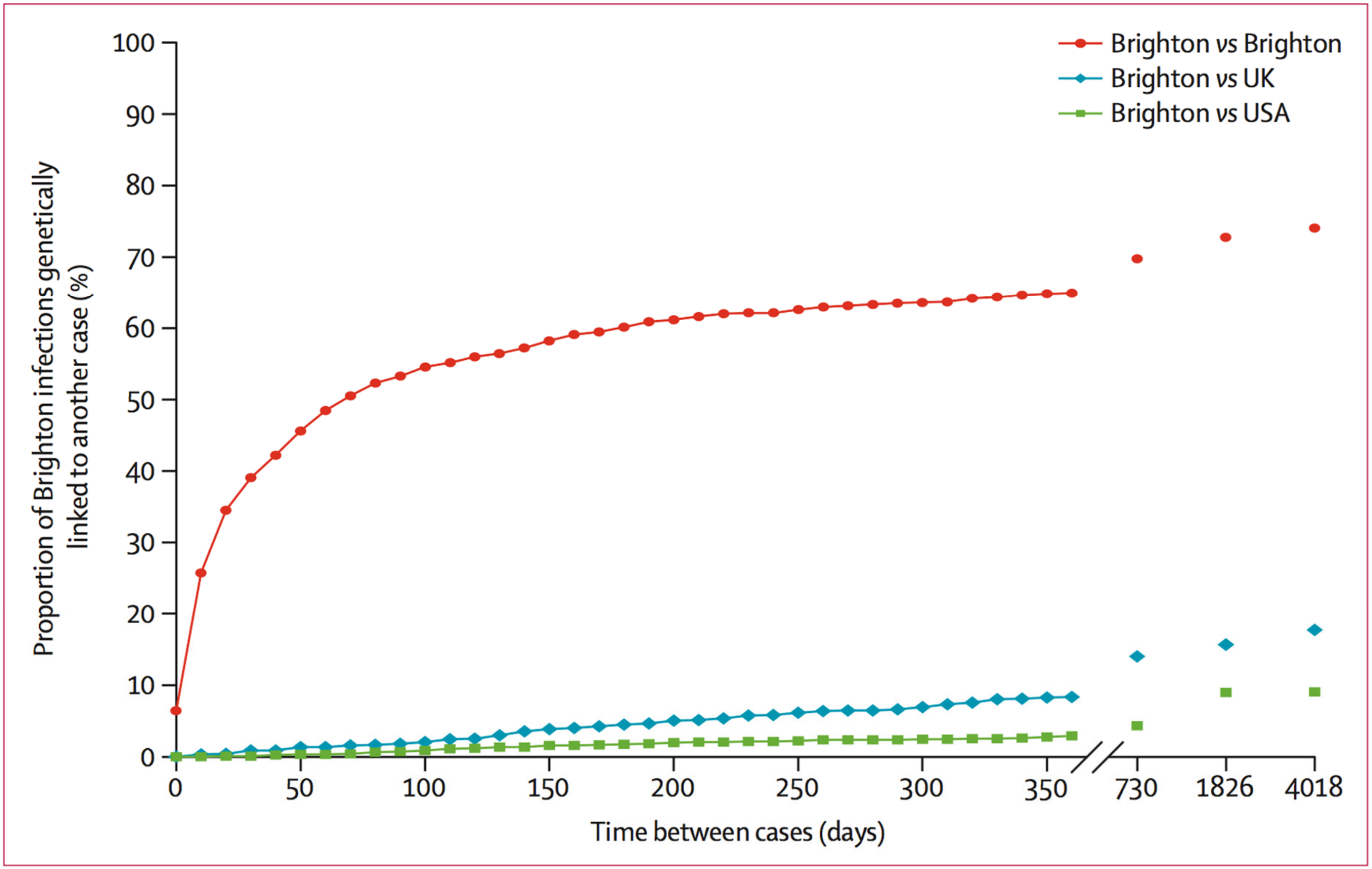
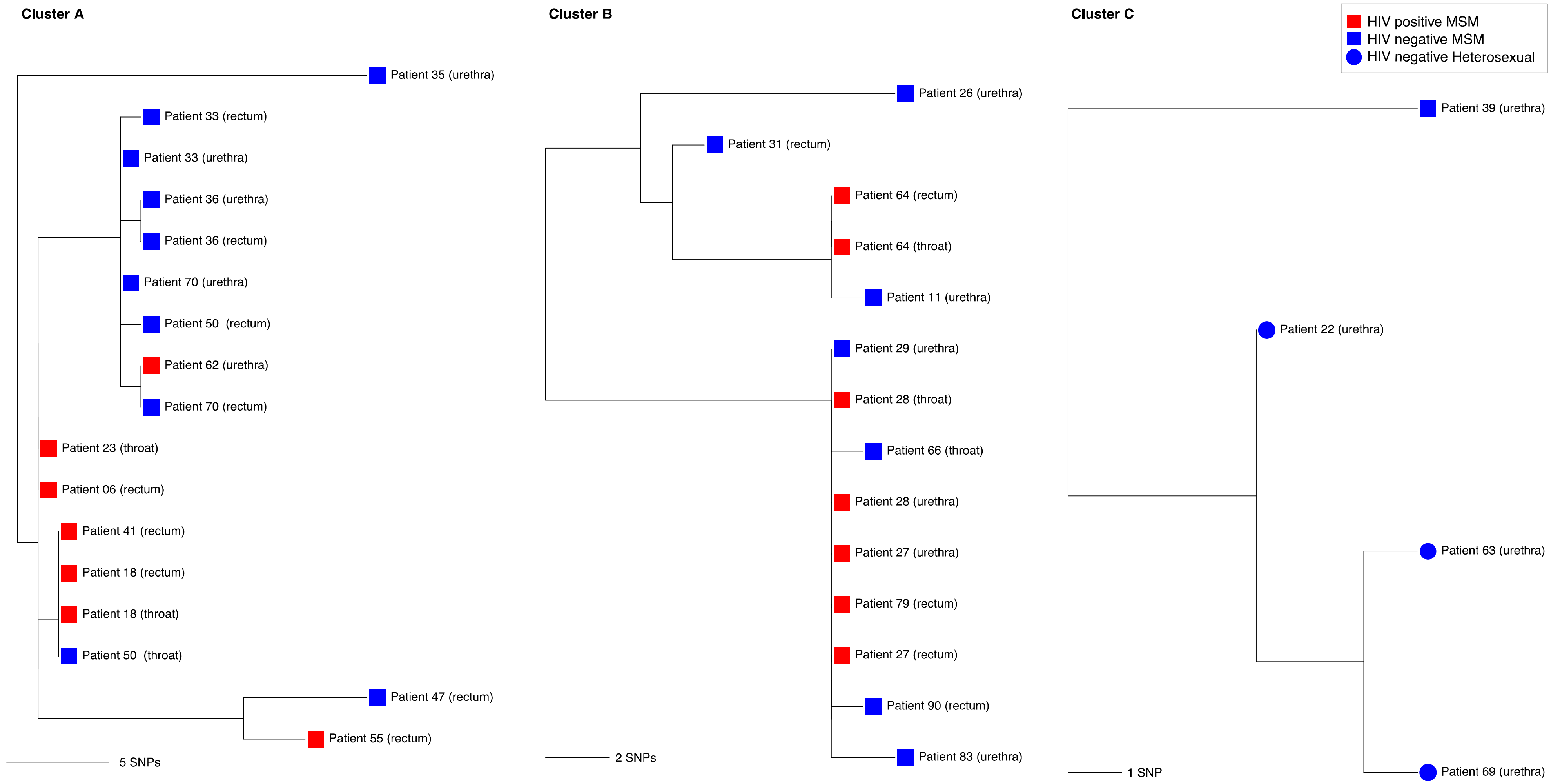


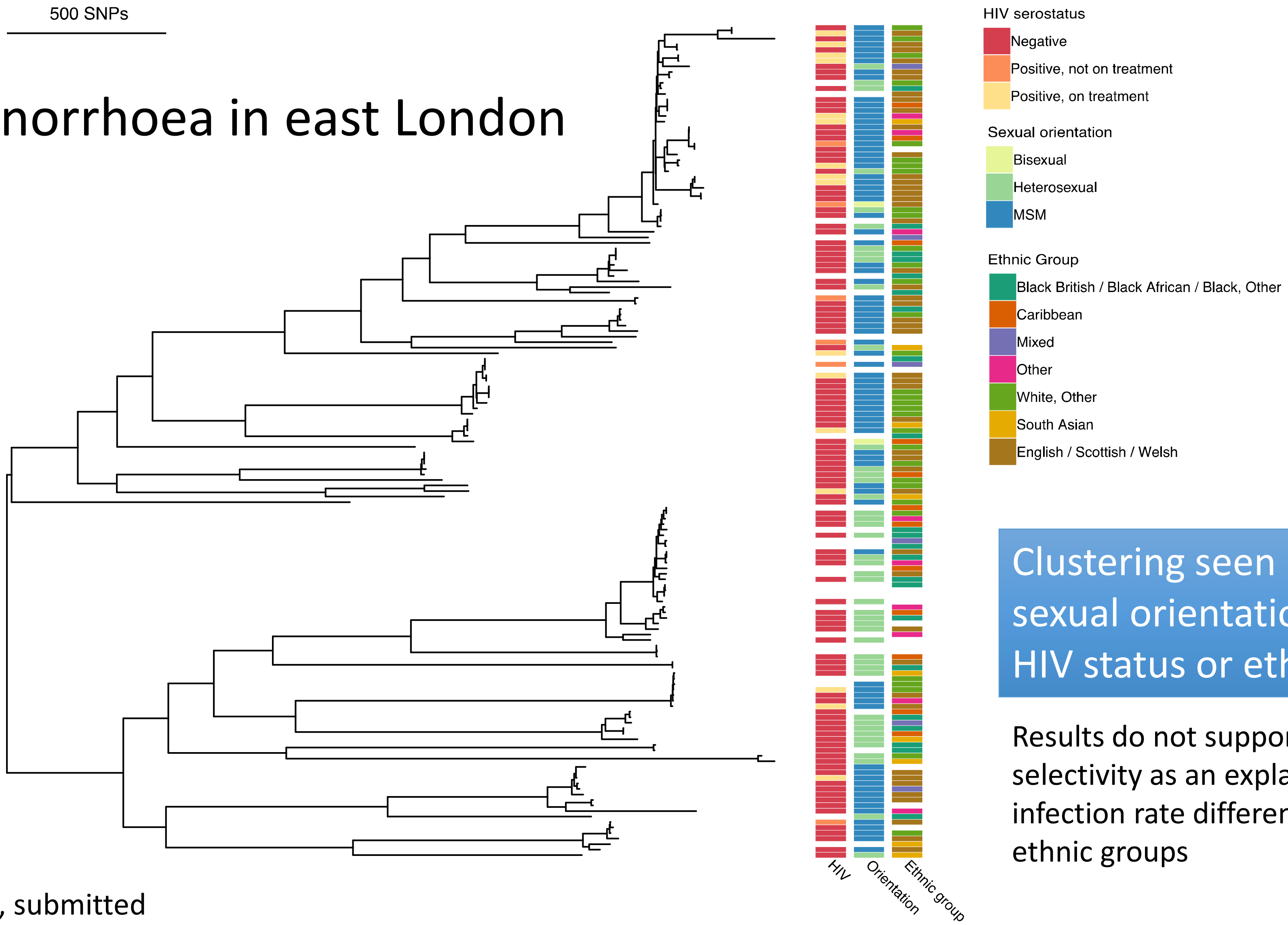
Figure 3: **Proportion of Brighton gonorrhoea infections genetically linked to another sampled case**

Evidence of mixed HIV sero-status transmission clusters



Peters *et al*, 2017, STI

Gonorrhoea in east London



Clustering seen by reported sexual orientation, but not HIV status or ethnicity

Results do not support assortative selectivity as an explanation for infection rate differences between ethnic groups

Dave *et al*, submitted

Detecting antibiotic resistance mechanisms

- 681 samples sequenced from UK, USA, Canada, and WHO reference collections
- MICs determined by gold standard agar dilution in national surveillance
- Catalogue of common resistance determinants identified

Overall predict MIC to nearest doubling dilution 54% of assays and to within 1 doubling dilution 93% and to within 2 doubling dilutions 98%

Eyre et al, 2018 JAC

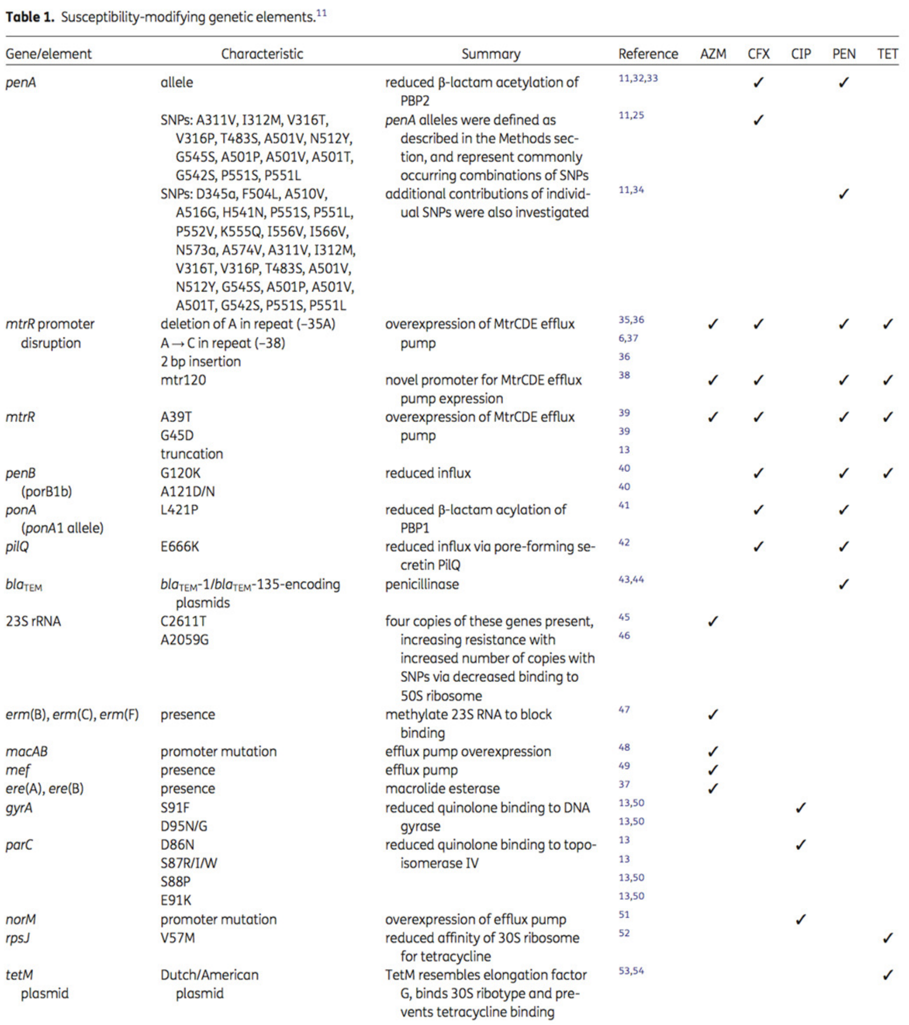
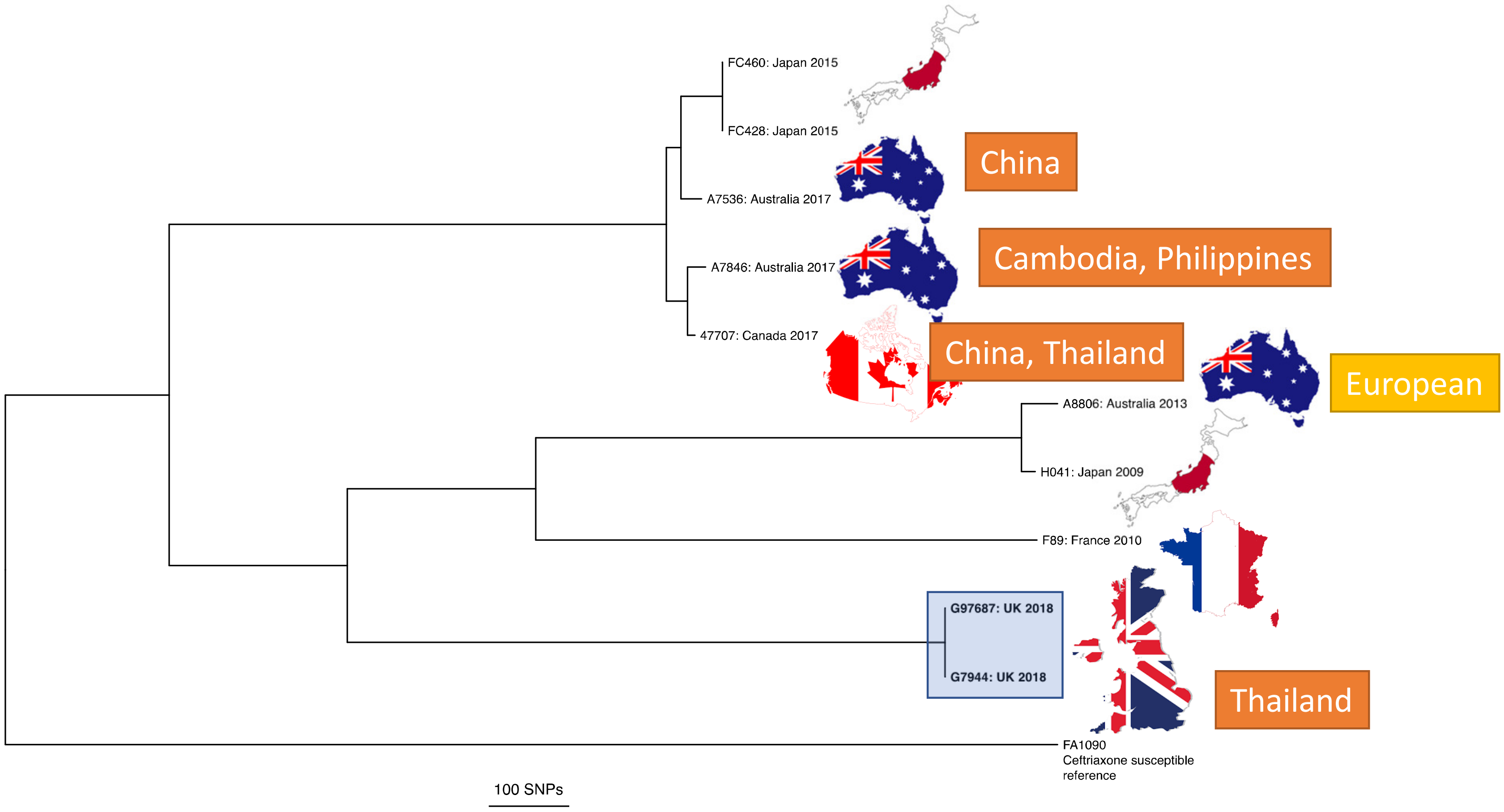


Table 1. Susceptibility-modifying genetic elements.[11]

Gene/element	Characteristic	Summary	Reference	AZM	CFX	CIP	PEN	TET
penA	allele	reduced β-lactam acetylation of PBP2	11,32,33		✓		✓	
	SNPs: A311V, I312M, V316T, V316P, T483S, A501V, N512Y, G545S, A501P, A501V, A501T, G542S, P551S, P551L	*penA* alleles were defined as described in the Methods section, and represent commonly occurring combinations of SNPs	11,25		✓			
	SNPs: D345a, F504L, A510V, A516G, H541N, P551S, P551L, P552V, K555Q, I556V, I566V, N573a, A574V, A311V, I312M, V316T, V316P, T483S, A501V, N512Y, G545S, A501P, A501V, A501T, G542S, P551S, P551L	additional contributions of individual SNPs were also investigated	11,34				✓	
mtrR promoter disruption	deletion of A in repeat (−35A) A → C in repeat (−38) 2 bp insertion	overexpression of MtrCDE efflux pump	35,36 6,37 36	✓	✓		✓	✓
	mtr120	novel promoter for MtrCDE efflux pump expression	38	✓	✓		✓	✓
mtrR	A39T G45D truncation	overexpression of MtrCDE efflux pump	39 39 13	✓	✓		✓	✓
penB (porB1b)	G120K A121D/N	reduced influx	40 40		✓		✓	✓
ponA (*ponA*1 allele)	L421P	reduced β-lactam acylation of PBP1	41		✓		✓	
pilQ	E666K	reduced influx via pore-forming secretin PilQ	42		✓		✓	
$\mathit{bla}_{\mathrm{TEM}}$	$\mathit{bla}_{\mathrm{TEM}}$-1/$\mathit{bla}_{\mathrm{TEM}}$-135-encoding plasmids	penicillinase	43,44				✓	
23S rRNA	C2611T A2059G	four copies of these genes present, increasing resistance with increased number of copies with SNPs via decreased binding to 50S ribosome	45 46	✓				
erm(B), *erm*(C), *erm*(F)	presence	methylate 23S RNA to block binding	47	✓				
macAB	promoter mutation	efflux pump overexpression	48	✓				
mef	presence	efflux pump	49	✓				
ere(A), *ere*(B)	presence	macrolide esterase	37	✓				
gyrA	S91F D95N/G	reduced quinolone binding to DNA gyrase	13,50 13,50			✓		
parC	D86N S87R/I/W S88P E91K	reduced quinolone binding to topoisomerase IV	13 13 13,50 13,50			✓		
norM	promoter mutation	overexpression of efflux pump	51			✓		
rpsJ	V57M	reduced affinity of 30S ribosome for tetracycline	52					✓
tetM plasmid	Dutch/American plasmid	TetM resembles elongation factor G, binds 30S ribotype and prevents tetracycline binding	53,54					✓

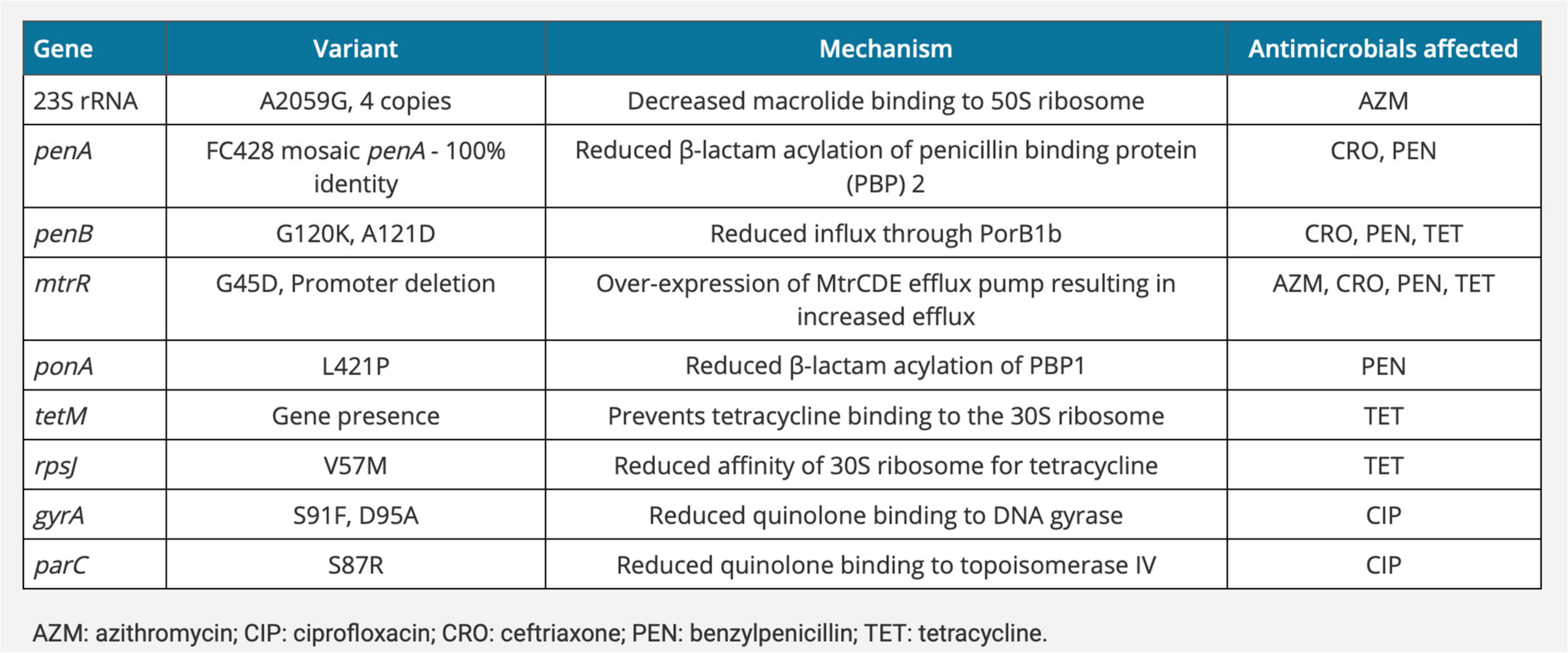
UK XDR case vs. previous ceftriaxone resistant cases



Eyre et al, 2018 Eurosurveillance

What about resistance?

Ceftriaxone resistance from the most successful penA gene variant to date, azithromycin from the most common high-level resistance mutation



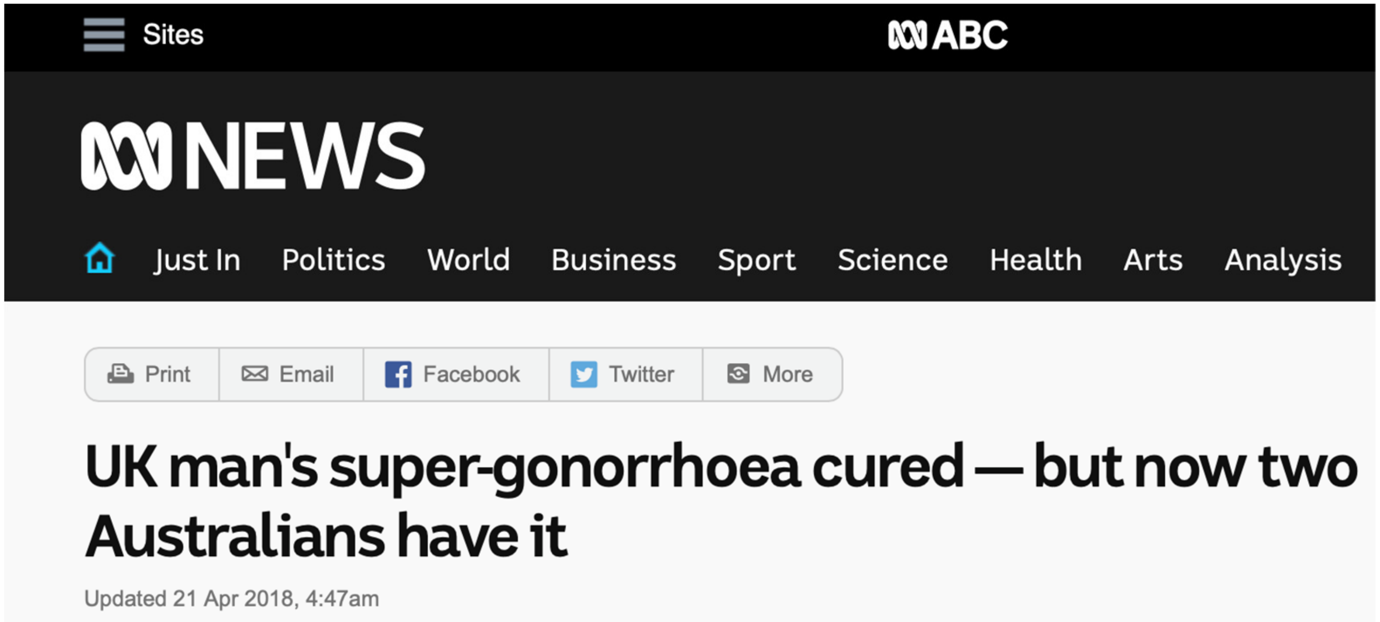
Gene	Variant	Mechanism	Antimicrobials affected
23S rRNA	A2059G, 4 copies	Decreased macrolide binding to 50S ribosome	AZM
penA	FC428 mosaic *penA* - 100% identity	Reduced β-lactam acylation of penicillin binding protein (PBP) 2	CRO, PEN
penB	G120K, A121D	Reduced influx through PorB1b	CRO, PEN, TET
mtrR	G45D, Promoter deletion	Over-expression of MtrCDE efflux pump resulting in increased efflux	AZM, CRO, PEN, TET
ponA	L421P	Reduced β-lactam acylation of PBP1	PEN
tetM	Gene presence	Prevents tetracycline binding to the 30S ribosome	TET
rpsJ	V57M	Reduced affinity of 30S ribosome for tetracycline	TET
gyrA	S91F, D95A	Reduced quinolone binding to DNA gyrase	CIP
parC	S87R	Reduced quinolone binding to topoisomerase IV	CIP

AZM: azithromycin; CIP: ciprofloxacin; CRO: ceftriaxone; PEN: benzylpenicillin; TET: tetracycline.

Comparing the UK isolate to all 7,812 sequences available for *N. gonorrhoeae*

- Rapid search to find 100 most closely related
- Precision tree for these 100

A further development…



UK man's super-gonorrhoea cured — but now two Australians have it

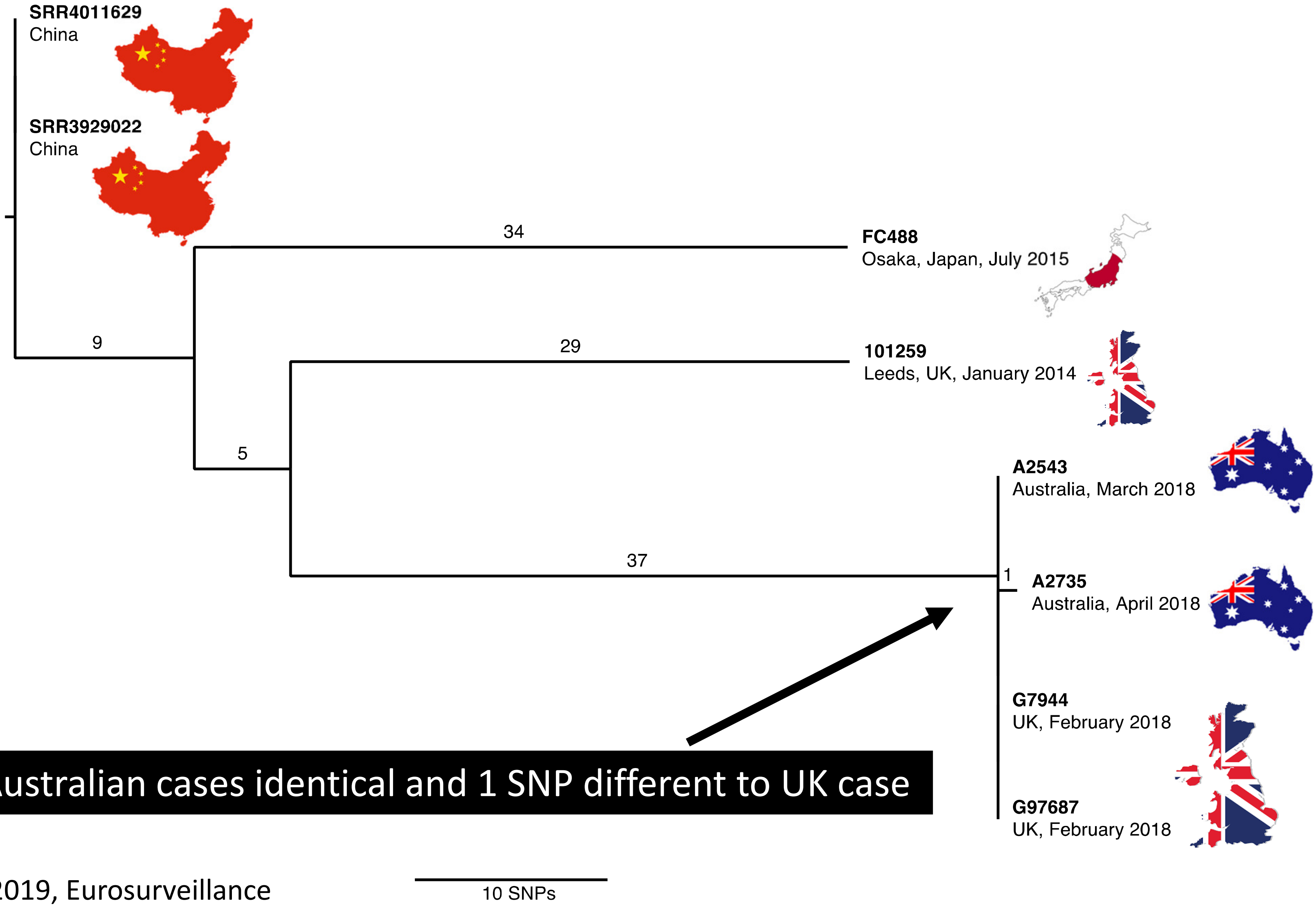
Updated 21 Apr 2018, 4:47am

ABC AUSTRALIA

Two Australian residents

- A male who reported sexual contact with a female in South East Asia
- A female who had no travel history outside of Australia

Both had ceftriaxone-resistant gonorrhoea with high-level azithromycin resistance



Australian cases identical and 1 SNP different to UK case

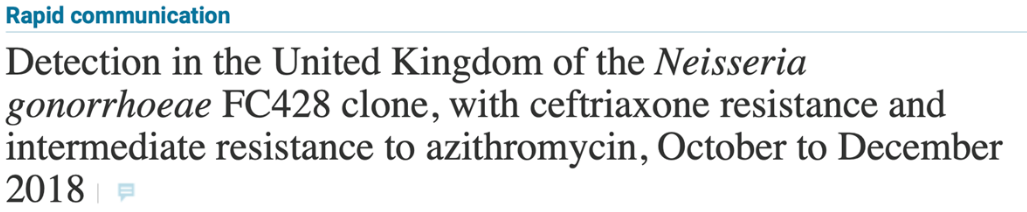
Jennison et al, 2019, Eurosurveillance

European transmission of ceftriaxone resistant gonorrhoea October – December 2018

- Two female patients developed gonorrhoea following contact with UK-resident men from the same sexual network linked to travel to Ibiza, Spain
- Ceftriaxone resistant, intermediate susceptible to azithromycin

Both cases were genetically identical – suggesting recent common source

Eurosurveillance

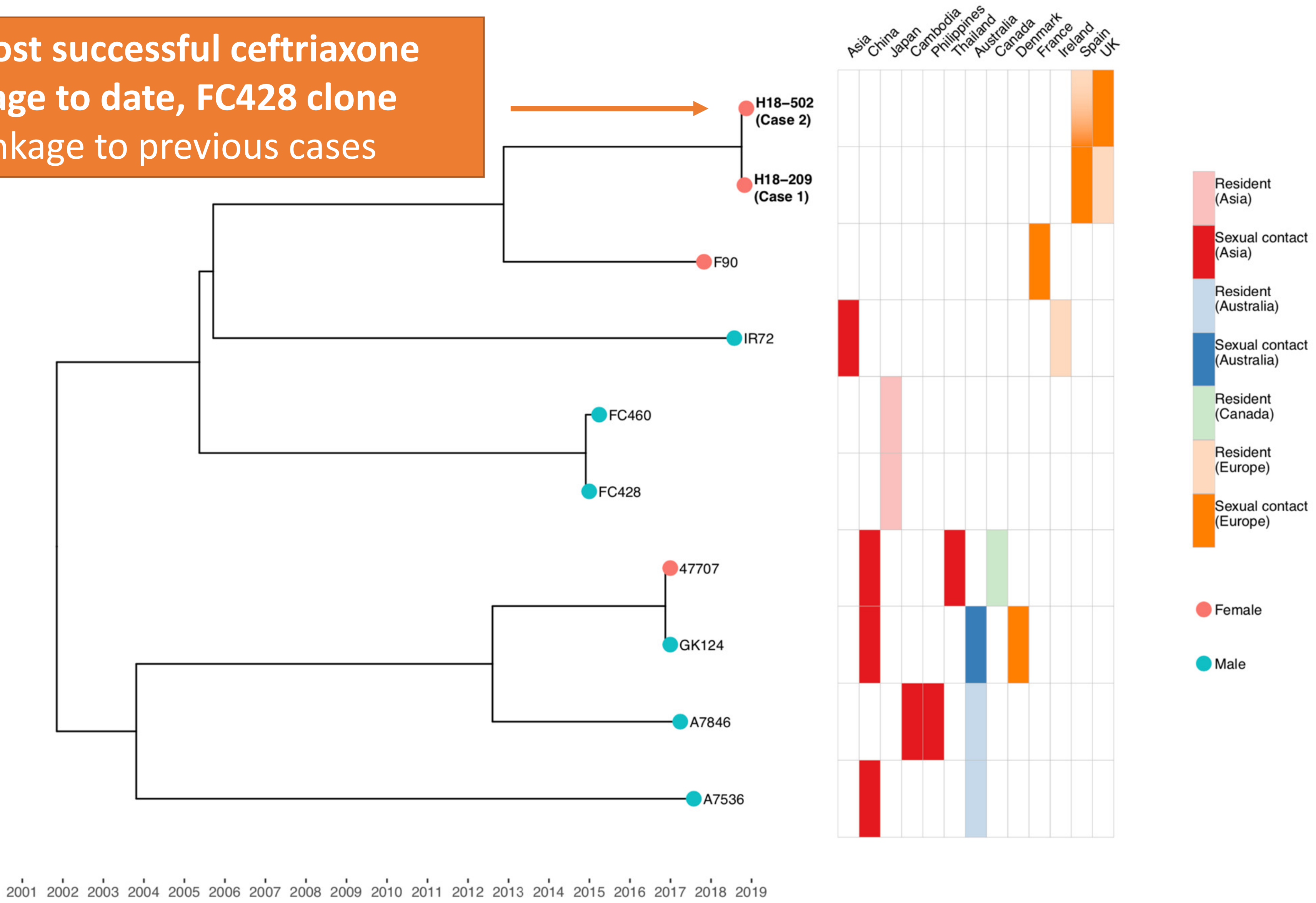


Rapid communication

Detection in the United Kingdom of the *Neisseria gonorrhoeae* FC428 clone, with ceftriaxone resistance and intermediate resistance to azithromycin, October to December 2018

Part of the most successful ceftriaxone resistant lineage to date, FC428 clone

- No direct linkage to previous cases



Potential impacts

- Able to confirm likely transmission of ceftriaxone resistant gonorrhoea in Europe → public health response and raised public awareness
- Data from all cases from the FC428 clone suggest South East Asia or China, is the likely reservoir → need for enhanced surveillance in region
- Highlights the need for multifactorial interventions – new drugs, ?vaccine, access to diagnostics, surveillance, test of cure, effective partner notification

What about sequencing as a routine diagnostic tool?

- Potential to
  - Detect presence of infection
  - Detect antibiotic resistance determinants
  - Enhance partner notification

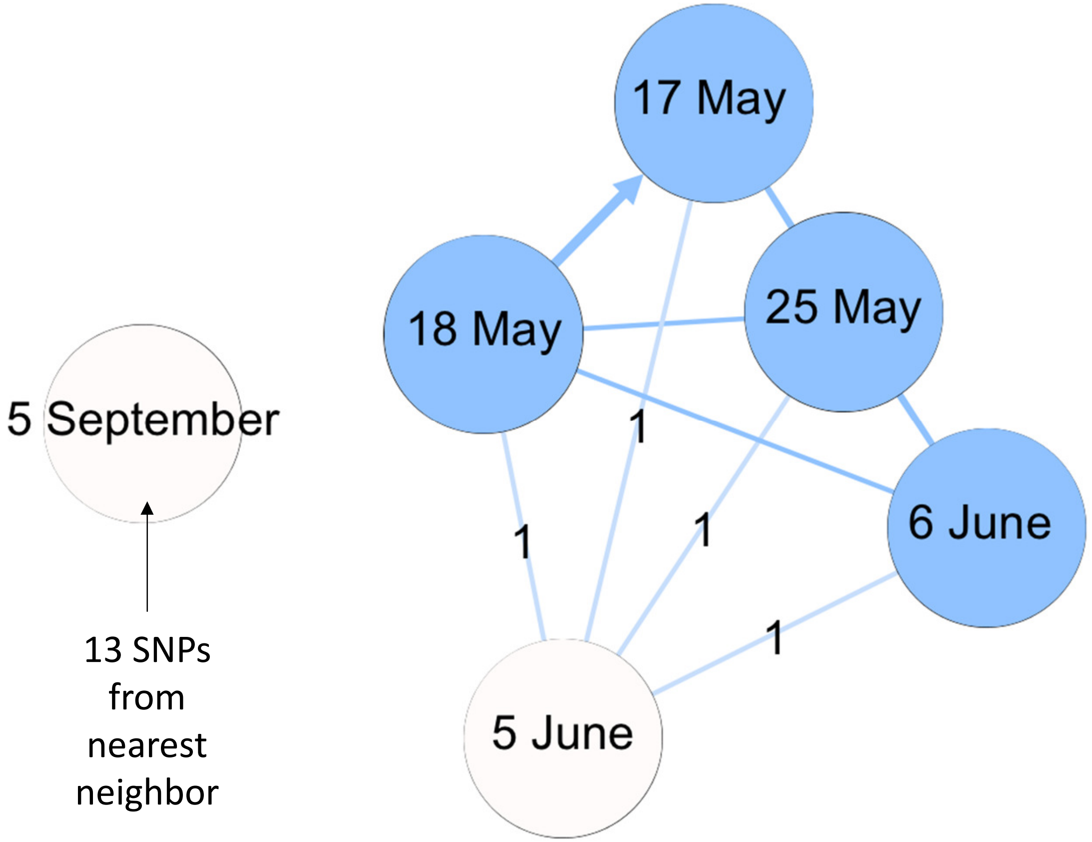
Leeds real-time sequencing study

- Illumina based sequencing of positive cultures
- Investigate the utility of WGS for routine use alongside partner notification
- Aiming to return final report based by 14 day test of cure visit

WGS enables links to be made between cases not revealed by existing partner notification

- Cluster of 6 MSM
- 4 are identical isolates
- Only one link made on the basis of partner notification

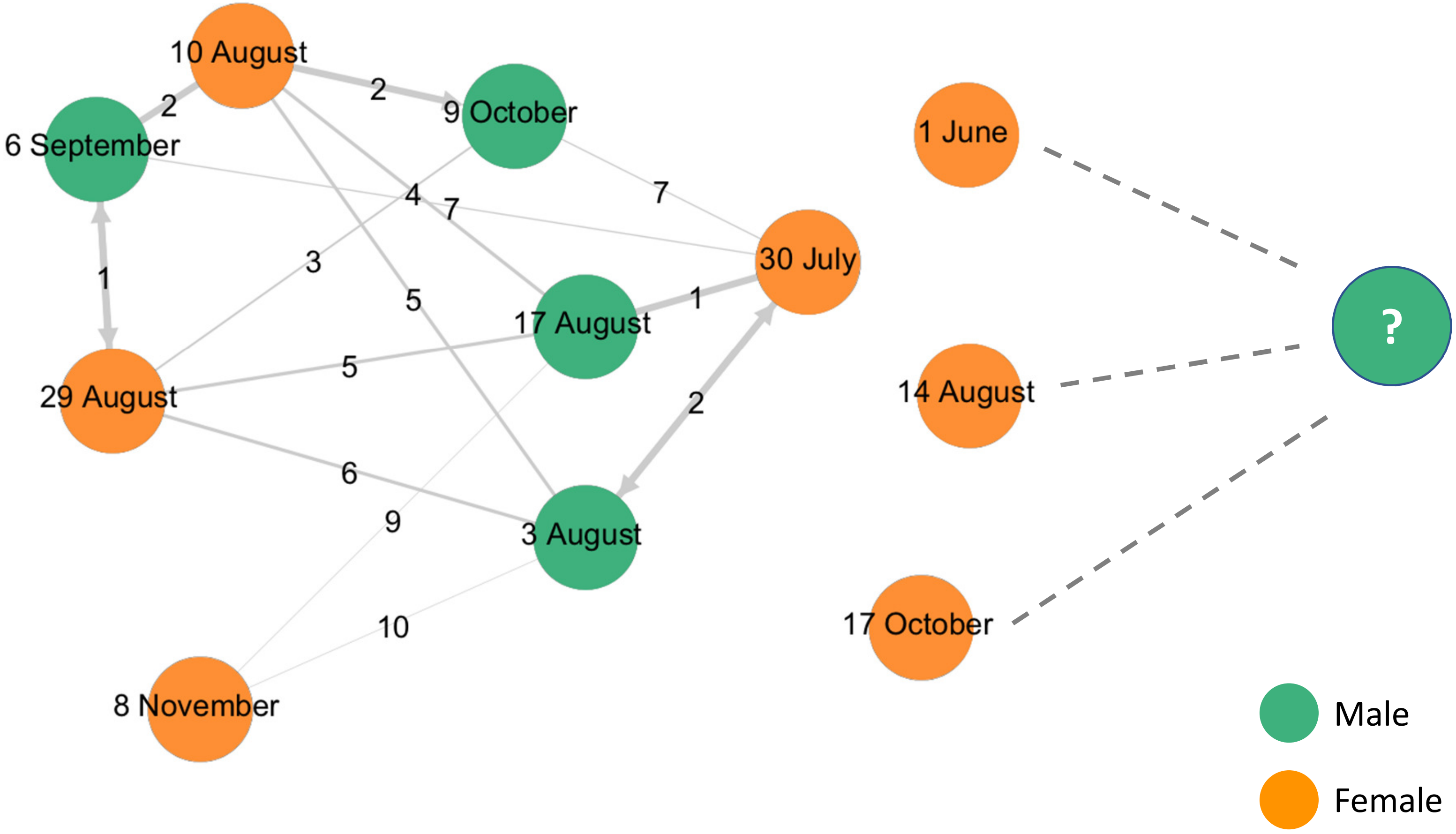
←	Link confirmed by routine PN and WGS
—	WGS linked



13 SNPs from nearest neighbor

WGS suggest the presence of unsampled common sources

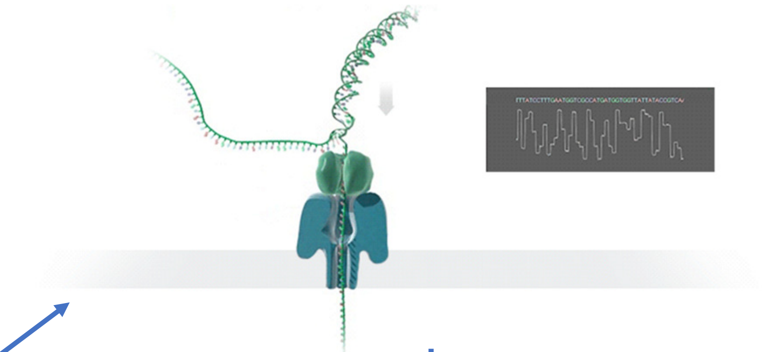
Cluster containing 3 verified couples and 3 heterosexual females without matches



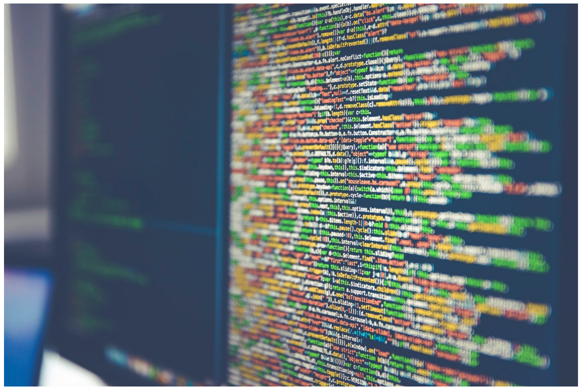
Direct from sample sequencing

Extract DNA

Sequence
20 mins → 8 hours



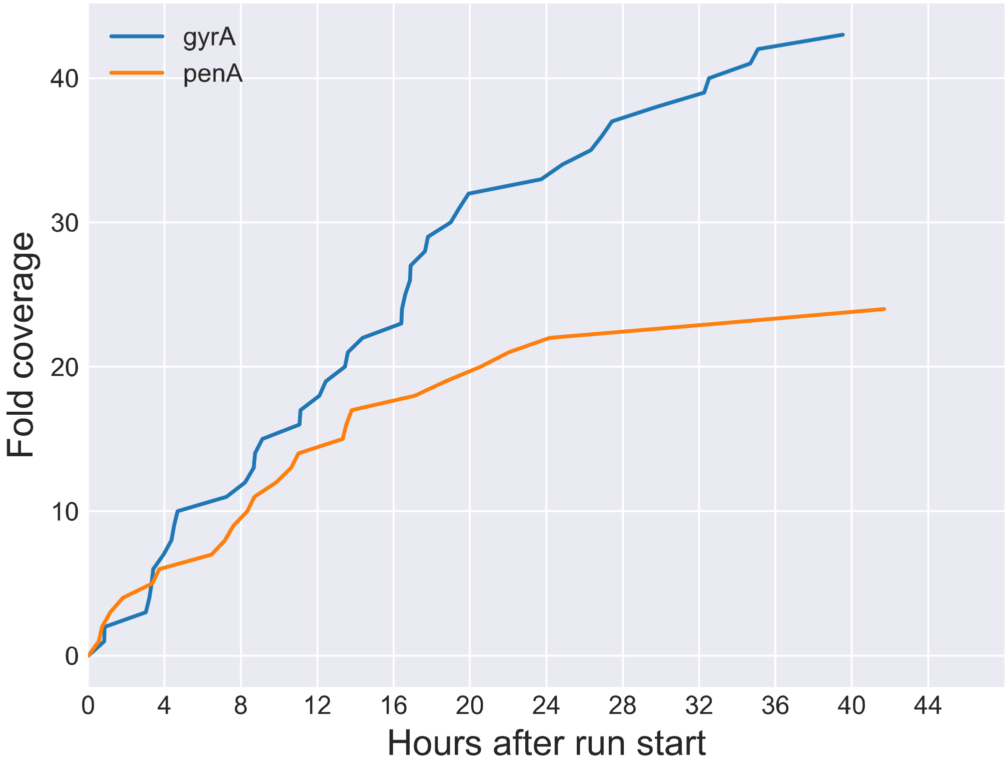
Bioinformatics



Analysis → Final report, using database of antibiotic resistance determinants and previous sequences

Direct from sample sequencing

ONT sequencing direct from a patient urine sample:
Urine sample A



Proof of principle: Resistance determinants identified within 8-12 hours

Conclusions

- Whole-genome sequencing can inform about historical and recent transmission events at a global and national scale
- Whole-genome sequencing can identify antibiotic resistant determinants and track their spread
- There is a challenge to know how best to use whole-genome sequencing in the clinic
  - It may be very helpful to identify antibiotic resistance
  - Genomic links allow the reach of partner notification to be potentially quantified
  - Active question about how to approach genomically linked cases not linked by routine partner notification

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BIG DATA INSTITUTE

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