Clinical and epidemiological characteristics of new epidemic *Clostridium difficile* strains

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Introduction

C. difficile infections (CDI) are a major antibiotic-associated clinical problem.

- **Antimicrobials can trigger and exacerbate CDI by:**
  - Disrupting the protective normal gut flora.
  - Conferring a selective advantage on resistant strains.

- **Challenge of controlling (multiply) resistant strains is evident in:**
  - Association with nosocomial outbreaks.
  - Worldwide spread of PCR-ribotype 027 after acquisition of fluoroquinolone resistance (He et al., 2013).

- **The significance of fluoroquinolones as a risk factor for outbreaks**
  - Exemplified by FQ resistant genotypes eg. 001, 017, 106 (UK) 053 (USA), 018 (Italy) which pre or post-date 027.

The problem is dynamic; fluoroquinolone resistance is a common phenotypic trait of epidemic or outbreak strains.

Fluoroquinolone Resistance in *C. difficile*

Fluoroquinolones (FQ)

Fluoroquinolones inhibit DNA replication by targeting DNA gyrase
Enzyme comprised of two subunits, encoded by *gyrA* and *gyrB.*
Functions to nick double-stranded chromosomal DNA, introduces negative supercoils, then seals the nicked DNA.

High level fluoroquinolone resistance
Results when specific amino acid substitutions occur within the DNA gyrase **q**uinolone **r**esistance **d**etermining **r**egion (QRDR).
Conferred by a single non-synonymous SNP in either *gyrA* or *gyrB* gene:

GyrA Threonine 82 to Isoleucine
GyrB Aspartic acid 426 to Asparagine or Valine.

Fluoroquinolone resistance is an easily acquired, outbreak-associated phenotypic trait

To what extent can the emergence of FQ resistant strains, combined with high levels of prescribing, explain the problem of nosocomial CDI?

Examined the question using three unique data sets:

- **UK FQ usage data (DDD) collected longitudinally (2000-2014).**
  Spans a period when antimicrobial stewardship targeting FQ use was introduced (October 2007). One of multiple policies targeting CDI.
- ***C. difficile* incidence data, concurrent with prescribing data.**
  A marked decline in UK CDI incidence began in 2007, however the contribution of reduced FQ use is unclear; multiple interventions were introduced simultaneously.
- ***C. difficile* WGS data from isolates collected longitudinally/concurrently.**
  Provide a definitive answer, allowing incidence to be examined by genetic lineage and FQ susceptibility; also phylogenetic insights.

C. difficile Whole Genome Sequences

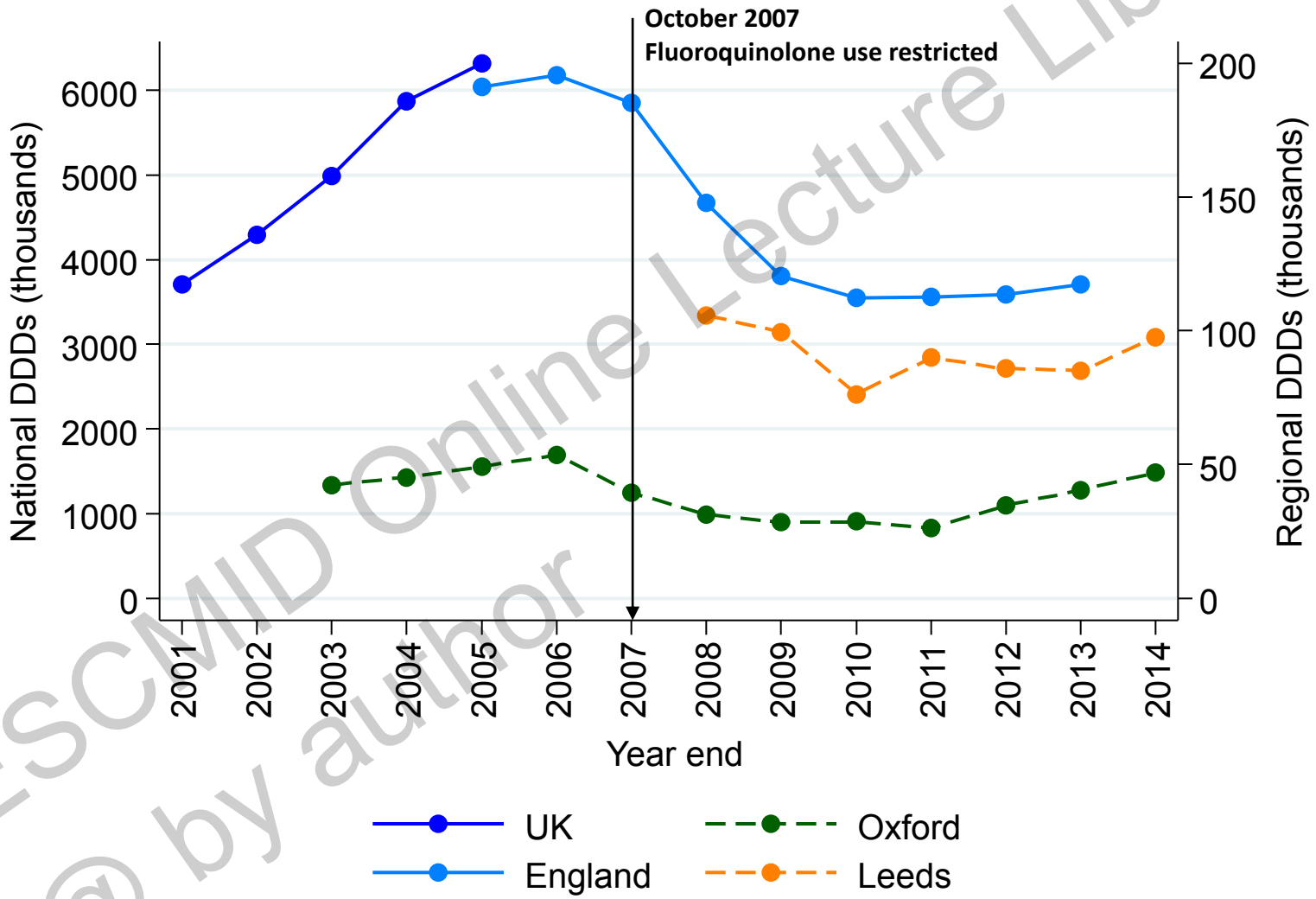
Main focus *C. difficile* Isolates from:

- Oxford clinical cases **n=2,049** (September 2006 - August 2013)
- Leeds clinical cases **n=1,024** (August 2010 - May 2015)

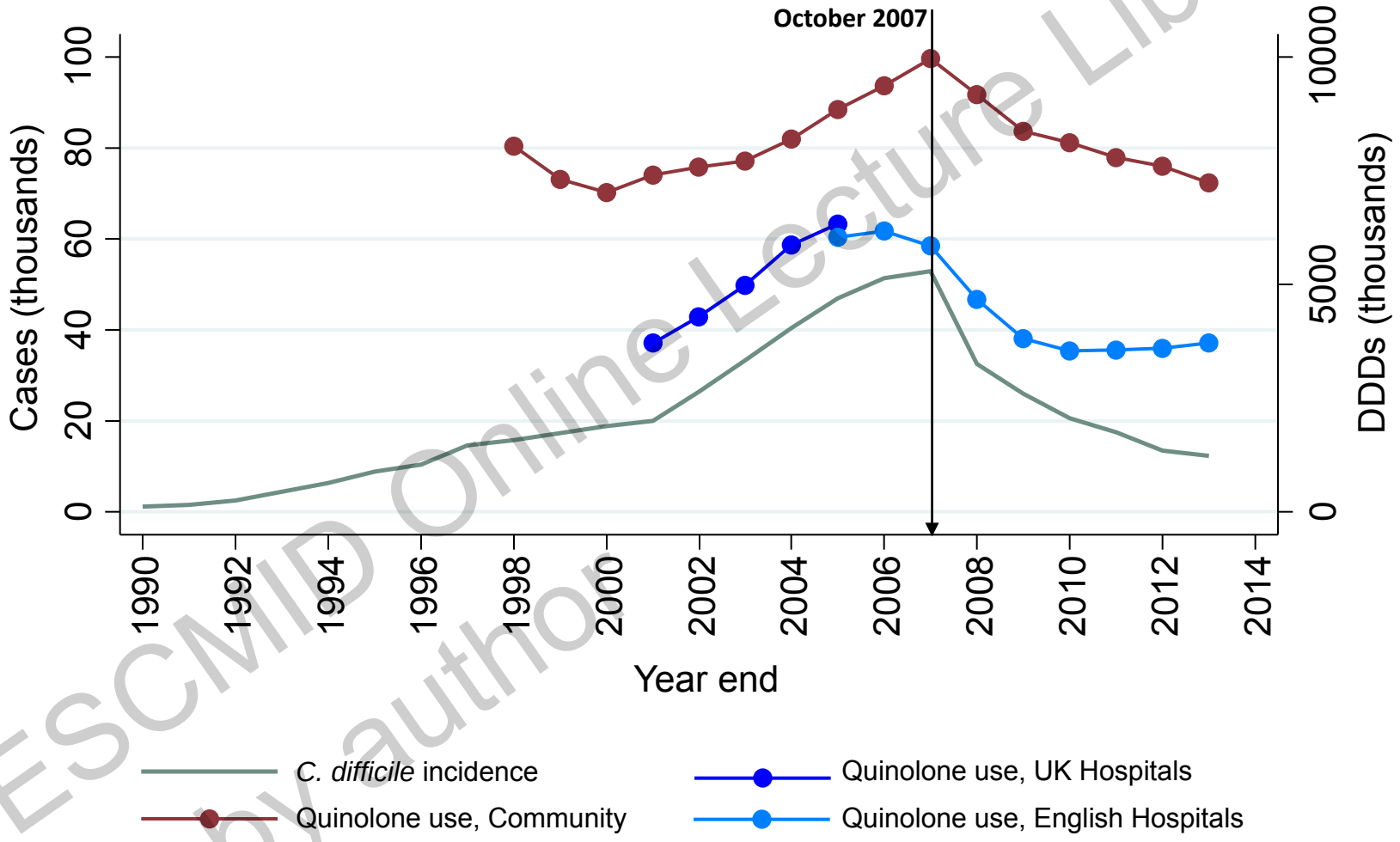
A subset of isolates from:

- Optimer fidaxomicin clinical trial, **n=830** from North America and Europe (May 2006 - Nov 2009).
- Oxford ELISA (toxin) negative 'cases' **n=183** and asymptomatic carriers **n=218** (October 2010 - April 2013).
- Oxford healthy infants **n=201** (October 2008 - July 2013).

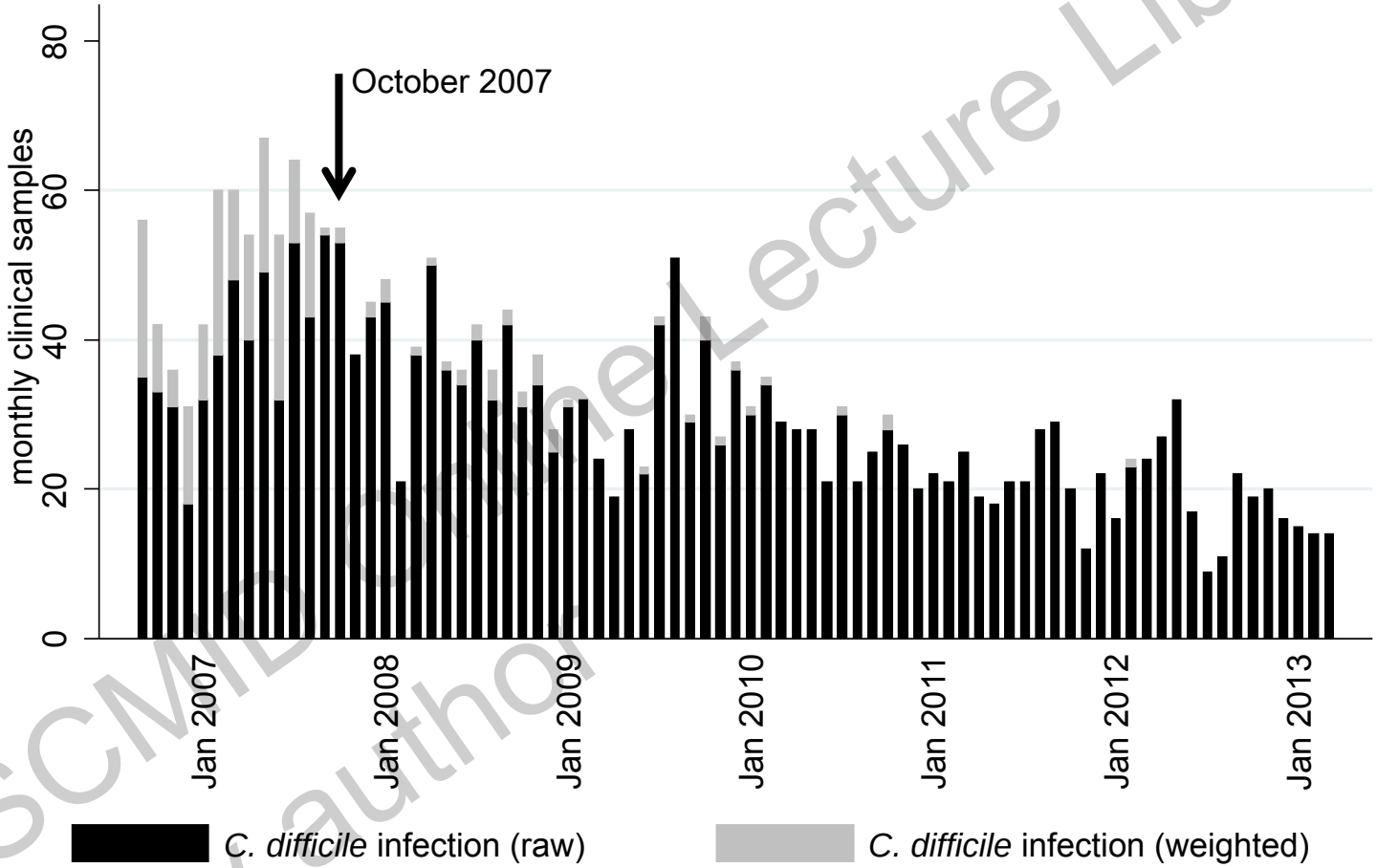
National and Regional Quinolone Use (Hospitals)



Quinolone use and CDI Incidence (National)



CDI Incidence (Oxford)



Oxford incidence consistent with national decline.

Key Observations

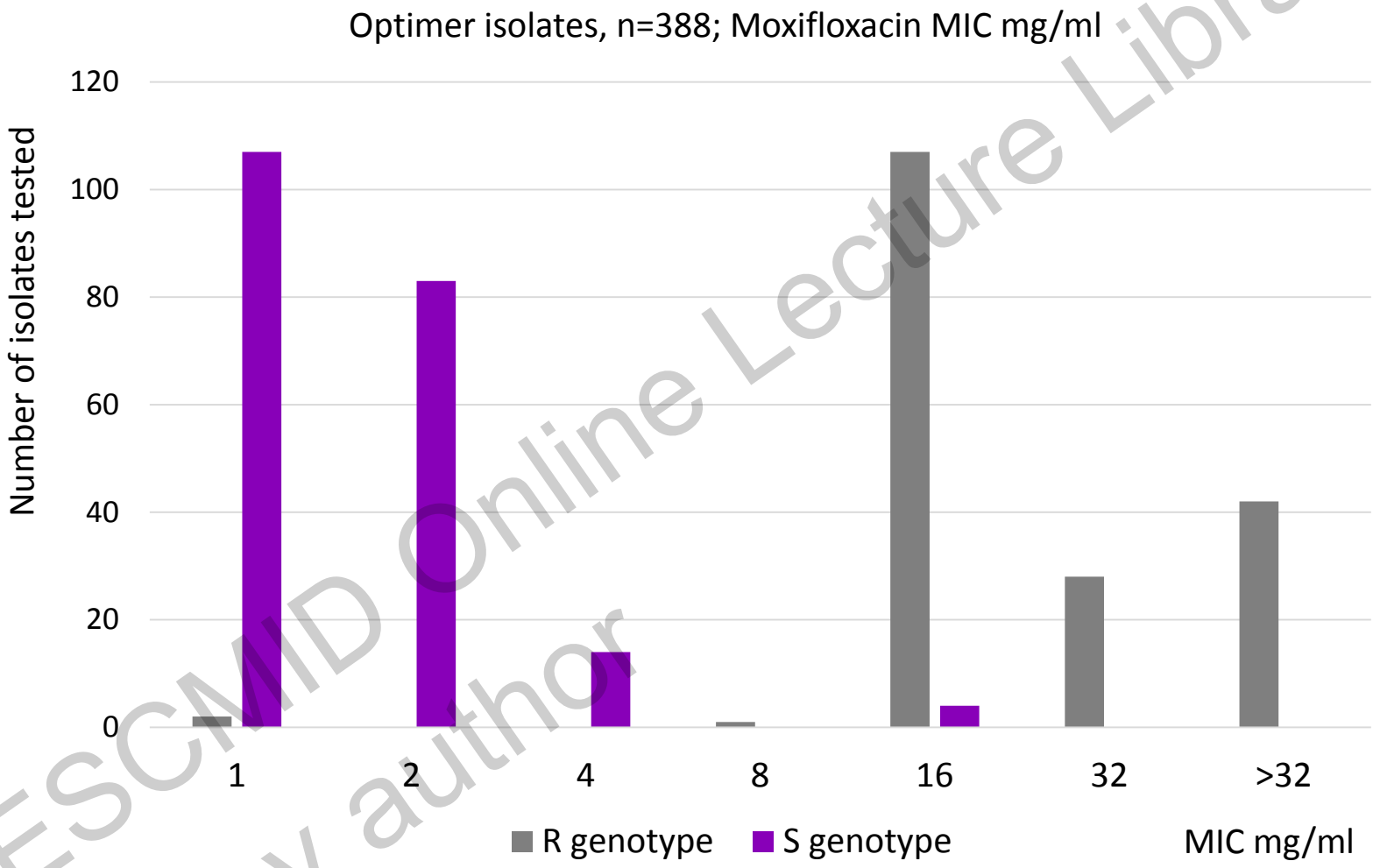
- National and regional **fluoroquinolone** prescribing data follow national and regional ***C. difficile*** **incidence** data.
- **Is there a causal link between fluoroquinolone prescribing and *C. difficile* incidence?**

- **Investigated using genetic data**; first deriving two characteristics from WGS:

  Fluoroquinolone susceptibility genotype

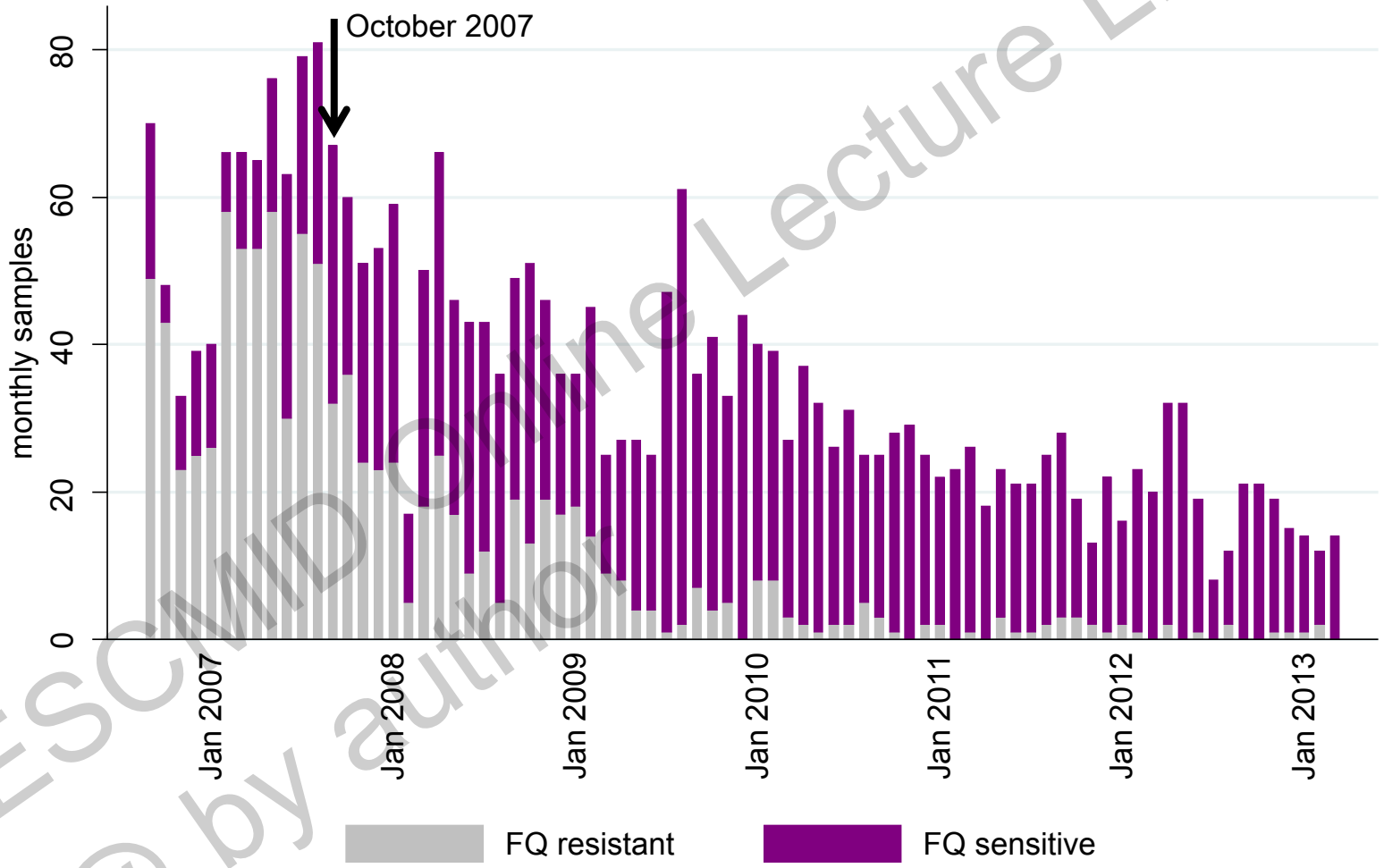
  Genetic lineage - Multilocus Sequence Type (ST)

Resistance Genotype Predicts Phenotype

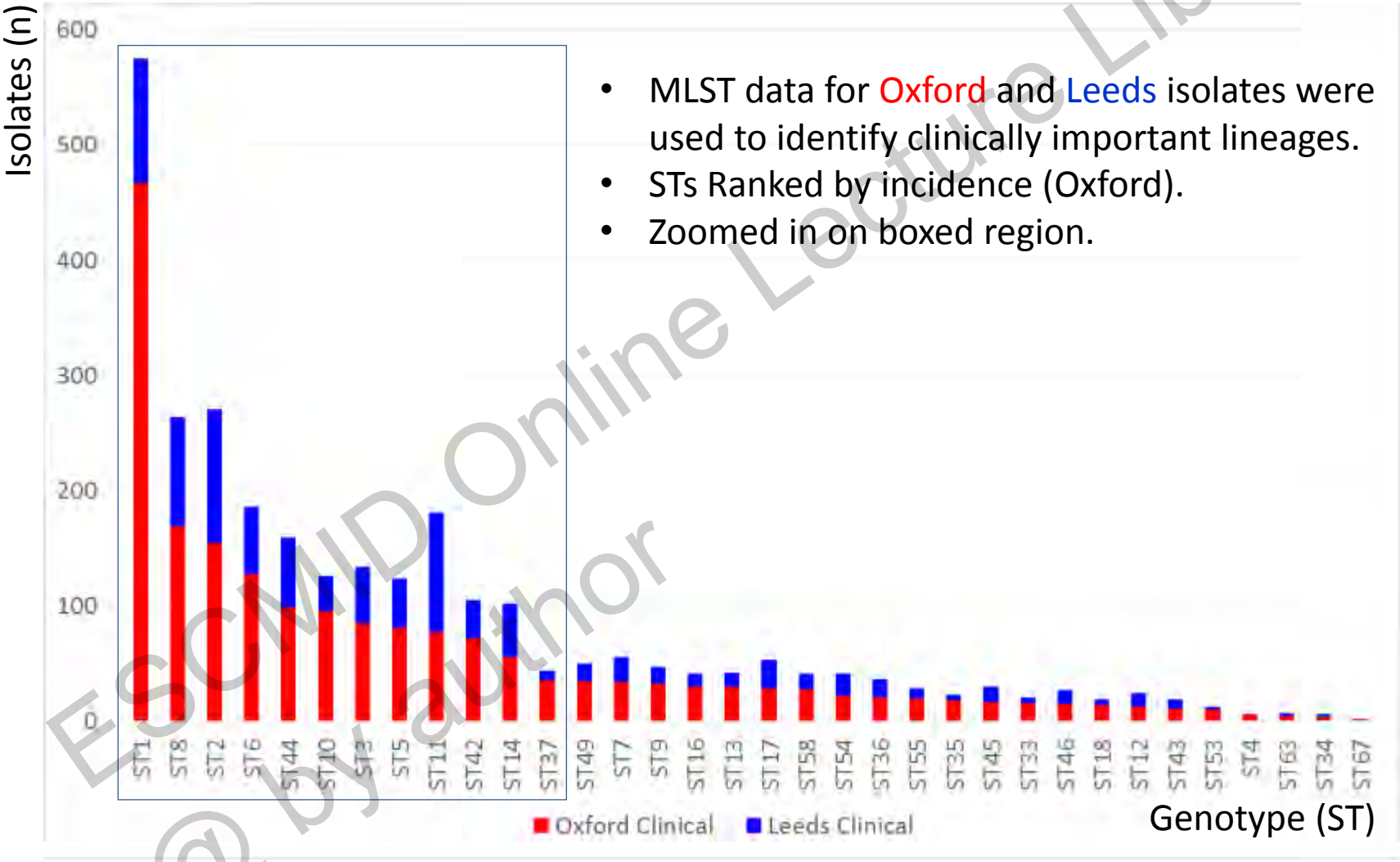


Resistant genotype: Classical mutations within the QRDR of *gyrA* or *gyrB*

Re-examine Oxford Incidence by Fluoroquinolone susceptibility

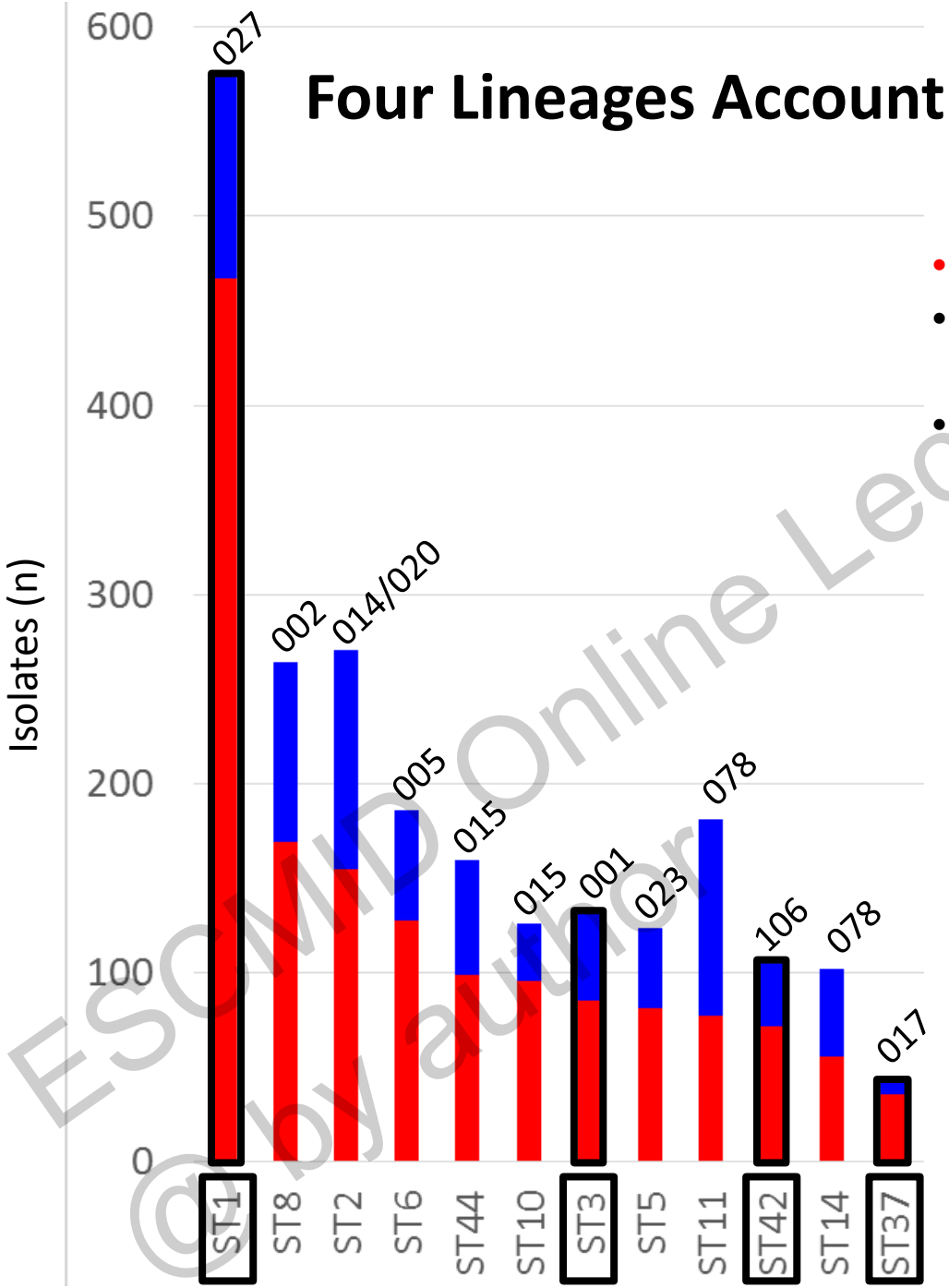


Clinically Important Lineages defined by WGS-derived MLST



- MLST data for Oxford and Leeds isolates were used to identify clinically important lineages.
- STs Ranked by incidence (Oxford).
- Zoomed in on boxed region.

Four Lineages Account for most FQ resistance



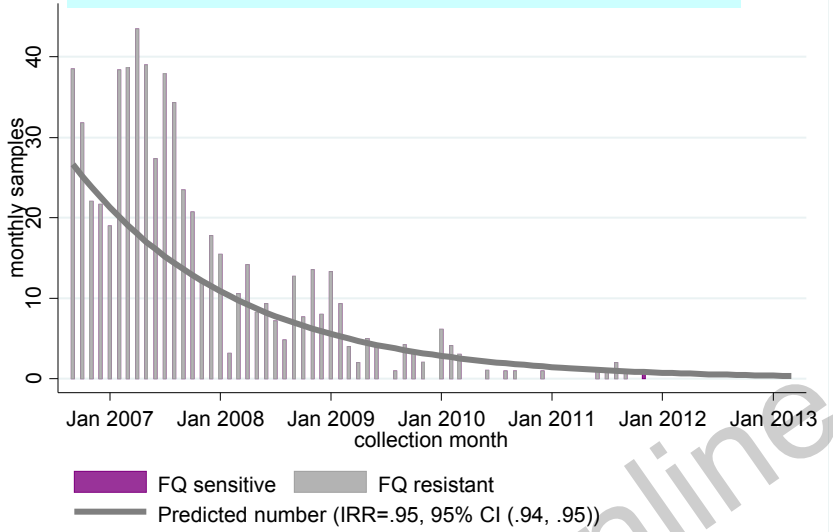
- **Oxford and Leeds clinical isolates**
- PCR-ribotype data added, good correlation with MLST.
- FQ resistance genotype data identified four lineages as accounting for the majority of resistance in the population.

FQ resistant lineages

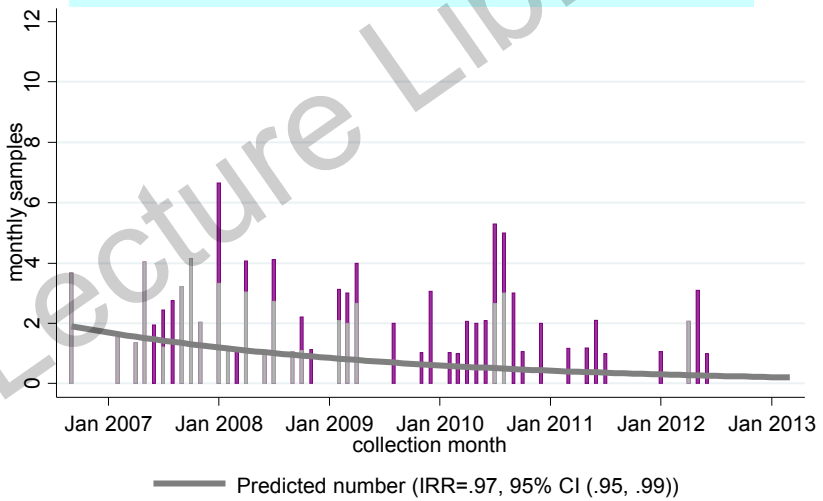
Incidence of four FQ Resistant Lineages

Grey trend line refers to resistant isolates only

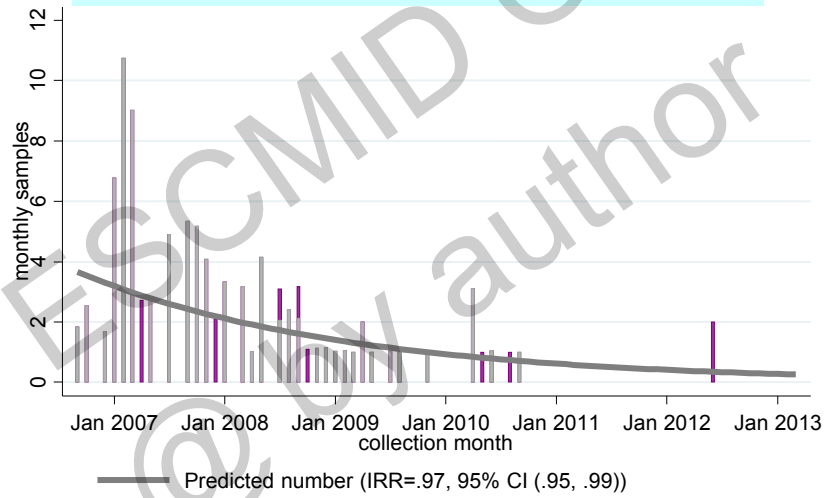
ST1(027) Oxford clinical by FQ sensitivity



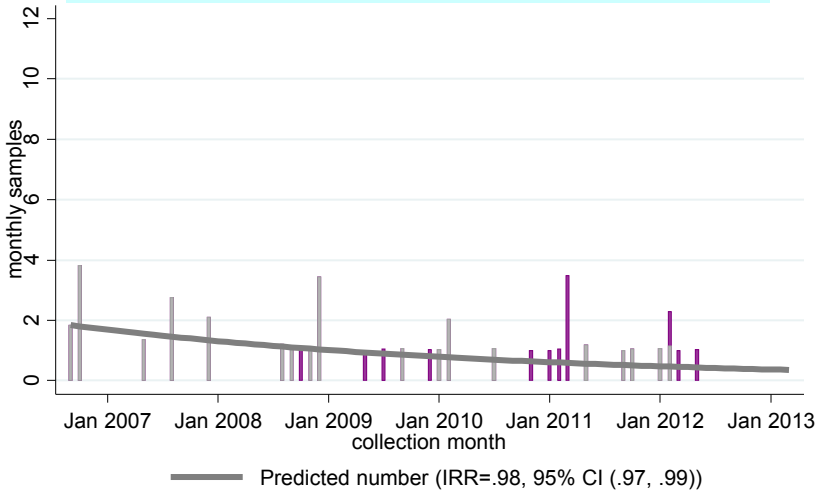
ST3(001) Oxford clinical by FQ sensitivity



ST42(106) Oxford clinical by FQ sensitivity

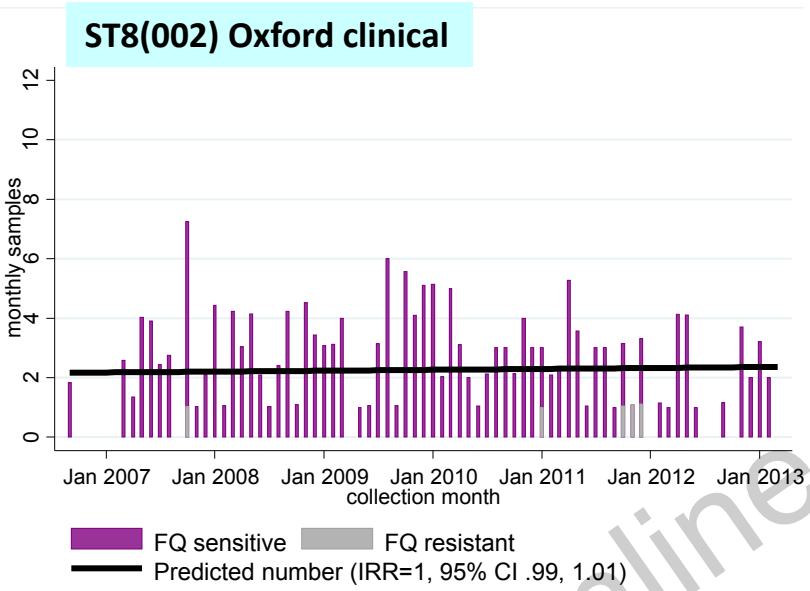


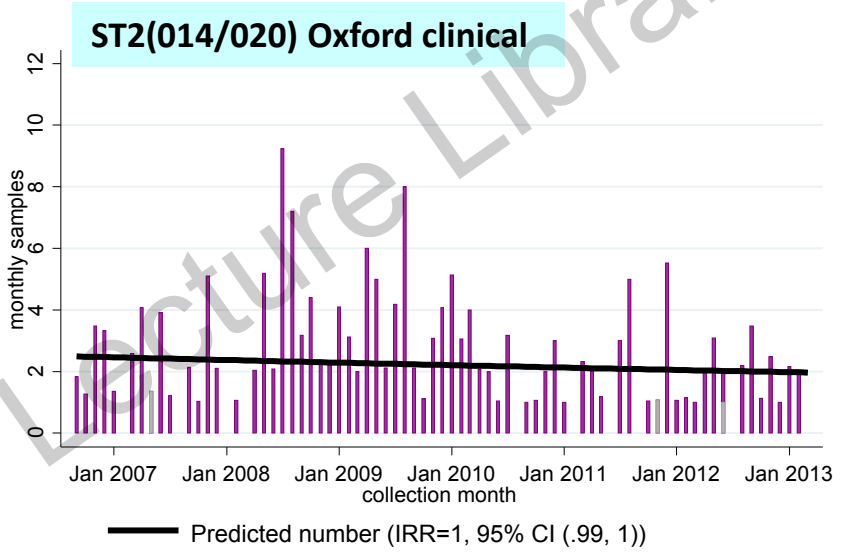
ST37(017) Oxford clinical by FQ sensitivity

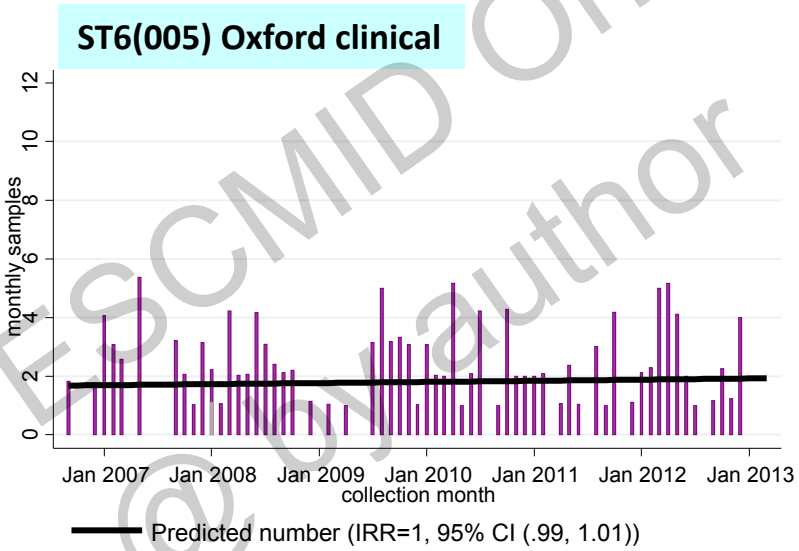


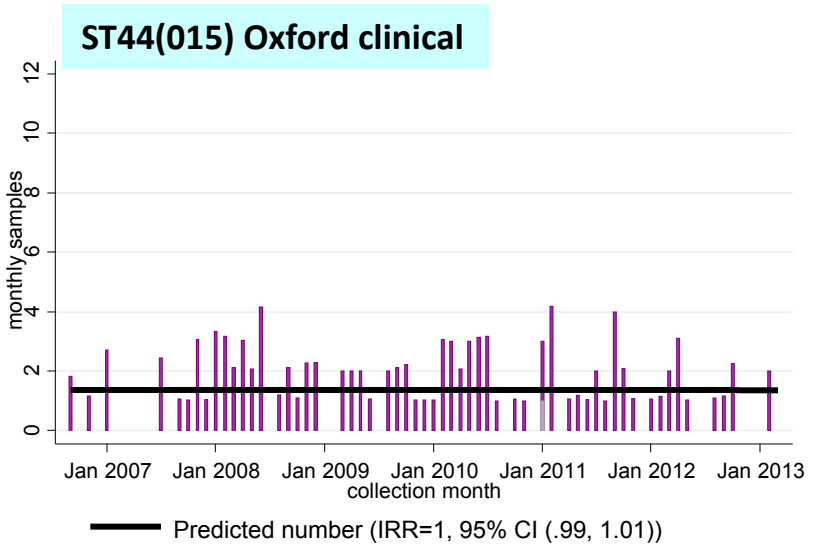
Incidence of FQ Sensitive lineages

Black trend line refers to all isolates (sensitive and resistant)









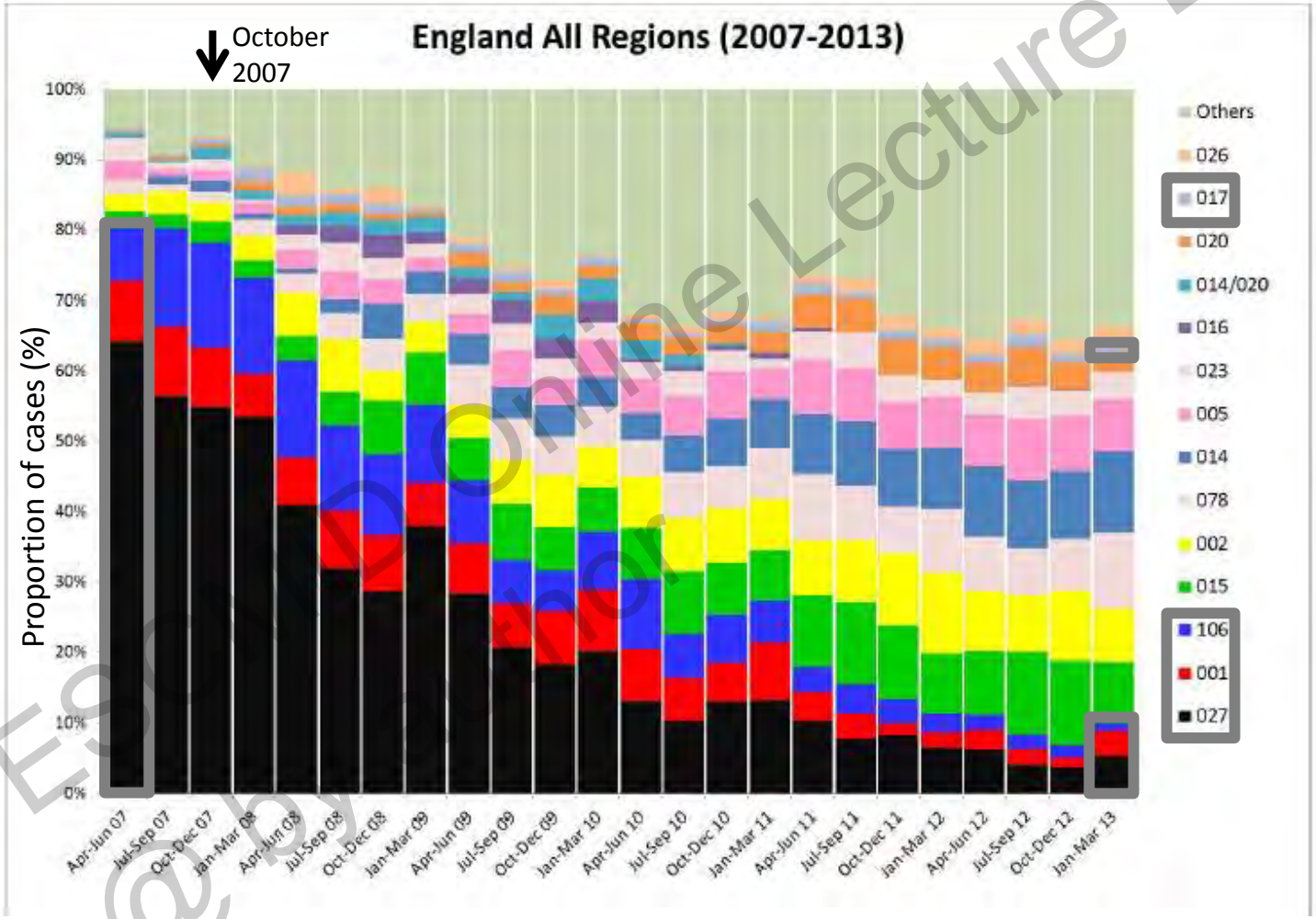
UK *C. difficile* Ribotyping Network

Infer FQ resistance

Public Health England

Clostridium difficile Ribotyping Network (CDRN) for England and Northern Ireland

2011–13 Report



Key Findings so far:

- 1. National and Regional UK CDI incidence increased and declined following the rise and fall of fluoroquinolone prescribing.
- 2. Four genetic lineages account for the majority of quinolone resistant isolates. Their disappearance accounts for the decline in CDI incidence regionally and nationally.
- 3. The incidence of CDI caused by sensitive lineages is unchanged regionally and nationally.

Phylogenetic Analysis

- **Historical perspective**

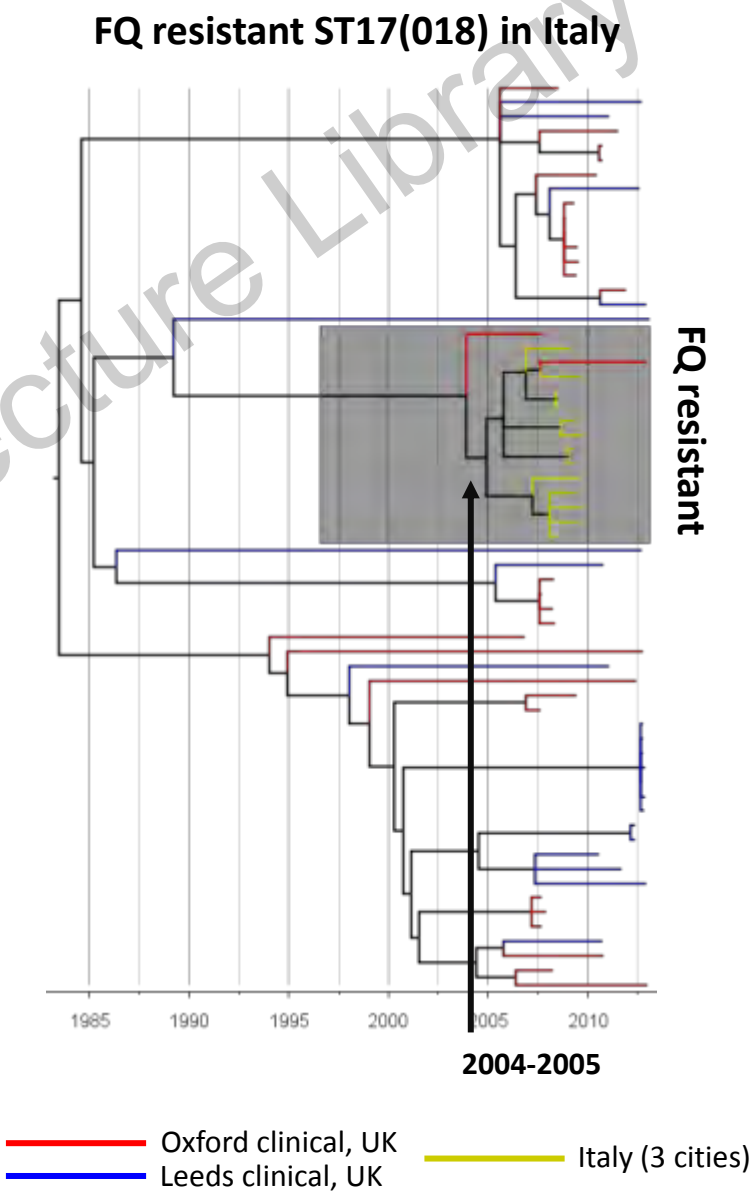
Isolate collections began only one year before UK fluoroquinolone use was restricted.
Constructing time scaled trees allows evolutionary insights into events before sampling began.
For example, **dating the emergence of fluoroquinolone resistance**. Just before a lineage emerges as a clinical problem?

- **Allows inferences concerning transmission**

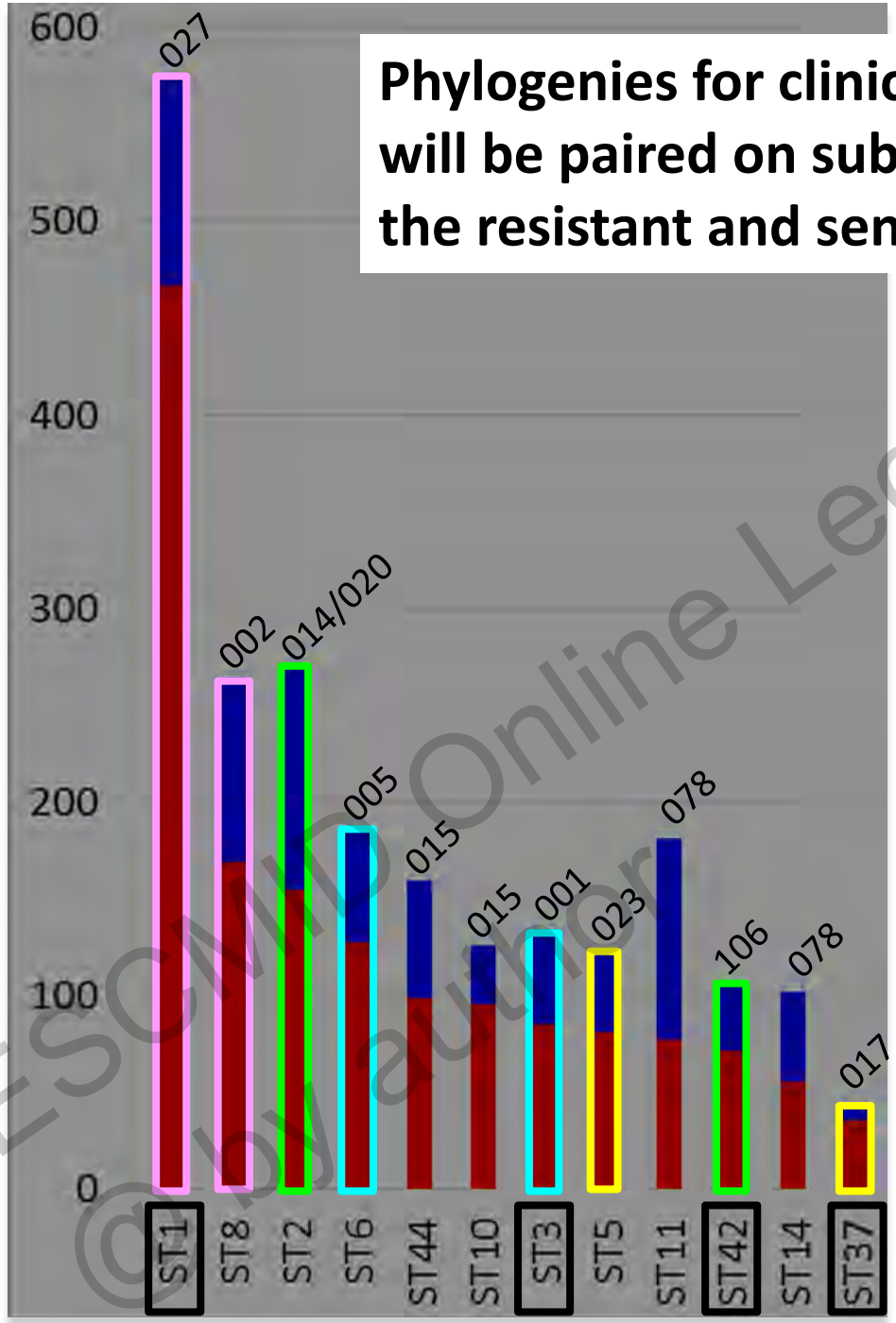
Greater geographic structure with shorter branches suggests more rapid, localised (nosocomial) transmission.

Time scaled phylogenies constructed for each lineage (defined by Sequence Type) using ClonalFrameML.

Didelot X, Wilson DJ (2015) ClonalFrameML: Efficient Inference of Recombination in Whole Bacterial Genomes. PLoS Comput Biol 11(2): e1004041. doi:10.1371/journal.pcbi.1004041



Phylogenies for clinically abundant lineages will be paired on subsequent slides to contrast the resistant and sensitive lineages.



Legend

Oxford isolates

Leeds isolates

Phylogenies shown side by side on subsequent slides

Resistant lineages

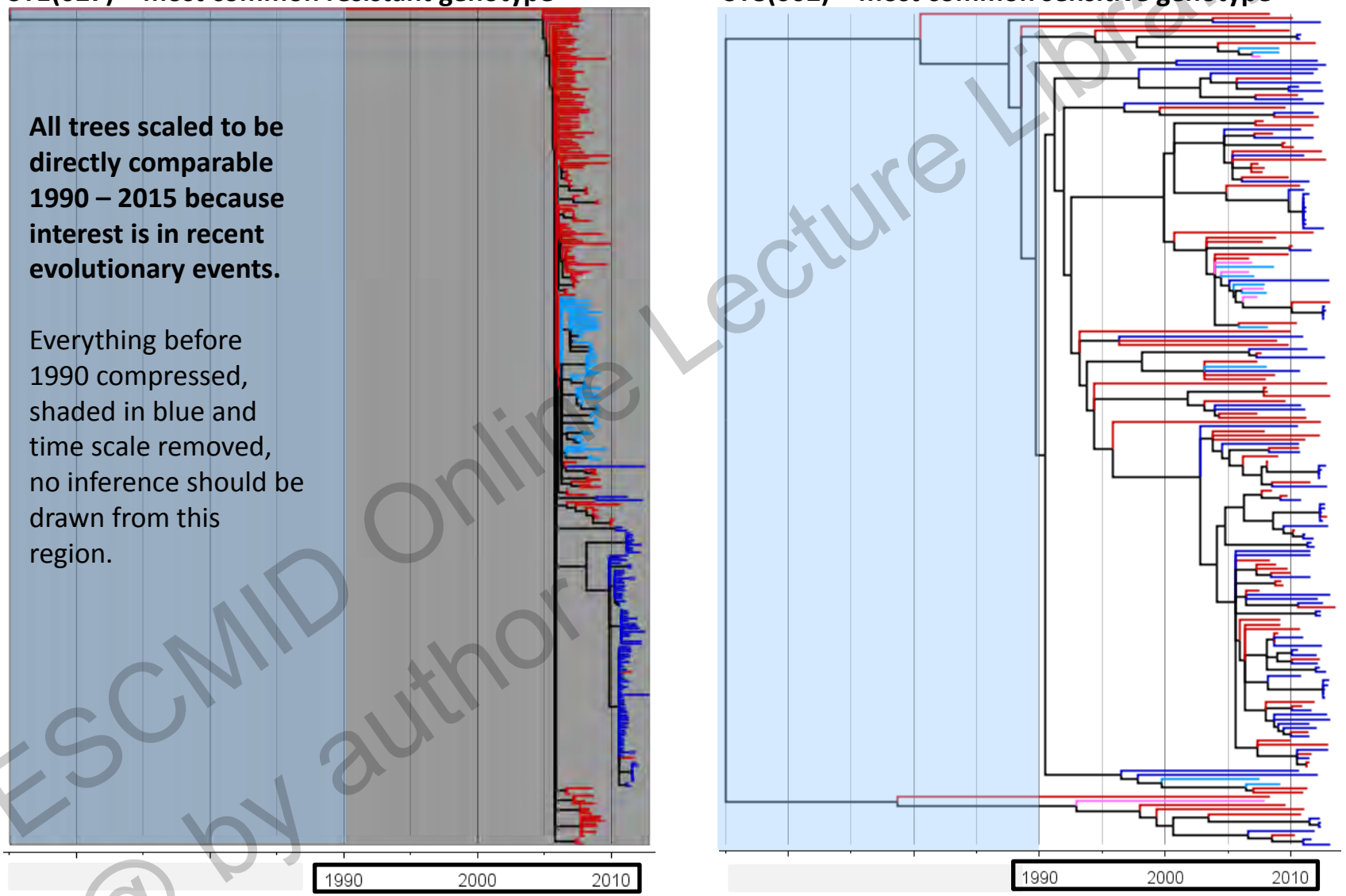
Resistant ST1(027) vs Sensitive ST8(002)

ST1(027) – most common resistant genotype

ST8(002) – most common sensitive genotype

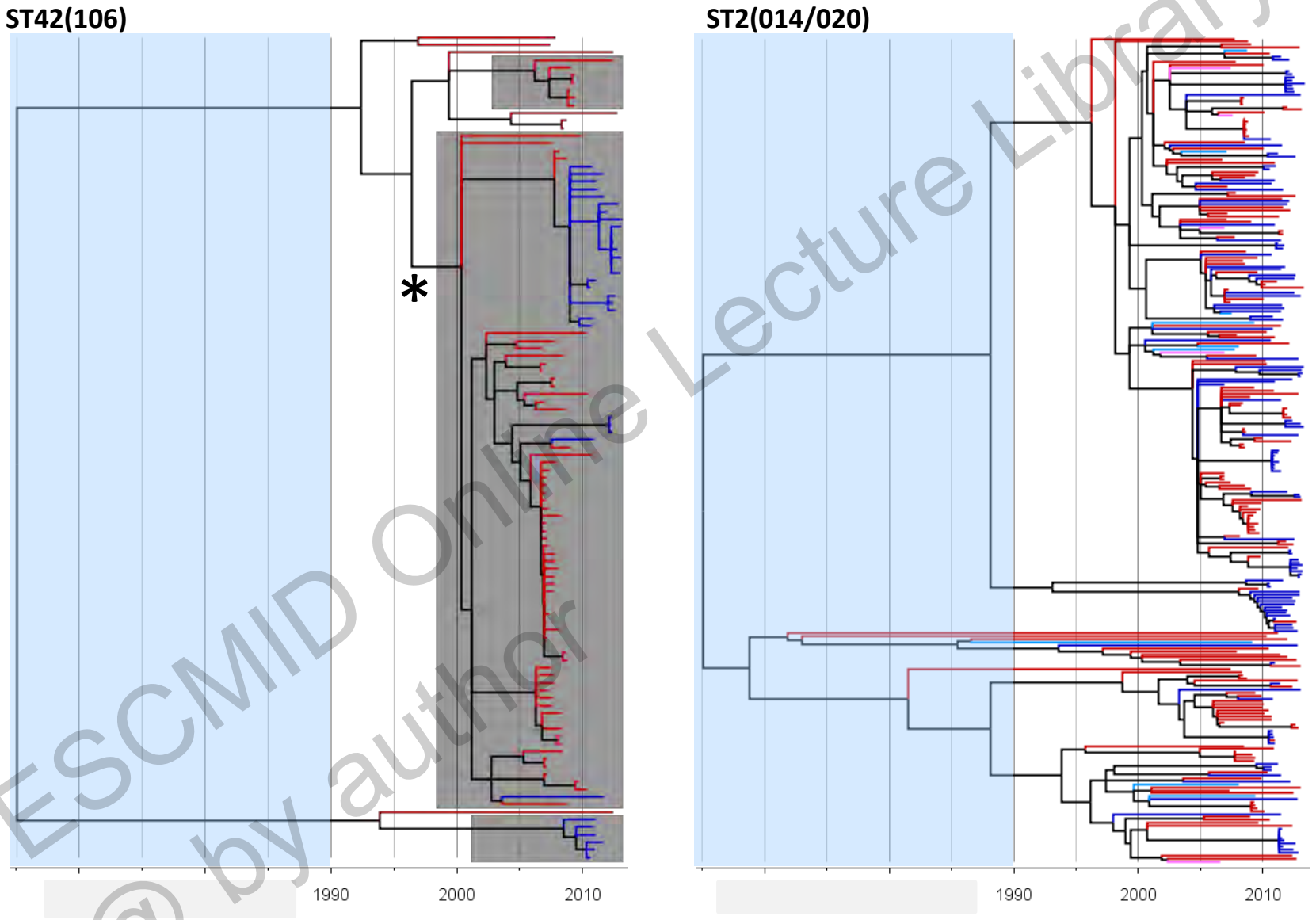
All trees scaled to be directly comparable 1990 – 2015 because interest is in recent evolutionary events.

Everything before 1990 compressed, shaded in blue and time scale removed, no inference should be drawn from this region.

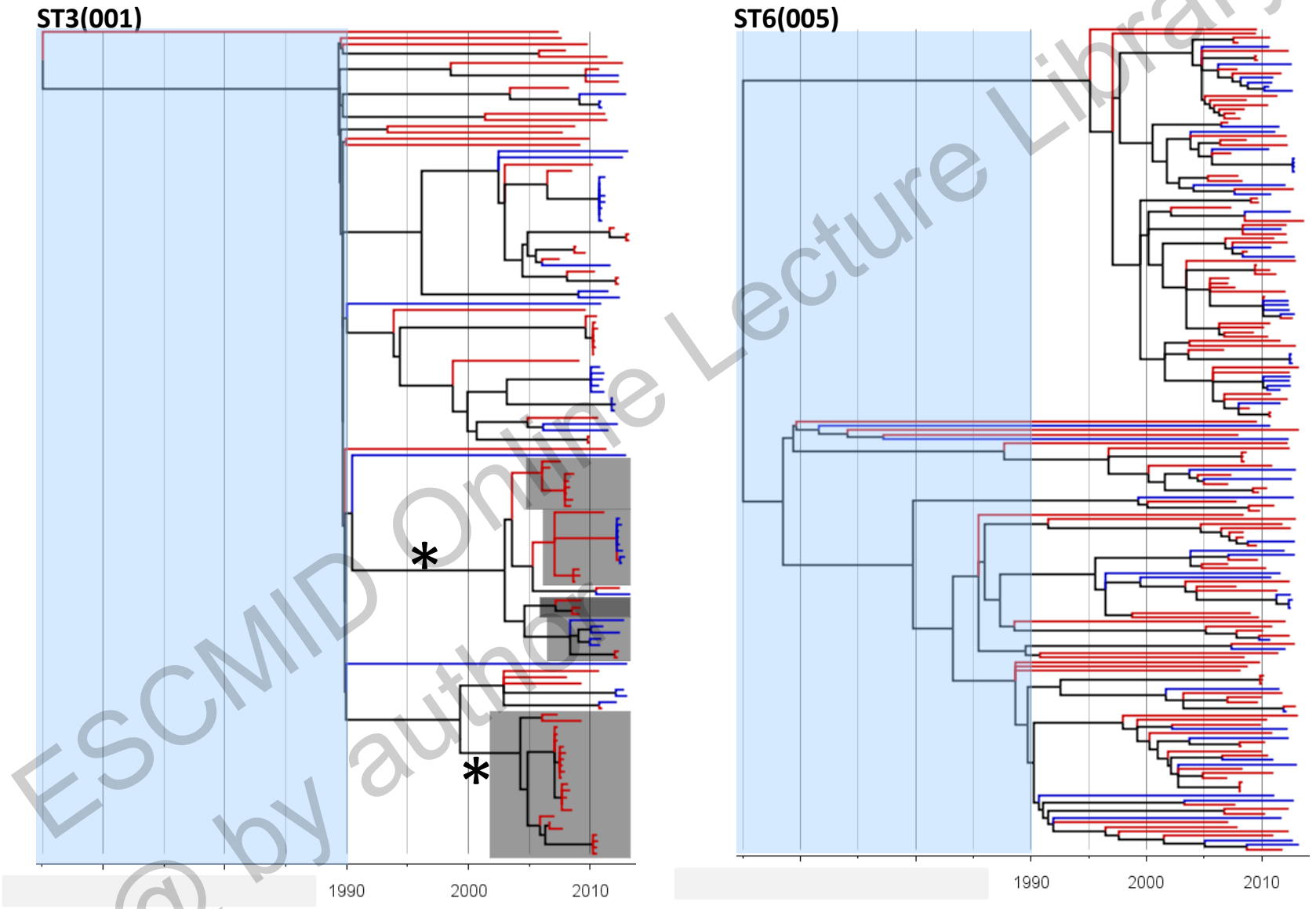


ST1(027) every 3rd isolate, ST8(002) every 2nd isolate.

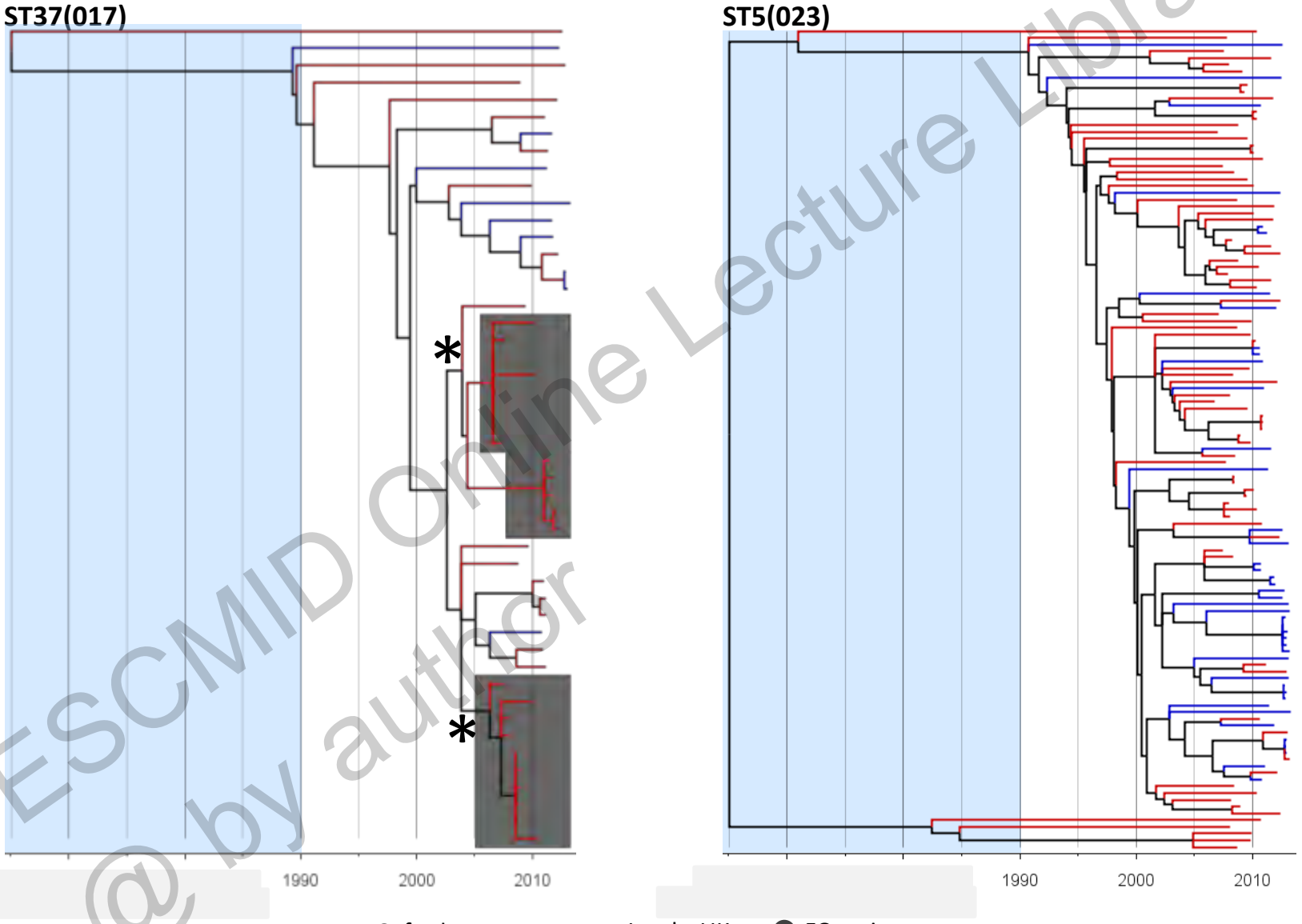
Resistant ST42(106) vs Sensitive ST2(014/020)



Resistant ST3(001) vs Sensitive ST6(005)



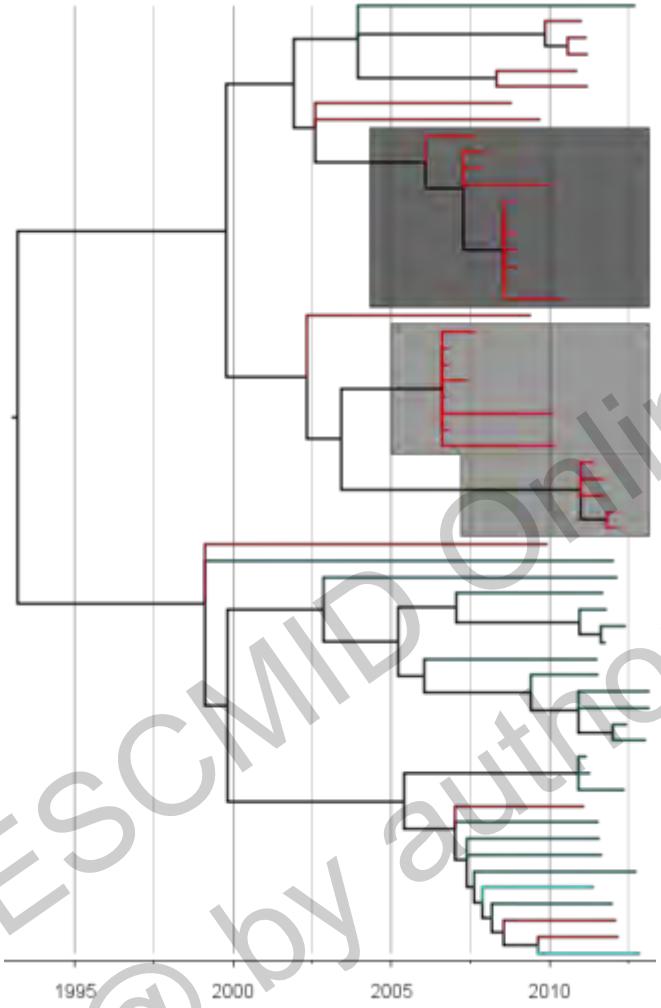
Resistant ST37(017) vs Sensitive ST5(023)



Key Findings

- Phylogenies of FQ resistant lineages exhibit greater geographic structure and shorter branches than sensitive lineages, consistent with more frequent nosocomial transmission.
- Approximate emergence dates of FQ resistant lineages are consistent with literature reports.
- **Does the fluoroquinolone sensitive *C. difficile* population represent a natural, non-clinically adapted population as found in asymptomatic carriers and healthy infants?**

ST37(017) Oxford Clinical Cases and Oxford Healthy Infants



Oxford clinical, UK

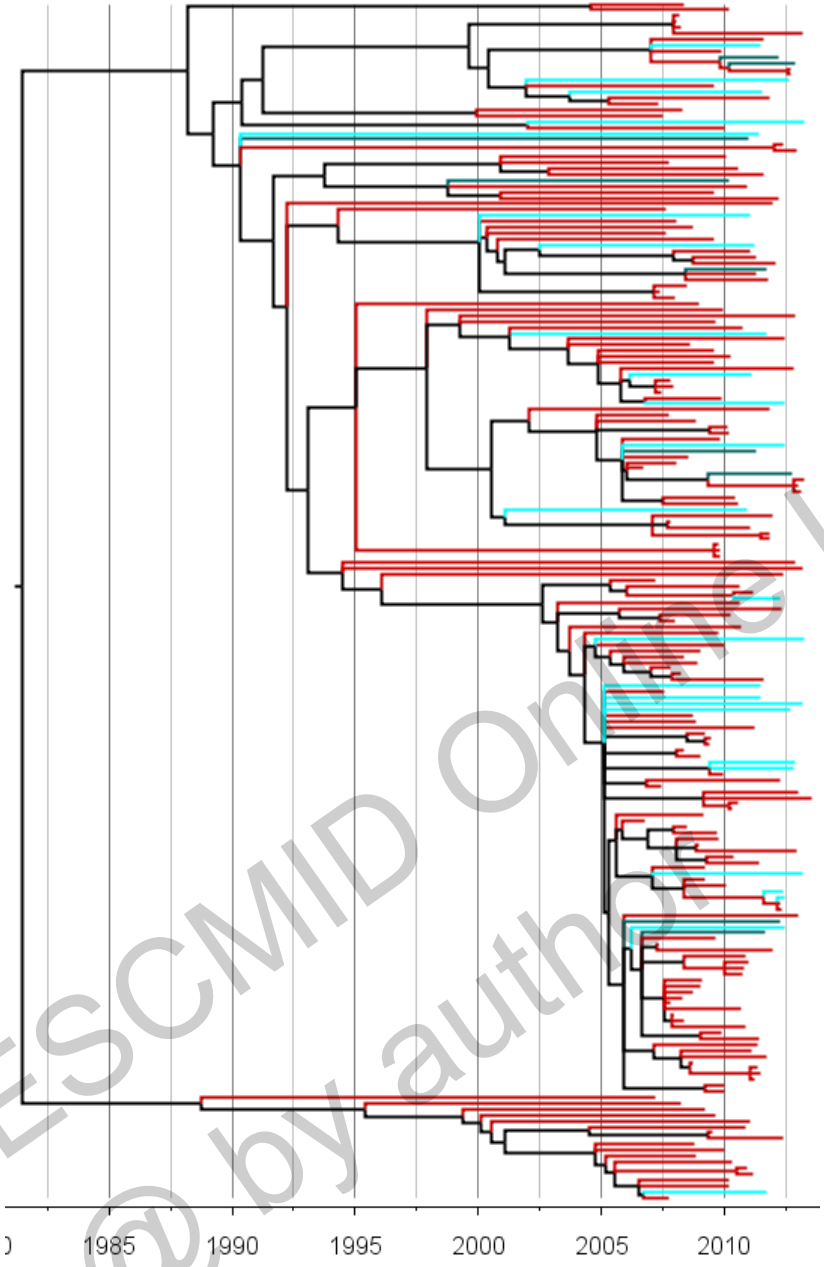
Oxford Asymptomatic or ELISA negative

Oxford Healthy Infants

FQ resistant

Sensitive clinical isolates mix with those from healthy infants and have longer branches, consistent with less frequent introductions to clinical environment from background wild type population.

Phylogenetic ‘isolation’ and short branches of resistant clinical isolates consistent with their being confined to and transmitted frequently within the healthcare environment.



ST8(002): Oxford clinical, ELISA negative, asymptomatic and healthy infants.

Oxford clinical, UK

Oxford Asymptomatic or ELISA negative

Oxford Healthy Infants

The three populations do not segregate phylogenetically. Phylogenies for ST2(014/020) and ST6(005) are very similar.

Conclusions (1)

- UK CDI incidence has followed the recent changes in fluoroquinolone prescribing.
- The virtual **disappearance of the four fluoroquinolone resistant lineages** accounted for the decline in CDI incidence.
- The incidence of CDI caused by sensitive lineages was unchanged.
- Phylogenies of **resistant lineages** exhibit greater **geographic structure** and shorter branches relative to sensitive lineages…suggests **more frequent nosocomial transmission**.
- **Sensitive** *C. difficile* population may represent a **wild type**, non-clinically adapted population.

Conclusions (2)

- **Consistent with previous findings**; symptomatic patient to patient transmission, point source or secondary spread in only a minority (35%) of CDI cases [Eyre et al., 2013 NEJM 369:1195-1205].
- **A national reduction in fluoroquinolone use of ~50%**; sufficient to remove resistant lineages from the UK healthcare environment, potentially indicating a threshold level of fluoroquinolone use which can be tolerated without triggering CDI outbreaks.
- The disappearance, rather than reversion and continued spread of lineages 027, 001, 106, 017 argues against the possibility that these lineages have other properties favouring their transmission.
- **Data indicate importance of maintaining UK fluoroquinolone restriction** (at least in high risk patients), and suggest that this **policy could be useful elsewhere.**

Acknowledgements

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