Restricting fluoroquinolone prescribing appears to explain the decline in incidence of group of cases caused by fluoroquinolone-susceptible isolates (p > 0.2). Cases caused by fluoroquinolone-resistant isolates with or without hospital contact (p < 0.0001) versus no change in either fluoroquinolone restriction over infection control was shown by significant declines in inferred secondary (transmitted) were short-branched and geographically structured, consistent with selection and rapid transmission. The importance of decline regardless of susceptibility.

Methods Regional (Oxfordshire and Leeds, UK) and national data for the incidence of C difficile infections and antimicrobial prescribing data (1998–2014) were combined with whole genome sequences from 4045 national and international C difficile isolates. Genotype (multilocus sequence type) and fluoroquinolone susceptibility were determined from whole genome sequences. The incidence of C difficile infections caused by fluoroquinolone-resistant and fluoroquinolone-susceptible isolates was estimated with negative-binomial regression, overall and per genotype. Selection and transmission were investigated with phylogenetic analyses.

Findings National fluoroquinolone and cephalosporin prescribing correlated highly with incidence of C difficile infections (cross-correlations > -0.88), by contrast with total antibiotic prescribing (cross-correlations < -0.59). Regionally, C difficile decline was driven by elimination of fluoroquinolone-resistant isolates (approximately 67% of Oxfordshire infections in September, 2006, falling to approximately 3% in February, 2013; annual incidence rate ratio 0.52, 95% CI 0.48–0.56 vs fluoroquinolone-susceptible isolates: 1.02, 0.97–1.08). C difficile infections caused by fluoroquinolone-resistant isolates declined in four distinct genotypes (p < 0.01). The regions of phylogenies containing fluoroquinolone-resistant isolates were short-branched and geographically structured, consistent with selection and rapid transmission. The importance of fluoroquinolone restriction over infection control was shown by significant declines in inferred secondary (transmitted) cases caused by fluoroquinolone-resistant isolates with or without hospital contact (p < 0.0001) versus no change in either group of cases caused by fluoroquinolone-susceptible isolates (p > 0.2).

Interpretation Restricting fluoroquinolone prescribing appears to explain the decline in incidence of C difficile infections, above other measures, in Oxfordshire and Leeds, England. Antimicrobial stewardship should be a central component of C difficile infection control programmes.

Funding UK Clinical Research Collaboration (Medical Research Council, Wellcome Trust, National Institute for Health Research); NIHR Oxford Biomedical Research Centre; NIHR Health Protection Research Unit on Healthcare Associated Infection and Antimicrobial Resistance (Oxford University in partnership with Public Health England [PHE]), and on Modelling Methodology (Imperial College, London in partnership with PHE); and the Health Innovation Challenge Fund.

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Introduction Closstridium difficile infection is a major clinical challenge worldwide. At least three antimicrobial classes are deemed to be high-risk C difficile infection triggers, including most cephalosporins, to which C difficile is inherently resistant, and clindamycin, to which genotypes causing early outbreaks were resistant. Global dispersion of hypervirulent NAP1/PCR-ribotype-027 C difficile revealed an association between fluoroquinolone resistance and epidemic spread. Accordingly, clindamycin or fluoroquinolone use has been restricted, and combined with other measures aiming to control localised C difficile infection outbreaks. Most cases of C difficile infection are temporally associated with health care, reflecting a combination of health-care-associated acquisition, and health-care-related
This observational study tested two hypotheses. First, if C difficile infection declines in England were driven by reductions in use of particular antibiotics, then incidence of C difficile infection caused by resistant isolates should decline faster than that caused by susceptible isolates across several genotypes (defined by multilocus sequence type). Second, if decreases in C difficile infection were driven by improvements in hospital infection control, then transmitted (secondary) cases should decline regardless of susceptibility.

To confirm that national policies17,18 affected antibiotic prescribing and C difficile infection incidence, we first compared national antimicrobial prescribing data for hospitals and the community (obtained respectively from IMS Health [Danbury, CT, USA] and the Health & Social Care Information Centre [appendix]) with national incidence of C difficile infection (ie, infections per 100,000 population per year, using data from Public Health England).

The primary study dataset comprised whole genome sequences from clinical C difficile isolates cultured from consecutive stool samples from symptomatic, unique patients submitted to the Oxford University Hospitals NHS Trust between Sept 12, 2006, and Aug 19, 2013 (n=2021; appendix). A further 261 isolates between Sept 1, 2006, and Feb 26, 2013, where only the sequence type was available were also included. The hospital did all C difficile testing in Oxfordshire, serving general practices, which included an integrated and shared C difficile infection surveillance system, managing data to 2013.

Methods

Study design

This observational study tested two hypotheses. First, if C difficile infection declines in England were driven by reductions in use of particular antibiotics, then incidence of C difficile infection caused by resistant isolates should...
community hospitals, and other providers, so incidence is per Oxfordshire population (approximately 600 000) per year. This culture-positive C difficile infection incidence was compared with Oxfordshire’s nationally submitted EIA-positive incidence (incorporating changes in mandatory reporting requirements in 2008) to confirm representativeness of whole genome sequences. The latter was compared with English incidence of C difficile infection to assess generalisability.

Generalisability of Oxfordshire data was also assessed with similar information from Leeds Teaching Hospitals NHS Trust, UK. This comprised whole genome sequences for consecutive clinical, toxin-positive (cytotoxin assay) isolates from symptomatic patients (Aug 2, 2010, to May 1, 2013; n=1020; appendix), Leeds regional incidence of C difficile infection data (nationally submitted) and ribotype prevalence, and antibiotic prescribing data.

Additional genetic context was provided by further regional and international C difficile whole genome sequences (May 9, 2006, to July 12, 2013) of isolates from toxin-EIA-negative clinical samples of symptomatic Oxfordshire patients (n=395), toxin-positive samples representing two clinical trials of fidaxomicin in North America and Europe (n=803),19,20 and from healthy Oxfordshire infants (n=200; appendix).

### Genome sequences and multilocus sequence type identification
Genomes were sequenced using Illumina technology. Velvet de novo assemblies and reference-based assemblies were generated, the latter mapped to C difficile 630 (GenBank AM180355.1; reads submitted to National Center for Biotechnology Information, BioProjectID PRJNA304087; appendix). The sequences of loci defining C difficile sequence types were identified and extracted with BiGSdB;21 sequence types were assigned with the C difficile PubMLST database. The notation ST1(027) indicates, for example, sequence-type-1 (PCR-ribotype-027).

### Whole genome sequence-derived fluoroquinolone susceptibility
Isolates were designated fluoroquinolone-susceptible or fluoroquinolone-resistant based on specific non-synonymous substitutions within the quinolone resistance-determining region of gyrA/B genes22–23 extracted from whole genome sequences.24 gyrA C(245)T[T(82)I] and gyrB G(1276)A[D(426)N] confer high-level fluoroquinolone resistance in C difficile and other species.25–26 Susceptibility predictions were validated phenotypically for 387 fidaxomicin trial isolates27–28 (n=191 Canada, n=196 USA), with agar dilution (moxifloxacin minimum inhibitory concentration; appendix).

### Statistical analysis
We made univariable comparisons between English antimicrobial prescribing and incidence of C difficile infection with bivariate cross-correlations (appendix). Genotype (sequence type)-specific incidence rates for C difficile infection caused by toxin EIA-positive, culture-positive isolates were calculated with negative binomial regression accounting for missing data by probability weights (appendix). For genotypes with more than 10% fluoroquinolone-resistant isolates, rates were calculated separately for fluoroquinolone-susceptible and fluoroquinolone-resistant isolates. These data were available for isolates from April 2008 to March 2011. Rates were also calculated separately for infections that could plausibly have arisen from secondary spread (transmission) inferred by close genetic relationships to previous infections (two or fewer single nucleotide variants from the original case),27 and also separately for fluoroquinolone-susceptible and fluoroquinolone-resistant isolates. Phylogenetic trees were constructed for each sequence type (or several closely related sequence types), with maximum likelihood, then corrected for recombination.
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C difficile infection declines occurred while total antibiotic prescribing was increasing (by 4-4% per year in the community [p=0.0001, 2006–13], but only 0.5% per year in hospitals [p=0.053, 2006–12]; figure 1B). Between 2005 and 2012 (when data were complete for England), the cross-correlations (CCs) between English incidence of C difficile infection and total English antibiotic prescribing were –0.57 (95% CI −0.67 to −0.41) for hospital and community, –0.59 (−0.68 to −0.44) for community, and 0.29 (−0.19 to 0.60) for hospital prescribing (optimum CC using a 1-year lag; appendix). During the same period, the strongest univariable associations between English incidence of C difficile infection and individual antimicrobials were with cephaplospirins (CC=−0.97, 95% CI 0.82–0.98 for hospital and community; 0.94, 0.68–0.97 for community; and 0.97, 0.81–0.99 for hospital prescribing; optimum 0-year lag) and fluoroquinolones (CC=1.00, 0.84–1.00 for hospital and community; 0.88, 0.48–0.95 for community; and 0.93, 0.66–0.97 for hospital prescribing; optimum 0-year lag; appendix), although hospital fluoroquinolone prescribing began to decline slightly earlier than community prescribing (p=0.0001 from 2005 to 2009 vs in the community p=0.0001 from 2007 to 2012; figure 1A). Other antibiotics were more weakly associated (appendix).

Similar to English incidence of C difficile infection, Oxfordshire rates also decreased from 2007 (when isolate-level fluoroquinolone-susceptibility could be determined; p=0.0001; figure 2A). Fluoroquinolone prescribing in Oxfordshire hospitals declined from a peak in 2005 until 2010 (p=0.0001), when use began to increase again (p=0.0001 from 2010 to 2013). Hospital cephalosporin and fluoroquinolone prescribing were also positively associated with incidence of C difficile infection (CC=0.73, 0.15 to 0.86, and 0.62, −0.09 to 0.81; appendix), but associations were estimated much less precisely given the much smaller population (approximately 1% of England). Positive associations were also observed between C difficile infection decline and decline in extended spectrum penicillins (0.84, 0.24 to 0.90) and beta-lactamase-resistant penicillins (0.67, −0.04 to 0.81; appendix). Community prescribing data were not available.

Paired fluoroquinolone susceptibility phenotype and gyrA/B DNA sequences were assessed for 387 isolates from the two clinical trials of fidaxomicin in North America and Europe,\textsuperscript{11,12} representing 53 sequence types. Phenotype and whole genome sequences were 98.7%-concordant (appendix; sensitivity 97.8%, specificity 99.5%); only one of 185 isolates predicted as resistant by whole genome sequencing\textsuperscript{11,12} lacked an elevated minimum inhibitory concentration (MIC). Conversely, only four of 202 isolates lacking resistance-associated substitutions\textsuperscript{11,12} had raised MICs (16 mg/L). gyrA/B sequence therefore reliably predicts the fluoroquinolone resistance phenotype.

The decrease in Oxfordshire C difficile infections was solely due to a decline in C difficile infection caused by using ClonalFrameML (version 1.0–6).\textsuperscript{23} Trees were time-scaled and made directly comparable post-1990 (appendix). In each tree, the evolutionary distinctiveness (ED) score of each genome was calculated;\textsuperscript{24} low ED scores indicate closely related genomes, whereas high scores indicate their relative absence (appendix).

**Role of the funding source**

The study sponsor had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all study data and had final responsibility for the decision to submit for publication.

**Results**

Incidence of C difficile infection in England increased from 1998 to 2006 (p<0.0001) then declined rapidly over the years that followed to 2013 (p<0.0001; figure 1A).

**Figure 2:** Incidence of Clostridium difficile infections together with fluoroquinolone and cephaplospirin prescribing for Oxfordshire (A) and incidence of C difficile infections by fluoroquinolone susceptibility for Oxfordshire (B).

(A) Mandatory incidence of C difficile infections corresponds to all cases reported for individuals older than 2 years (from 2004 to 2007); cases were only reported for individuals older than 65 years, and are upweighted to provide similar estimates in individuals older than 2 years (appendix). Only toxin-positive culture-positive samples were used in the genotype-specific and phylogenetic analyses. (B) C difficile is inherently resistant to most cephalosporins.\textsuperscript{4} IRR=annual incidence rate ratio.
For genotypes with more than 10% resistant isolates (denoted FQR), rates were calculated separately for C difficile infections caused by fluoroquinolone-susceptible and resistant isolates. To show that the difference in IRR for resistant and susceptible isolates is not driven solely by the decline in ST1(027), rates were also calculated for all non-ST1(027) genotypes together, as well as for all genotypes with more than 10% resistant isolates (excluding ST1(027)) and for all genotypes with 10% or less resistant isolates (FQS). Heterogeneity between IRRs in non-ST1(027) genotypes together, as well as for all genotypes with more than 10% resistant isolates (excluding ST1(027)) and for all genotypes with 10% or less resistant isolates (FQS) was assessed using the heterogeneity statistic and the Q test. The significance level of each statistical test was set at p<0·05.

19 phylogenies were constructed representing the 22 most common C difficile genotypes in Oxfordshire and Leeds (figure 4D–F, appendix). The phylogeny of each genotype containing more than 10% fluoroquinolone-resistant isolates (figure 4D, E, appendix) showed rapid, geographically structured clonal expansions associated with resistance. This observation was reproduced internationally in parts of the phylogenies representing Calgary, Canada (figure 4D, E) and in isolates from three cities in northern Italy: Modena, Turin, and Arsizio (appendix). We recorded significantly lower ED scores for resistant versus susceptible areas of phylogenies containing both fluoroquinolone-resistant and fluoroquinolone-susceptible isolates (eg, ST3 p<0·0001, figure 4E; ST37 p<0·0001, appendix). By contrast, the phylogenies of genotypes consisting primarily of susceptible isolates (figure 4F, appendix) were geographically unstructured and had longer branches. This was also seen internationally in susceptible isolates from Calgary and Montreal, Canada (figure 4E, appendix). In fluoroquinolone-susceptible genotypes, the ED scores (and, by inference, transmission) did not differ significantly between Oxfordshire and Leeds clinical isolates (p>0·1; appendix).

![Figure 3: Oxfordshire Clostridium difficile IRR by fluoroquinolone resistance and genotype](image-url)

For genotypes with more than 10% resistant isolates (denoted FQR), rates were calculated separately for C difficile infections caused by fluoroquinolone-susceptible and resistant isolates. To show that the difference in IRR for resistant and susceptible isolates is not driven solely by the decline in ST1(027), rates were also calculated for all non-ST1(027) genotypes together, as well as for all genotypes with more than 10% resistant isolates (excluding ST1(027)) and for all genotypes with 10% or less resistant isolates (FQS). Heterogeneity between IRRs in non-ST1(027) genotypes together, as well as for all genotypes with more than 10% resistant isolates (excluding ST1(027)) and for all genotypes with 10% or less resistant isolates (FQS) was assessed using the heterogeneity statistic and the Q test. The significance level of each statistical test was set at p<0·05.
Additional phylgelies for three prevalent fluoroquinolone-susceptible genotypes revealed similar branch lengths irrespective of sampling region size (appendix). Oxfordshire phylogenies (appendix), containing genomes from toxin EIA-positive and EIA-negative samples, plus genomes from healthy, asymptomatic, community infants, showed a lack of structure by source, even within a single region. ED scores were generally lower for clinical toxin EIA-positive genomes than for infant and EIA-negative genomes, especially in ST8(002) (p=0.0033) and

Figure 4: Contrasting incidence of Clostridium difficile infections (Oxfordshire) and whole-genome sequence phylogenies representing the fluoroquinolone-resistant genotype ST1(027), the mixed resistant and susceptible genotype ST3(001), and the almost entirely fluoroquinolone-susceptible genotype ST8(002)

(A) Incidence of C difficile infections by fluoroquinolone susceptibility for genotype ST1(027) in Oxfordshire. Red bars indicate fluoroquinolone-resistant isolates, blue bars indicate fluoroquinolone-susceptible isolates, grey bars indicate resistance not determined. (B) Incidence of C difficile infections by fluoroquinolone susceptibility for genotype ST3(001) in Oxfordshire. (C) Incidence of C difficile infections by fluoroquinolone susceptibility for genotype ST8(002) in Oxfordshire. (D) Time-scaled phylogeny for ST1(027) generated with ClonalFrameML. Every third Oxfordshire isolate (by date) is shown. Phylogenies were scaled to be directly similar post-1990; the grey shaded regions before 1990 represent the regions of the phylogenies that should not be compared because they are not scaled identically. Background colour indicates fluoroquinolone susceptibility; branch colour indicates geographic location. (E) Time-scaled phylogeny for the mixed fluoroquinolone-resistant or susceptible genotype, ST3(001), generated using ClonalFrameML. Two fluoroquinolone-resistant areas of the phylogeny are indicated by red shading within the blue susceptible region. Rapid clonal expansion after resistance emergence is supported by significantly lower ED scores for resistant versus susceptible areas. (F) Time-scaled phylogeny for ST8(002) generated using ClonalFrameML. Two fluoroquinolone-resistant isolates are indicated at the bottom of the panel. IRR=annual incidence rate ratio. ED=evolutionary distinctiveness. R=fluoroquinolone resistant. S=fluoroquinolone susceptible.
ST2(014/020) \((p=0.0014; \text{appendix})\), consistent with greater transmission in the former.

Fluoroquinolone restriction and multiple enhanced infection control measures were introduced simultaneously in England in 2007.\(^7\) Therefore, we investigated the hypothesis that infection control, not antimicrobial stewardship, reduced incidence of \(C.\) difficile infection by reducing transmission (eg, that fluoroquinolone-resistant isolates were simply more prevalent in hospitals where infection control efforts were concentrated). Secondary spread (transmission) was inferred when subsequent infections had closely genetically related isolates. We estimated the Oxfordshire incidence of inferred secondary cases separately for fluoroquinolone-resistance versus fluoroquinolone-susceptibility, and also for infections where hospital-based contact occurred between primary and secondary cases.\(^7\) There was strong evidence for declines in secondary \(C.\) difficile infections caused by fluoroquinolone-resistant isolates, both with hospital contact with a previous case (aIRR \(0.21, 95\%\ CI 0.13–0.34, p=0.0001\)) and without (0.45, 0.29–0.71, \(p=0.0001; \text{figure 5}\)). Declines occurred in secondary cases caused by fluoroquinolone-resistant isolates of ST1(027) and non-ST1(027) genotypes (ps0.012, \text{appendix}). By contrast, there was no evidence of declines in secondary cases caused by fluoroquinolone-susceptible isolates, either with hospital contact with a previous infection (0.87, 0.67–1.13, \(p=0.29\)) or without (1.14, 0.92–1.42, \(p=0.23\)), supporting the importance of fluoroquinolone restriction over infection control interventions.

**Discussion**

Our analysis of multiple whole genome sequence datasets shows that reductions in the incidence of \(C.\) difficile infections caused by fluoroquinolone-resistant isolates (of multiple genotypes) plausibly has driven the decline in \(C.\) difficile infections in Oxfordshire and Leeds, England, from 2007. Declines occurred alongside significant reductions in fluoroquinolone use in hospitals and the community. Extensive whole genome sequence phylogenies show that acquisition of fluoroquinolone resistance preceded the emergence of multiple, prevalent genotypes (figure 4, \text{appendix}); after fluoroquinolone prescribing was controlled, incidence declines were specific to \(C.\) difficile infections caused by fluoroquinolone-resistant isolates of these same genotypes (figure 3, figure 4, \text{appendix}). By contrast, the incidence of \(C.\) difficile infections from multiple fluoroquinolone-susceptible genotypes remained constant (figure 3, figure 4C, \text{appendix}), unaffected by changes in fluoroquinolone use or other national policy measures, such as restricted cephalosporin prescribing and enhanced infection control interventions, irrespective of genotype (figure 5, \text{appendix}).\(^7\) Crucially, there was no evidence of a decline in plausibly nosocomially transmitted secondary cases caused by fluoroquinolone-susceptible \(C.\) difficile, which would be expected if improved infection control had made a major contribution to \(C.\) difficile infection declines, whereas secondary cases caused by fluoroquinolone-resistant \(C.\) difficile decreased markedly (figure 5, \text{appendix}).

The phylogenetically estimated date of fluoroquinolone resistance emergence preceded the clinical emergence of several problematic \(C.\) difficile genotypes of different phylogenetic clades: \(^7\) ST1(027), \(^9\) ST42(106), ST3(001), and ST37(017) (figure 4, \text{appendix}).\(^28,29\) The recent emergence of fluoroquinolone-resistant ST17(018) in Italy (\text{appendix}) also followed high fluoroquinolone use.\(^9\) Our greater sampling density revealed short-branched, geographically structured phylogenies of fluoroquinolone-resistant \(C.\) difficile consistent with rapid spread within hospitals, and occasional transmission between them (figure 4D–F, \text{appendix}). Inclusion of international isolates allowed us to show generalisability of our findings outside of the UK. Although fluoroquinolone-susceptible, limited ST8(002) and ST2(014/020) transmission plausibly occurred, as indicated by small, short-branched clusters, and lower ED scores for clinical-toxin EIA-positive isolates than for infant/EIA-negative isolates (\text{appendix}). However, the absence of large-scale geographic structure in the long-branched phylogenies of all fluoroquinolone-susceptible isolates was inconsistent with widespread geographic spread.

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**Figure 5:** Incidence trends of inferred secondary \(C.\) difficile cases in Oxfordshire from April 2008 to March 2011

Inferred secondary cases are those caused by \(C.\) difficile isolates that are genetically closely related (\(\leq\)two single nucleotide variants) to isolates recovered from a previous case, and therefore potentially transmitted. Incidence trends were calculated separately for inferred secondary cases caused by fluoroquinolone-resistant ST1(027), fluoroquinolone-resistant non-ST1(027), and fluoroquinolone-susceptible isolates, stratified by with versus without hospital-based contact. Horizontal dotted line shows an IRR per year of 1 (ie, no change over time) against which the 95% CI bars are compared to determine statistical significance of any change. The \(p\) values are a test of the IRR against the null hypothesis of no change over time (IRR=1). IRR=annual incidence rate ratio.
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...genotypes (appendix) suggests that most were introduced independently into the clinical environment from alternative potential reservoirs. Antibiotics, and when taking community prescribing with previously successful small-scale interventions national restriction of high-risk antimicrobials is consistent reduced, although this change could still predominantly total antimicrobial (not only fluoroquinolone) use was...ST1(027) outbreak control has also been achieved when they were instead used in combinations, such as co-

...penicillins (mostly amoxicillin alone) and beta-lactamase resistant penicillins (mostly fluoxacinil alone) were stronger than for fluoroquinolones in Oxfordshire, the use of many antibiotics in these groups actually rose because they were instead used in combinations, such as co-amoxiclav. Penicillins generally have a lesser risk of infection and hospital-prescribed extended-spectrum penicillins (mostly amoxicillin alone) and beta-lactamase resistant penicillins (mostly fluoxacinil alone) were stronger than for fluoroquinolones in Oxfordshire, the use of many antibiotics in these groups actually rose because they were instead used in combinations, such as co-amoxiclav. Penicillins generally have a lesser risk of fluoroquinolones-susceptibility-specific declines in incidence observed. Similarly, if an ST1(027)-specific factor had led to its decline, there would be no reason for...C difficile infection and hospital-prescribed extended-spectrum penicillins (mostly amoxicillin alone) and beta-lactamase resistant penicillins (mostly fluoxacinil alone) were stronger than for fluoroquinolones in Oxfordshire, the use of many antibiotics in these groups actually rose because they were instead used in combinations, such as co-amoxiclav. Penicillins generally have a lesser risk of...C difficile infections also persisted in carriers, outbreak control procedures were strengthened. This finding supports the greater importance of fluoroquinolone restriction in both hospitals and the community over enhanced infection control in recent reductions in English incidence of C difficile infection. Antimicrobial stewardship targeted all patients in hospitals and the community, so clinically adapted resistant C difficile might conceivably have been eliminated from asymptomatic carriers and cases. If fluoroquinolone-resistant C difficile persisted in carriers, outbreak conditions should have returned rapidly once fluoroquinolone prescribing increased. This did not occur even after post-2010 increases in hospital fluoroquinolone prescribing in Oxford and Leeds (figure 2A, appendix). However, whereas before 2007 fluoroquinolones were prescribed widely, including in elderly people, increases after 2010 do not necessarily equate to increased exposure of patients with high risk of C difficile infection. Instead, these increases might reflect new, specific indications such as neutropenic prophylaxis (see appendix for Leeds; equivalent data not available in Oxford), consistent with observations that fluoroquinolone use is not a risk factor under non-outbreak conditions. The lack of national rise in fluoroquinolone-resistant C difficile infections also...patients infected with transmitted strains, irrespective of fluoroquinolone susceptibility. Analysis of closely related C difficile genomes from different patients (ie, representing possible transmissions potentially preventable by infection control measures) clearly showed that incidence only fell for secondary cases caused by fluoroquinolone-resistant C difficile, irrespective of hospital contact with a previous closely genetically related case, with no change in secondary cases caused by fluoroquinolone-susceptible isolates (figure 5, appendix). This finding is consistent with previous work finding no change in incidence of C difficile infection after infection control procedures were strengthened. This finding supports the greater importance of fluoroquinolone restriction in both hospitals and the community over enhanced infection control in recent reductions in English incidence of C difficile infection. Antimicrobial stewardship targeted all patients in hospitals and the community, so clinically adapted resistant C difficile might conceivably have been eliminated from asymptomatic carriers and cases. If fluoroquinolone-resistant C difficile persisted in carriers, outbreak conditions should have returned rapidly once fluoroquinolone prescribing increased. This did not occur even after post-2010 increases in hospital fluoroquinolone prescribing in Oxford and Leeds (figure 2A, appendix). However, whereas before 2007 fluoroquinolones were prescribed widely, including in elderly people, increases after 2010 do not necessarily equate to increased exposure of patients with high risk of C difficile infection. Instead, these increases might reflect new, specific indications such as neutropenic prophylaxis (see appendix for Leeds; equivalent data not available in Oxford), consistent with observations that fluoroquinolone use is not a risk factor under non-outbreak conditions. The lack of national rise in fluoroquinolone-resistant C difficile infections also...C difficile infections also supports their almost complete eradication from both symptomatic patients and asymptomatic carriers in England, consistent with regional (Oxfordshire) findings that by late 2011, fluoroquinolone-resistant isolates of the commonest incidence genotype (ST1(027)) had disappeared from asymptomatic colonisation as well as infection. The genotypes ST1(027), ST42(106), ST3(001), and ST37(017), accounting for most fluoroquinolone-resistant isolates, represent three divergent C difficile clades, each with a genetically distinct, toxin-encoding pathogenicity locus. These genotypes could therefore differ in virulence or transmissibility due to varying gene content. ST1(027), for example, is almost four times likelier than other genotypes to cause symptomatic infection (although this could reflect its fluoroquinolone-resistant phenotype in settings with high fluoroquinolone prescribing). It seems unlikely that other gene content should be completely confounded with fluoroquinolone...
resistance, particularly within the large clade 1* (containing ST42(106), ST3(001), and Italian ST17(018)). However, even if additional virulence factors are associated with ST1(027), the overall diversity of outbreak-associated genetic backgrounds in which fluoroquinolone resistance is found suggests that this phenotype alone might be sufficient to confer outbreak potential.

A few sporadic fluoroquinolone-resistant isolates were identified in otherwise susceptible genotypes (appendix), suggesting that chance, combined with regional antibiotic prescribing policies, could trigger localised spread. ST11(078) was unusual, in that fluoroquinolone resistance occurred in 24 (13%) of 182 isolates, distributed throughout the phylogeny (appendix). ST11(078) can be transmitted zoonotically,26 and the unstructured pattern of fluoroquinolone resistance within this phylogeny could reflect the sporadic emergence of resistance either during agricultural fluoroquinolone use, or after human colonisation and antibiotic exposure.

The main study limitation was being primarily based in one, albeit large (population of approximately 600 000 people) region, where 7 years of individual-isolate whole genome sequences enabled us to predict fluoroquinolone susceptibility. Whole genome sequence data from Leeds were available for less than 3 years, precluding a similar analysis to figure 2 in another region. Different datasets from different sources were used for incidence of C difficile infections and antibiotic use because no one dataset was collected consistently across the entire period from a single source. Comparisons of incidence of C difficile infections and antibiotic use are ecological, and therefore prone to unmeasured confounding. English hospital-level antibiotic data are not available before 2013 (only subsequently),24 so we were unable to investigate associations between fluoroquinolone use and C difficile infections across Trusts in a broader ecological analysis. However, our key characteristics, fluoroquinolone susceptibility and genotype, were unknown when the C difficile infections occurred and were not part of the inclusion or exclusion criteria. Therefore, the phylogenetic analyses are representative of the genotypes circulating in the locations studied when sampled.

In summary, fluoroquinolone resistance occurs in several genetically divergent C difficile genotypes.26 The contrasting phylogenies of fluoroquinolone-resistant and fluoroquinolone-susceptible C difficile probably reflect increased potential for health-care-associated selection and epidemic spread of fluoroquinolone-resistant bacteria. Thus, the C difficile genotypes causing infections at any given time and location, and the relative importance of different transmission routes (nosocomial person-to-person versus multiple introductions) might be a direct consequence of antimicrobial prescribing policies. The multifaceted approach to C difficile infection control adopted by England successfully curtailed transmission. Whole genome sequence data suggest that fluoroquinolone restriction plausibly played the most important part in this success. Appropriate antimicrobial stewardship therefore is, and will likely remain, central to the control of C difficile infections.

Contributors
MHW, TEAP, ASW, and DWC contributed equally to the work. KED, XD, and TPQ contributed equally to the work. KED, XD, TPQ, MHW, TEAP, ASW, and DWC designed the study with input from DWE, NS, TG, RMH, and DJW. KED, DWE, NS, DG, AW, SHO, WNF, JM, and JM collected specimens from Oxfordshire and Leeds, cultured C difficile, and extracted chromosomal C difficile DNA for whole genome sequences. SG, EJCG, and DMC contributed the fidaxomicin clinical trial isolate collection. EJCG and DMC did fluoroquinolone susceptibility testing. The Modernising Microbiology Informatics Group, JMF, TG, and DJW did the assembly of short DNA sequence reads. KED derived genotype data from whole genome sequences and identified genomes for the construction of ClonalFrameML, dual-scaled phylogenies. All phylogenies were done by XD. KED combined phylogenetic and fluoroquinolone resistance genotype data. PH, SH, MHW, and DWC obtained antimicrobial prescribing data, RH and APJ obtained national incidence data for C difficile infection. TPQ, ASW, and TEAP did the biostatistical analysis of Oxfordshire and national incidence and antimicrobial prescribing data. WNF, TPQ, ASW, and MHW did the biostatistical analysis of Leeds incidence and antimicrobial prescribing data. DWE, TPQ, ASW, and TEAP did the inferred secondary cases analysis. KED, XD, TPQ, MHW, TEAP, ASW, and DWC wrote the first draft of the article and all authors contributed to and had final approval of the Article.

Declaration of interests
Relevant to the submitted work, MHW has received both grants and personal fees from Actelion, Cubist, Astellas, Merck, Sanofi-Pasteur, Summit, Bionerieux, and Quagen; personal fees only from Optimer and Synthetic Biologics; and grants, personal fees, and other funding were received from Alere (the latter including consulting fees, research funding; and a grant to department). Outside the submitted work, MHW received grants and personal fees from Cereza, Abbott, Da Volterra, and European Tissue Symposium; and personal fees only from AstraZeneca, Durata, Nabriva, Pfizer, Roche, The Medicines Company, V H Squared, Basilea, Bayer, MotifBio, and Pareake. EJCG reports the following relationships: 2016 advisory boards for Merck & Co, Bayer Pharmaceuticals, BioK+, Sanofi-Aventis, Summit Corp PLC, Kindred Healthcare Corp, Novartis, Sanlysou-Daichi, Rempe; speakers’ bureau for Bayer Inc and Merck & Co; and research grants from Merck & Co, Theraave Inc, Pfizer Inc, Astellas Inc, Cereza, Forrest Pharmaceuticals, ImpeX Pharmaceuticals, Novartis, Clinical Microbiology Institute, Genzyme, Nanopacific Holdings Inc, Romark Laboratories LC, Vicrox Corp, Warner Chilcott, AvidiBiotics Corp, GLSyntesis Inc, Immunome Inc, Toliche Pharma LLC, Salix, Summit Corp PLC, GlassSmithKline, Rempe Pharmaceuticals, Synbiotic Therapeutics, Tolbec Pharmaceuticals LLC, Anicrobio Inc, Durata, Gynuity Health Projects, and Medicines Company. SH is affiliated with the National Institute for Health Research Health Protection Research Units (NIHR HPRU) in Healthcare Associated Infection and Antimicrobial Resistance at Imperial College London in partnership with Public Health England and the NIHR HPRU in Healthcare Associated Infection and Antimicrobial Resistance at University of Oxford in partnership with Public Health England. The views expressed are those of the author and not necessarily those of the NHS, the NIHR, the Department of Health, or Public Health England. JF reports grants from Astellas Pharma Europe, Melinta Therapeutics, and Morphochern AG, outside the submitted work. PH has received speaker’s fees from Astellas, advisory board fee from Merck Sharp & Dohme, conference and travel fees from Euromedica, and speaker’s fees from Gilead, outside the submitted work. SG reports previous employment with Optimer Pharmaceuticals and Cubist, as well as several patents with Optimer Pharmaceuticals (mostly expired, no income). ASW reports grants from Welcome Trust, grants from National Institutes of Health UK, grants from Medical Research Council UK, grants from Department of Health UK, during the conduct of the study. The other authors declare no competing interests.

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