1   **Effects of control interventions on *Clostridium difficile* infection in England: an**

2   **observational study**

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43   **Research in Context**

44   **Evidence before this study**

45   England is almost unique in experiencing a marked, recent decline in the incidence of

46   healthcare-associated *C. difficile* infections (CDI). Previous reports showed the decline of one

47   epidemic genotype (PCR-ribotype 027), whereas other genotypes appeared to persist. These

48   changes followed the implementation of a multifaceted national CDI control policy in 2007.

49   However, the relative contributions made by the different interventions that were introduced

50   simultaneously is unknown.

51   **Added value of this study**

52   This study is the first to investigate the contribution of specific public health interventions to

53   the marked national decline in CDI. Our novel approach involved the integrated analysis of

54   multiple, large, concurrent data sets concerning CDI incidence, antimicrobial prescribing,

55   and, crucially, the whole genome sequences (WGS) of over 4000 human *C. difficile* isolates.

56   Our key finding was that the post-interventions decline in CDI reflected the disappearance of

57   fluoroquinolone-resistant isolates (predominantly from four, genetically distinct genotypes),

58   while the incidence of CDI caused by fluoroquinolone-susceptible isolates (of many different

59   genotypes) remained unchanged. WGS-based phylogenetic analyses of the entire *C. difficile*

60   population, with one phylogeny constructed for each genotype, identified shorter,

61   geographically clustered branches, specific to the fluoroquinolone-resistant regions. This is

62   consistent with rapid nosocomial transmission preceding the disappearance of

63   fluoroquinolone-resistant isolates. Among the susceptible isolates the numbers that were

64   closely genetically related (and by inference transmitted, either directly or indirectly), did not

65   change over time. This was despite the implementation of comprehensive infection

66   prevention and control measures, which would have targeted fluoroquinolone-resistant and

67   susceptible *C. difficile* equally. These data suggest that it was the restriction of

68 fluoroquinolone prescribing, above other interventions, (including cephalosporin restriction

69 and infection control precautions), that appears to explain the decline in CDI incidence.

70 **Implications of all the available evidence**

71 This powerful population genetic and biostatistical analysis supports the restriction of

72 fluoroquinolone prescribing as a cornerstone in the control of epidemic CDI in the UK and

73 worldwide.

74 **SUMMARY**

75 **Background**

76 The control of *Clostridium difficile* infections (CDI) is an international clinical challenge.

77 Uniquely, CDI incidence in England declined by ~80% after 2006, following implementation

78 of national control policies; we investigated their role in this decline. This study tested two

79 hypotheses. First, if CDI declines in England were driven by changes in use of particular

80 antibiotics, then incidence of CDI caused by resistant isolates should decline faster than that

81 caused by susceptible isolates across multiple genotypes (defined by multilocus sequence

82 type (ST)). Second, if CDI declines were driven by improvements in hospital infection

83 control, then transmitted (secondary) cases should decline regardless of susceptibility.

84 **Methods**

85 Regional and national CDI incidence and antimicrobial prescribing data (1998-2014) were

86 combined with whole genome sequences (WGS) from 4045 national and international *C.*

87 *difficile* isolates. Genotype (multilocus sequence type) and fluoroquinolone susceptibility

88 were determined from WGS. The incidence of CDI caused by fluoroquinolone-resistant and -

89 susceptible isolates was estimated using negative-binomial regression, overall and per

90 genotype. Selection and transmission were investigated using phylogenetic analyses.

91 **Findings**

92 National fluoroquinolone and cephalosporin prescribing correlated highly with CDI incidence

93 (cross-correlations>0·88), in contrast to total antibiotic prescribing (cross-correlations<0·59).

94 Regionally, CDI decline was driven by elimination of fluoroquinolone-resistant isolates

95 (~67% of Oxfordshire cases in September 2006, ~3% in February 2013; annual incidence rate

96 ratio: 0·52, (95%CI 0·48,0·56), versus fluoroquinolone-susceptible isolates: 1·02,

97 (0·97,1·08)). CDI caused by fluoroquinolone-resistant isolates declined in four distinct

98 genotypes (p<0.01). The regions of phylogenies containing fluoroquinolone-resistant isolates

99   were short-branched and geographically-structured, consistent with selection and rapid

100   transmission. The importance of fluoroquinolone restriction over infection control was

101   demonstrated by significant declines in inferred secondary (transmitted) cases caused by

102   fluoroquinolone-resistant isolates with or without hospital contact ($p<0.0001$), versus no

103   change in either group of cases caused by fluoroquinolone-susceptible isolates ($p>0.2$).

104   **Interpretation**

105   Restricting fluoroquinolone prescribing appears to explain the decline in CDI incidence,

106   above other measures, in Oxfordshire and Leeds, England. Antimicrobial stewardship should

107   be a central component of CDI control programs.

108   **Funding**

INTRODUCTION

Clostridium difficile infection (CDI) remains a major clinical challenge worldwide.[1,2] At least

three antimicrobial classes are considered high-risk CDI triggers,[3] including most

cephalosporins, to which *C. difficile* is inherently resistant[4] and clindamycin, to which

genotypes causing early outbreaks had acquired resistance.[5,6,7] Recent global dispersion of

'hypervirulent' NAP1/PCR-ribotype-027 CDI revealed an association between

fluoroquinolone resistance and epidemic spread.[8,9] Accordingly, restricting clindamycin or

fluoroquinolone use has been employed, with other measures, to control localised CDI

outbreaks.[7,10,11]

Most CDI cases are temporally associated with healthcare,[2] reflecting a combination of

healthcare-associated acquisition, and healthcare-related triggers including antibiotics. Three

UK studies using highly discriminatory whole genome sequencing (WGS),[12,13,14] and a US

study using alternative high-resolution typing,[15] found as few as one-third of CDIs involved

recent acquisition from an active case, leaving the source for two-thirds unexplained.

Contrasting with other countries,[1,2] CDI incidence in England declined markedly over the last

decade,[16] following the introduction of national CDI prevention and management policies

from June 2007.[17,18] These included recommendations to avoid clindamycin and

cephalosporins, minimise fluoroquinolone, carbapenem and aminopenicillin use, and improve

infection prevention and control activities (Supplementary Methods).[17] We investigated the

impact of these interventions on *C. difficile* evolution, selection and transmission, to inform

future CDI control policies for this global challenge.

METHODS

This study tested two hypotheses. First, if CDI declines in England were driven by changes in use of particular antibiotics, then incidence of CDI caused by resistant isolates should decline faster than that caused by susceptible isolates across multiple genotypes (defined by multilocus sequence type (ST)). Second, if CDI declines were driven by improvements in hospital infection control, then transmitted (secondary) cases should decline regardless of susceptibility.

To confirm that national policies[17,18] affected antibiotic prescribing and CDI incidence, we first compared national antimicrobial prescribing data for hospitals and the community (obtained respectively from IMS Health, (Danbury, CT) and the Health & Social Care Information Centre (Supplementary Methods)) with national CDI incidence i.e. cases per English population per year (data from Public Health England).

The primary study dataset comprised WGS from clinical *C. difficile* isolates cultured from consecutive toxin enzyme immunoassay (EIA)-positive stool samples from symptomatic patients submitted to the Oxford University Hospitals NHS Trust between September 12, 2006-August 19, 2013 (n=2,021) (Supplementary Methods; Table S1). The hospital conducts all *C. difficile* testing in Oxfordshire, serving general practices, community hospitals and other providers, so incidence is per Oxfordshire population (~600,000) per year. This culture-positive CDI incidence was compared to Oxfordshire's nationally-submitted EIA-positive incidence (incorporating changes in mandatory reporting requirements in 2008) to confirm representativeness of WGS. The latter was compared to English CDI incidence to assess generalisability.

8

162   Generalisability of Oxfordshire data were also assessed using comparable information from

163   Leeds Teaching Hospitals NHS Trust, UK. This comprised WGS for consecutive clinical,

164   toxin-positive (cytotoxin assay) isolates from symptomatic patients (August 2 2010 to May 1

165   2013; n=1,020) (Table S1), Leeds regional CDI incidence data (nationally-submitted) and

166   ribotype prevalence, and antibiotic prescribing data.

167

168   Additional genetic context was provided by further regional and international *C. difficile*

169   WGS (May 9, 2006-July 12, 2013) for isolates from: toxin-EIA-negative clinical samples of

170   symptomatic Oxfordshire patients (n=395); toxin-positive samples representing two clinical

171   trials of fidaxomicin in North America and Europe (n=803),[19,20] and from healthy

172   Oxfordshire infants (non-clinical, n=200) (Table S1).

173

174   **Genome Sequencing and Multilocus Sequence Type Identification**

175   Genomes were sequenced using Illumina technology. Velvet *de novo* assemblies and

176   reference-based assemblies were generated, the latter mapped to *C. difficile* 630 (GenBank

177   AM180355.1) (Supplementary Methods; reads submitted to NCBI, BioProjectID

178   PRJNA304087, accession numbers Table S1). The sequences of loci defining *C. difficile* STs

179   were identified and extracted using BIGSdb;[21] STs were assigned using

180   http://pubmlst.org/cdifficile/. The notation ST1(027) indicates, for example, Sequence-Type-

181   1 (PCR-ribotype-027).

182

183   **WGS-derived Fluoroquinolone Susceptibility**

184   Isolates were designated fluoroquinolone-susceptible or -resistant based on specific non-

185   synonymous substitutions within the quinolone resistance-determining region of *gyrA/B*

186   genes[22,23] extracted from WGS.[21] *gyrA* C(245)T [T(82)I] and *gyrB* G(1276)A [D(426)N]

187   confer high-level fluoroquinolone resistance in *C. difficile* and other species.[16,17]

188   Susceptibility predictions were validated phenotypically for 387 fidaxomicin trial isolates[19,20]

189   (n=191 Canada, n=196 USA), using agar dilution (moxifloxacin minimum inhibitory

190   concentration, (MIC)) (Figure S1, Supplementary Material).

191

192   **Statistical Analysis**

193   Univariable comparisons between English antimicrobial prescribing and CDI incidence were

194   made using bivariate cross-correlations (Supplementary Methods). Genotype(ST)-specific

195   incidence rates for CDI caused by toxin EIA-positive, culture-positive isolates were

196   calculated using negative binomial regression accounting for missing data by probability

197   weights (Supplementary Methods). For genotypes with >10% fluoroquinolone-resistant

198   isolates, rates were calculated separately for fluoroquinolone-susceptible and

199   fluoroquinolone-resistant isolates. Rates were also calculated separately for cases that could

200   plausibly have arisen from secondary spread (transmission) inferred by close genetic

201   relationships to prior cases (≤2 single nucleotide variants (SNVs) from the original case[12]),

202   and also separately for fluoroquinolone-susceptible and fluoroquinolone-resistant isolates.

203   Phylogenetic trees were constructed for each ST (or several closely related STs), using

204   maximum likelihood, then corrected for recombination using ClonalFrameML version 1.0-

205   6.[24] Trees were time-scaled and made directly comparable post-1990 (Supplementary

206   Methods). In each tree, the Evolutionary Distinctiveness (ED) score of each genome was

207   calculated;[25] low ED scores indicate closely related genomes, whereas high scores indicate

208   their relative absence (Supplementary Methods).

209

210   **Role of the Funding Source**

214 **RESULTS**

215 **CDI Incidence and Antibiotic Prescribing**

216 CDI incidence in England increased from 1998-2006 (p<0.0001) then declined rapidly to

217 2013 (p<0.0001) (Figure 1A). Declines occurred while total antibiotic prescribing was

218 increasing (by 4.4%/year in the community (p<0.0001, 2006-2013) but only 0.5%/year in

219 hospitals (p=0.053, 2006-2012)) (Figure 1B). Between 2005-2012 (when data were complete

220 for England), the cross-correlations (CC) between English CDI incidence and total English

221 antibiotic prescribing were -0.57 (95% CI -0.67,-0.41), -0.59 (-0.68,-0.44) and 0.29 (-

222 0.19,0.60) (for hospital+community, community, and hospital prescribing respectively,

223 optimum CC using a 1-year lag, Table S2, Supplementary Methods). During the same period,

224 the strongest univariable associations between English CDI incidence and individual

225 antimicrobials were with cephalosporins (CC=0.97 (0.82,0.98), 0.94 (0.68,0.97) and 0.97

226 (0.81,0.99) respectively (optimum 0-year lag)) and fluoroquinolones (CC=1.00 (0.84,1.00),

227 0.88 (0.48,0.95) and 0.93 (0.66,0.97) respectively (optimum 0-year lag)) (Figure S2A, Table

228 S2), although hospital fluoroquinolone prescribing began to decline slightly earlier than

229 community prescribing (p<0.0001 from 2005-2009, vs in the community p<0.0001 from

230 2007-2012; Figure 1A). Other antibiotics were more weakly associated (Figure S2A-F, Table

231 S2).

232

233 Similar to English CDI incidence, Oxfordshire rates also decreased from 2007 (when isolate-

234 level fluoroquinolone-susceptibility could be determined) (p<0.0001) (Figure 2A).

235 Fluoroquinolone prescribing in Oxfordshire hospitals declined from a peak in 2005 until 2010

236 (p<0.0001), when usage began to increase again (p<0.0001 from 2010-2013). Hospital

237 cephalosporin and fluoroquinolone prescribing were also positively associated with CDI

238 incidence (CC=0.73 (0.15,0.86) and 0.62 (-0.09,0.81),, Table S2), but associations were

239   estimated much less precisely given the much smaller population (~1% of England). Positive

240   associations were also observed between CDI decline and decline in extended spectrum

241   penicillins (CC=0.84 (0.24,0.90)) and beta-lactamase resistant penicillins (CC=0.67 (-

242   0.04,0.81), Figure S9. Community prescribing data was not available.

243

244   **Predicting *C. difficile* Fluoroquinolone Susceptibility from WGS**

245   Paired fluoroquinolone susceptibility phenotype and *gyrA*/*B* DNA sequences were assessed

246   for 387 isolates representing 53 STs. Phenotype and WGS were 98.7% concordant (Figure

247   S1; sensitivity 97.8%, specificity 99.5%); only 1/185 isolates predicted resistant by WGS[22,23]

248   lacked an elevated MIC. Conversely, only 4/202 isolates lacking resistance-associated

249   substitutions[22,23]) had raised MICs (16 mg/L). *gyrA*/*B* sequence therefore reliably predicts

250   fluoroquinolone resistance phenotype.

251

252   **Regional CDI Incidence and Fluoroquinolone Susceptibility**

253   The decrease in Oxfordshire cases was due solely to a decline in CDI caused by

254   fluoroquinolone-resistant isolates (estimated at ~67% of all Oxfordshire CDI September

255   2006, falling to ~3% February 2013, annual incidence rate ratio (aIRR)=0.52 (95% CI 0.48-

256   0.56) $p<0.0001$ (Figure 2B)). The majority (62%) of fluoroquinolone-resistant isolates were

257   from genotype ST1(027), but the decline persisted even when excluding ST1(027) and

258   pooling the remaining fluoroquinolone-resistant isolates together, (aIRR=0.73 (0.66-0.81),

259   $p<0.0001$ for all non-ST1; aIRR=0.66 (0.59-0.75), $p<0.0001$ for all non-ST1 with >10%

260   resistant isolates, Figures 3, S3D). Considering genotypes containing >10% resistant isolates

261   separately, CDI caused by fluoroquinolone-resistant isolates declined significantly for four

262   genotypes from three distinct chromosomal backgrounds[26]: clade 1 ST42(106) (p=0.00076),

263   ST3(001) (p=0.0054); clade 2 ST1(027) (p<0.0001) and clade 4 ST37(017) (p=0.0027),

264   Figures 3, 4A-B, S3A-C).

265

266   Notably, the incidence of CDI caused by fluoroquinolone-susceptible isolates remained

267   unchanged (aIRR=1.02 (95% CI 0.97-1.08) p=0.45, Figure 2B, heterogeneity p<0.0001 vs

268   fluoroquinolone-resistant), and actually increased in three of the five genotypes with >10%

269   but <99% resistant isolates (Figure 3, 4B, S3A-C). More limited data for Leeds, representing

270   a geographically independent region, were broadly similar (aIRR=0.55 (0.49-0.61) p<0.0001

271   pooling predominantly fluoroquinolone-resistant ribotypes, aIRR=1.03 (1.01-1.05) p=0.0031

272   pooling fluoroquinolone-susceptible ribotypes, Figure S4, Table S2), as were national

273   ribotyping data,[27] supporting generalisability.

274

275   **Phylogenetic evidence for fluoroquinolone-driven *C. difficile* transmission**

276   Nineteen phylogenies (Figures 4D-F, S5A-D), were constructed representing the 22 most

277   common *C. difficile* genotypes in Oxfordshire and Leeds. The phylogeny of each genotype

278   containing >10% fluoroquinolone-resistant isolates (Figure 4D,E, S5A) indicated rapid,

279   geographically structured clonal expansion(s) associated with resistance. This observation

280   was reproduced internationally among parts of the phylogenies representing Calgary, Canada

281   (Figure 4D,E) and among isolates from three cities in Northern Italy; Modena, Turin and

282   Arsizio (Figure S5A). It was supported by significantly lower ED scores for resistant versus

283   susceptible areas of phylogenies containing both fluoroquinolone-resistant and

284   fluoroquinolone-susceptible isolates (e.g. ST3 p<0.0001 Figure 4E, ST37 p<0.0001, Figure

285   S5A). By contrast, the phylogenies of genotypes consisting primarily of susceptible isolates

286   (Figure 4F, S5A-D) were geographically unstructured and had longer branches. This was also

287   seen internationally among susceptible isolates from Calgary and Montreal, Canada (Figure

288 4E, Figure S5B). Among fluoroquinolone-susceptible genotypes, the ED scores (and, by

289 inference, transmission) did not differ significantly between Oxfordshire and Leeds clinical

290 isolates (p>0.1) (Figure S5).

291

292 Additional phylogenies for three prevalent fluoroquinolone-susceptible genotypes revealed

293 similar branch lengths irrespective of sampling region size (Figure S6A,B). Oxfordshire

294 phylogenies (Figure S6B), containing genomes from toxin EIA-positive and -negative

295 samples, plus genomes from healthy, asymptomatic, community infants, demonstrated a lack

296 of structure by source, even within a single region. Interestingly, ED scores were generally

297 lower for clinical toxin EIA-positive genomes compared with infant and EIA-negative

298 genomes, especially in ST8(002) (p=0.0033) and ST2(014/020) (p=0.0014) (Figure S6A,B),

299 consistent with greater transmission in the former.

300

301 Fluoroquinolone restriction and multiple enhanced infection control measures were

302 introduced simultaneously in England in 2007.[17] Therefore, we investigated the hypothesis

303 that infection control, not antimicrobial stewardship, reduced CDI incidence by reducing

304 transmission; e.g. fluoroquinolone-resistant isolates were simply more prevalent in hospitals

305 where infection control efforts were concentrated. Secondary spread (transmission) was

306 inferred when subsequent cases had closely genetically related isolates. The Oxfordshire

307 incidence of inferred secondary cases was estimated separately for fluoroquinolone-resistance

308 versus fluoroquinolone-susceptibility, and also for cases where hospital-based contact

309 occurred between primary and secondary cases[12]. There was strong evidence for declines in

310 secondary CDI cases caused by fluoroquinolone-resistant isolates, both with (aIRR=0.21

311 (95% CI 0.13–0.34, p<0.0001) and without (aIRR=0.45 (0.29-0.71), p<0.0001) hospital

312 contact with a previous case. Declines occurred in secondary cases caused by

313   fluoroquinolone-resistant isolates from ST1(027) and non-ST1(027) genotypes (p≤0.012,

314   Figures 5, S8). By contrast, there was no evidence of declines in secondary CDI cases caused

315   by fluoroquinolone-susceptible isolates, either with (aIRR=0.87 (0.67–1.13), p=0.29), or

316   without (aIRR=1.14 (0.92–1.42), p=0.23) hospital contact with a previous case, supporting

317   the importance of fluoroquinolone restriction over infection control interventions.

DISCUSSION

Here, analysis of multiple WGS datasets demonstrates that reductions in CDI incidence

caused by fluoroquinolone-resistant isolates (of multiple genotypes) plausibly has driven the

CDI decline in Oxfordshire and Leeds, England from 2007. Declines occurred alongside

significant reductions in fluoroquinolone use in hospitals and the community. Extensive

WGS phylogenies show that acquisition of fluoroquinolone resistance preceded the

emergence of multiple, prevalent genotypes (Figures 4, S5A); after fluoroquinolone

prescribing was controlled, incidence declines were specific to CDI caused by

fluoroquinolone-resistant isolates of these same genotypes (Figures 3, 4, S3B, S4). By

contrast, the incidence of CDI from multiple fluoroquinolone-susceptible genotypes remained

constant (Figures 3, 4C, S3C), unaffected by changes in fluoroquinolone use or other national

policy measures, such as restricted cephalosporin prescribing and enhanced infection control

interventions (irrespective of genotype) (Figures 5, S2A, Table S2[17]). Critically, 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 CDI declines, whereas secondary cases caused by

fluoroquinolone-resistant *C. difficile* decreased markedly (Figure 5, S8).

The phylogenetically estimated date of fluoroquinolone resistance emergence preceded the

clinical emergence of multiple problematic *C. difficile* genotypes of different phylogenetic

clades[26]; not only ST1(027)[9] but also ST42(106), ST3(001) and ST37(017) (Figures 4,

S5A).[28,29.] The recent emergence of fluoroquinolone-resistant ST17(018) in Italy (Figure

S5A) also followed high fluoroquinolone usage.[30] Our greater sampling density[9] revealed

short branched, geographically structured phylogenies of fluoroquinolone-resistant *C. difficile*

consistent with rapid spread within hospitals, and occasional transmission between them

343   (Figures 4D-F, S5). Inclusion of international isolates allowed us to demonstrate

344   generalisability of our findings outside of the UK. Although fluoroquinolone-susceptible,

345   limited ST8(002) and ST2(014/020) transmission plausibly occurred, as indicated by small,

346   short branched clusters, and lower ED scores for clinical-toxin EIA-positive isolates versus

347   infant/EIA-negative isolates (Figure S6A,B)). However, the absence of large-scale

348   geographic structure in the long branched phylogenies of all fluoroquinolone-susceptible

349   genotypes (Figure S5B/C, S6) suggests that most were introduced independently into the

350   clinical environment from alternative potential reservoirs.[31,32] Fluoroquinolone-susceptible *C.*

351   *difficile* may therefore represent a population lacking large-scale adaptation to antimicrobial

352   selection pressures of clinical environments.

353

354   The CDI incidence decline following national restriction of high-risk antimicrobials is

355   consistent with previously-successful small-scale interventions restricting high-risk

356   antimicrobials as part of control packages.[7,10,11] However, our study demonstrated

357   conclusively that Oxfordshire CDI declines were due to the parallel disappearance of

358   fluoroquinolone-resistant isolates of multiple genotypes (Figures 2, 3) suggesting that any

359   selective advantage specific to resistant isolates may be lost when the antimicrobial is

360   withdrawn. In England, additional antimicrobials were also targeted for restriction.[17]

361   However, only cephalosporin use also fell (Figures 2A, S2). Since all *C. difficile* is inherently

362   resistant to most cephalosporins[4] their restriction cannot explain the fluoroquinolone-

363   susceptibility specific declines in incidence observed. Similarly, if an ST1(027)-specific

364   factor had led to its decline, there would be no reason for CDI caused by fluoroquinolone-

365   resistant isolates of several other genotypes (ST42(106), ST3(001), ST37(017)) in two other

366   *C. difficile* clades (1 and 3[26]) to decline concurrently (Figures 3, 5). While univariate cross-

367   correlations between CDI decline and hospital-prescribed extended-spectrum penicillins

368   (mostly amoxicillin alone) and beta-lactamase resistant penicillins (mostly flucloxacillin

369   alone) were stronger than for fluoroquinolones in Oxfordshire, the use of many antibiotics in

370   these groups actually rose because they were instead used in combinations such as co-

371   amoxiclav. Penicillins have generally been shown to have a lesser risk of provoking *C.*

372   *difficile* than other classes of antibiotics,[8,33] and when taking community prescribing into

373   account, (which forms a larger proportion of overall antimicrobial use than hospital

374   prescribing) the correlation between these penicillin groups and CDI incidence in England

375   disappears. Unfortunately, community prescribing data were not available for Oxfordshire for

376   comparison. Finally, the much smaller population size meant these univariate cross-

377   correlations were estimated imprecisely compared with England as a whole. Our study

378   therefore clarifies the issue of whether fluoroquinolone or cephalosporin restriction alone or

379   in combination is key to CDI control.[34,35,36] However, changes in dominant genotypes over

380   time have been reported in a single centre in the absence of antimicrobial restriction

381   policies.[37] ST1(027)-outbreak control has also been achieved when total antimicrobial (not

382   only fluoroquinolone) use was reduced,[38] although this could still reflect predominantly the

383   impact of fluoroquinolones.

384

385   Similar to cephalosporin restriction, enhanced infection control measures[17] such as isolation,

386   contact precautions, and enhanced environmental cleaning do not target specific *C. difficile*

387   genotypes and should therefore reduce numbers of symptomatic patients infected with

388   transmitted strains, irrespective of fluoroquinolone-susceptibility. Analysis of closely related

389   *C. difficile* genomes from different patients, i.e. representing possible transmissions[12]

390   potentially preventable by infection control measures, demonstrated clearly that incidence

391   only fell for secondary cases caused by fluoroquinolone-resistant *C. difficile*, irrespective of

392   hospital contact with a previous closely genetically related case, with no change in secondary

393   cases caused by fluoroquinolone-susceptible isolates (Figure 5, S8). This is consistent with

394   previous work[38] finding no change in CDI incidence after infection control procedures were

395   strengthened. This supports the greater importance of fluoroquinolone restriction in both

396   hospitals and the community over enhanced infection control in recent reductions in English

397   CDI incidence.

398

399   Antimicrobial stewardship targeted all patients in hospitals and the community,[17] so clinically

400   adapted resistant *C. difficile* may conceivably have been eliminated from asymptomatic

401   carriers and cases. If fluoroquinolone-resistant *C. difficile* persisted in carriers, outbreak

402   conditions should have returned rapidly once fluoroquinolone prescribing increased. This did

403   not occur even after post-2010 increases in hospital fluoroquinolone prescribing in Oxford

404   and Leeds (Figures 2A, S4). However, whereas pre-2007 fluoroquinolones were prescribed

405   widely, including among the elderly, increases post-2010 do not necessarily equate to

406   increased exposure of high-CDI-risk patients. Instead they may reflect new, specific

407   indications such as neutropenic prophylaxis (e.g. Figure S7 for Leeds; equivalent data not

408   available in Oxford), consistent with observations that fluoroquinolone use is not a risk factor

409   under non-outbreak conditions[39]. The lack of rise in fluoroquinolone-resistant CDI nationally

410   also supports their almost complete eradication from both symptomatic patients and

411   asymptomatic carriers in England, consistent with regional (Oxfordshire) findings that by late

412   2011, fluoroquinolone-resistant isolates of the commonest incidence genotype (ST1(027))

413   had disappeared from asymptomatic colonization as well as infection.[31]

414

415   The genotypes ST1(027), ST42(106), ST3(001), and ST37(017), accounting for most

416   fluoroquinolone-resistant isolates, represent three divergent *C. difficile* clades[26], each with a

417   genetically distinct, toxin-encoding Pathogenicity Locus.[26] These genotypes could therefore

418   differ in virulence and/or transmissibility due to varying gene content. ST1(027), for

419   example, is almost four times likelier than other genotypes to cause symptomatic infection

420   over colonization[40] (although this could reflect its fluoroquinolone-resistant phenotype in

421   settings with high fluoroquinolone prescribing). It seems unlikely that other gene content

422   should be completely confounded with fluoroquinolone resistance, particularly within the

423   large clade 1[26] (containing ST42(106), ST3(001) and (Italian) ST17(018)). However, even if

424   additional virulence factors are associated with ST1(027), the overall diversity of outbreak-

425   associated genetic backgrounds in which fluoroquinolone resistance is found, suggests that

426   this phenotype alone may unfortunately be sufficient to confer outbreak-potential.

427

428   A few sporadic fluoroquinolone-resistant isolates were identified in otherwise susceptible

429   genotypes (Figure S5), suggesting that chance, combined with regional antibiotic prescribing

430   policies, could trigger localised spread. ST11(078) was unusual, in that fluoroquinolone

431   resistance occurred in 24/182 (13%) of isolates, distributed throughout the phylogeny (Figure

432   S5B). ST11(078) can be transmitted zoonotically,[32] and the unstructured pattern of

433   fluoroquinolone resistance within this phylogeny could reflect the sporadic emergence of

434   resistance either during agricultural fluoroquinolone use, or following human colonisation

435   and antibiotic exposure.

436

437   The main study limitation is being primarily based in one, albeit large (c.600,000 population)

438   region, where 7 years of individual-isolate WGS enabled us to predict fluoroquinolone

439   susceptibility. Leeds WGS were available for only 2.7 years, precluding a similar analysis to

440   Figure 2 in another region. Different datasets from different sources were used for CDI

441   incidence and antibiotic use because no one dataset was collected consistently across the

442   entire period from a single source. Comparisons of CDI incidence and antibiotic use are

443 ecological, and therefore prone to unmeasured confounding. English hospital-level antibiotic

444 data are not available before 2013 (only subsequently[41]), so we were unable to investigate

445 associations between fluoroquinolone use and CDI across Trusts in a broader ecological

446 analysis. However, our key characteristics, fluoroquinolone-susceptibility and genotype, were

447 unknown when the CDI occurred and were not inclusion/exclusion criteria. Therefore, the

448 phylogenetic analyses are representative of the genotypes circulating in the locations studied

449 when sampled.

450

451 In summary, fluoroquinolone resistance occurs in multiple genetically divergent *C. difficile*

452 genotypes.[26] The contrasting phylogenies of fluoroquinolone-resistant and fluoroquinolone-

453 susceptible *C. difficile* likely reflect increased potential for healthcare-associated selection

454 and epidemic spread of fluoroquinolone-resistant bacteria. Thus the *C. difficile* genotypes

455 causing infections at any given time and location, and the relative importance of different

456 transmission routes (nosocomial person-to-person versus multiple introductions) may be a

457 direct consequence of antimicrobial prescribing policies. The multifaceted approach to CDI

458 control adopted by England successfully curtailed transmission. WGS data suggest that

459 fluoroquinolone restriction plausibly played the most important role in this success.

460 Appropriate antimicrobial stewardship therefore is, and will likely remain, central to the on-

461 going control of CDI.

463   **Figure 1**

464   **A.** National CDI incidence and fluoroquinolone prescribing. Mandatory CDI incidence

465   corresponds to all cases reported in over-2s (from 2004-2007 cases were only reported in

466   over 65-s, and are upweighted to provide comparable estimates in over 2s, see Supplementary

467   Methods). As mandatory reporting was only introduced in 2004, we have also included

468   voluntary-reported CDI to give an indication of trends prior to that date.

469   **B.** National antibiotic prescribing overall. Dotted lines are estimates (see Supplementary

470   Methods).

471   **Figure 2**

472   **A.** CDI incidence together with fluoroquinolone and cephalosporin prescribing for

473   Oxfordshire. Mandatory CDI incidence corresponds to all cases reported in over-2s (from

474   2004-2007 cases were only reported in over 65-s, and are upweighted to provide comparable

475   estimates in over 2s, see Supplementary Methods). Only toxin-positive culture-positive

476   samples were used in the genotype-specific and phylogenetic analyses.

477   **B.** CDI incidence by fluoroquinolone susceptibility for Oxfordshire. IRR=Annual incidence

478   rate ratio. *C. difficile* is inherently resistant to most cephalosporins.[4]

479   **Figure 3**

480   Oxfordshire *C. difficile* incidence trends by fluoroquinolone resistance and genotype.

481   IRR=Annual incidence rate ratio. For genotypes with >10% resistant isolates (denoted FQR),

482   rates were calculated separately for CDI caused by fluoroquinolone-susceptible and resistant

483   isolates. To show that the difference in trends for resistant and susceptible isolates is not

484   driven solely by the decline in ST1(027), rates were also calculated for all non-ST1(027)

485   genotypes together, as well as for all genotypes with >10% resistant isolates (excluding

486   ST1(027)) and for all genotypes with <=10% resistant isolates (FQS). Heterogeneity between

487   trends in CDI caused by fluoroquinolone-resistant vs fluoroquinolone-susceptible isolates:

488   All p<0.0001, Non-ST1 p<0.0001, Non-ST1 FQR p<0.0001, ST42 p<0.0001, ST37

489   p=0.00015, ST3 p=0.00070, ST35 p=0.92, ST11 p=0.0053.

490   **Figure 4**

491   Contrasting CDI incidence (Oxfordshire) and WGS phylogenies representing the

492   fluoroquinolone-resistant genotype ST1(027), the mixed resistant and susceptible genotype

493   ST3(001), and the almost entirely fluoroquinolone-susceptible genotype ST8(002).

494   **A.** CDI incidence by fluoroquinolone susceptibility for genotype ST1(027) in Oxfordshire.

495   IRR=Annual incidence rate ratio. Red bars indicate fluoroquinolone-resistant isolates, blue

496   bars indicate fluoroquinolone-susceptible isolates, black bars indicate resistance not

497   determined.

498   **B.** CDI incidence by fluoroquinolone susceptibility for genotype ST3(001) in Oxfordshire.

499   **C.** CDI incidence by fluoroquinolone susceptibility for genotype ST8(002) in Oxfordshire.

500   **D.** Time scaled phylogeny for ST1(027) generated using ClonalFrameML.[22] Every third

501   Oxfordshire isolate (by date) is shown. ED= Evolutionary Distinctiveness (ED),[23]

502   R=fluoroquinolone-resistant, S=fluoroquinolone-susceptible. Phylogenies were scaled to be

503   directly comparable post-1990; the grey shaded regions prior to 1990 represent the regions of

504   the phylogenies that should not be compared, since they are not scaled identically.

505   Background colour indicates fluoroquinolone susceptibility; branch colour geographic

506   location.

507   **E.** Time scaled phylogeny for the mixed fluoroquinolone resistant/susceptible genotype,

508   ST3(001), generated using ClonalFrameML.[22] Two fluoroquinolone-resistant areas of the

509   phylogeny are indicated by red shading within the blue 'susceptible' region. Rapid clonal

510   expansion after resistance emergence is supported by significantly lower ED scores for

511   resistant versus susceptible areas.

512   **F.** Time scaled phylogeny for ST8(002) generated using ClonalFrameML.[22] Every second

513   Oxfordshire isolate (by date) is shown. Two fluoroquinolone-resistant isolates are indicated at

514   the bottom of the panel.

515   **Figure 5**

516   The incidence of inferred secondary CDI cases in Oxfordshire, i.e. cases caused by *C.*

517   *difficile* isolates that are genetically closely related (≤2 single nucleotide variants) to isolates

518   recovered from a previous case, and therefore potentially transmitted. Incidence was

519   calculated separately for inferred secondary cases caused by fluoroquinolone-resistant

520   ST1(027), fluoroquinolone-resistant non-ST1(027) and fluoroquinolone-susceptible isolates,

521   stratified by 'with' versus 'without' hospital-based contact. IRR=Annual incidence rate ratio.

REFERENCES

1.   Davies KA, Longshaw CM, Davis GL, et al. Underdiagnosis of *Clostridium difficile*

across Europe: the European, multicentre, prospective, biannual, point-prevalence study of

Clostridium difficile infection in hospitalised patients with diarrhoea (EUCLID). *Lancet*

Infect Dis 2014; **14**: 1208–19.

2.   Lessa FC, Y Mu, Bamberg WM, et al. Burden of *Clostridium difficile* infection in the

United States. *N Engl J Med* 2015; **372**: 825–34.

3.   Owens RC Jr, Donskey CJ, Gaynes RP, LooVG, Muto CA. Antimicrobial-associated

risk factors for *Clostridium difficile* infection. *Clin Infect Dis* 2008; **46**: S19–31.

4.   Shuttleworth R, Taylor M, Jones DM. Antimicrobial susceptibilities of *Clostridium*

difficile. *J Clin Pathol* 1980; **33**: 1002–5.

5.   Spigaglia P. Recent advances in the understanding of antibiotic resistance in

Clostridium difficile infection. *Ther Adv Infect Dis* 2016; **3**: 23–42.

6.   Johnson S, Samore MH, Farrow KA, et al. Epidemics of diarrhea caused by a

clindamycin-resistant strain of *Clostridium difficile* in four hospitals. *N Engl J Med* 1999;

341: 1645–51.

7.   Pear SM, Williamson TH, Bettin KM, Gerding DN, Galgiani JN. Decrease in

nosocomial *Clostridium difficile*-associated diarrhea by restricting clindamycin use. *Ann*

Intern Med 1994; **120**: 272–7.

8.   Loo VG, Poirier L, Miller MA, et al. A predominantly clonal multi-institutional

outbreak of *Clostridium difficile*-associated diarrhea with high morbidity and mortality. *N*

Engl J Med 2005; **353**: 2442–9.

9.   He M, Miyajima F, Roberts P, et al. Emergence and global spread of epidemic

healthcare-associated *Clostridium difficile*. *Nat Genet* 2013; **45**: 109–13.

546 10.   Kallen AJ, Thompson A, Ristaino P, et al. Complete restriction of fluoroquinolone

547 use to control an outbreak of *Clostridium difficile* infection at a community hospital. *Infect*

548 *Control Hosp Epidemiol* 2009; **30**: 264–72.

549 11.   Muto CA, Blank MK, Marsh JW, et al. Control of an outbreak of infection with the

550 hypervirulent *Clostridium difficile* BI strain in a university hospital using a comprehensive

551 "bundle" approach. *Clin Infect Dis* 2007; **45**: 1266–73.

552 12.   Eyre DW, Cule ML, Wilson DJ, et al. Diverse sources of *C. difficile* infection

553 identified on whole-genome sequencing. *N Engl J Med* 2013; **369**: 1195–205.

554 13.   Martin J, Eyre DW, Fawley WN, Walker AS, Crook DW, Wilcox MH. 2016 *C.*

555 *difficile* (CD) ribotypes exhibit variable patient-to-patient transmission rates, as determined

556 by whole-genome sequencing (WGS), suggesting differing reservoirs and modes of

557 acquisition. Abstract O557: ECCMID 2016 Amsterdam 9-12 April.

558 14.   Kumar N, Miyajima F, He M, et al. Genome-Based Infection Tracking Reveals

559 Dynamics of *Clostridium difficile* Transmission and Disease Recurrence. *Clin Infect Dis*

560 2016; **62**: 746–52.

561 15.   Curry SR, Muto CA, Schlackman JL, et al. Use of multilocus variable number of

562 tandem repeats analysis genotyping to determine the role of asymptomatic carriers in

563 *Clostridium difficile* transmission. *Clin Infect Dis* 2013; **57**: 1094–102.

564 16.   Wilcox MH, Shetty N, Fawley WN, et al. Changing epidemiology of *Clostridium*

565 *difficile* infection following the introduction of a national ribotyping-based surveillance

566 scheme in England. *Clin Infect Dis* 2013; **55**: 1056–63.

567 17.   *Clostridium difficile* infection: How to deal with the problem. Public Health England

568 and the Department of Health, 2008.

569 http://www.hpa.org.uk/webc/HPAwebFile/HPAweb_C/1232006607827. Accessed February

570 16, 2016.

571   18.   Department of Health; Saving Lives: reducing infection, delivering clean and safe

572   care, antimicrobial prescribing, a summary of best practice, 2007.

573   http://webarchive.nationalarchives.gov.uk/20130107105354/http://www.dh.gov.uk/prod_cons

574   um_dh/groups/dh_digitalassets/@dh/@en/documents/digitalasset/dh_078117.pdf. Accessed

575   February 16, 2016.

576   19.   Cornely OA, Crook DW, Esposito R, et al. Fidaxomicin versus vancomycin for

577   infection with *Clostridium difficile* in Europe, Canada, and the USA: a double-blind, non-

578   inferiority, randomised controlled trial. *Lancet Infect Dis* 2012; **12**: 281–9.

579   20.   Louie TJ, Miller MA, Mullane KM, et al. Fidaxomicin versus vancomycin for

580   *Clostridium difficile* infection. *N Engl J Med* 2011; **364**: 422–431.

581   21.   Jolley KA, Maiden MC. BIGSdb: Scalable analysis of bacterial genome variation at

582   the population level. *BMC Bioinformatics* 2010; **11**: 595.

583   22.   Drudy D, Quinn T, O'Mahony R, Kyne L, O'Gaora P, Fanning S. High-level

584   resistance to moxifloxacin and gatifloxacin associated with a novel mutation in *gyrB* in toxin-

585   A-negative, toxin-B-positive *Clostridium difficile*. *J Antimicrob Chemother* 2006; **58**: 1264–

586   67.

587   23.   Spigaglia P, Barbanti F, Mastrantonio P, et al. Fluoroquinolone resistance in

588   *Clostridium difficile* isolates from a prospective study of *C. difficile* infections in Europe. *J*

589   *Med Microbiol* 2008; **57**: 784–9.

590   24.   Didelot X, Wilson DJ. ClonalFrameML: Efficient Inference of Recombination in

591   Whole Bacterial Genomes. *PLoS Comput Biol* 2015; **11**: e1004041.

592   25.   Isaac NJB, Turvey ST, Collen B, Waterman C, Baillie JEM. Mammals on the EDGE:

593   Conservation priorities based on threat and phylogeny. *PLoS One* 2007; **2**: e296.

594   26.   Dingle KE, Elliott B, Robinson E, et al. Evolutionary history of the *Clostridium*

595   *difficile* pathogenicity locus. *Genome Biol Evol* 2014; **6**: 36–52.

596   27.   *Clostridium difficile* Ribotyping Network (CDRN) for England and Northern Ireland,

597   2011-2013 Report. Public Health England, 2014.

598   28.   Drudy D, Fanning S, Kyne L. Toxin A-negative, toxin B-positive *Clostridium*

599   *difficile*. *Int J Infect Dis* 2007;11: 5–10.

600   29.   Borgmann S, Kist M, Jakobiak T, et al. Increased number of *Clostridium difficile*

601   infections and prevalence of *Clostridium difficile* PCR ribotype 001 in southern Germany.

602   *Euro Surveill* 2008; **13** pii: 19057.

603   30.   Spigaglia P, Barbanti F, Dionisi AM, Mastrantonio P. *Clostridium difficile* isolates

604   resistant to fluoroquinolones in Italy: emergence of PCR ribotype 018. *J Clin Microbiol*

605   2010; **48**: 2892–96.

606   31.   Eyre DW, Griffiths D, Vaughan A, et al. Asymptomatic *Clostridium difficile*

607   colonisation and onward transmission. *PLoS One*. 2013; **12**: e78445.

608   32.   Bauer MP, Kuijper EJ. Potential sources of *Clostridium difficile* in human infection.

609   *Infect Dis Clin North Am* 2015; **29**: 29–35.

610   33.   Brown KA, Khanafer N, Daneman N, Fisman DM. Meta-Analysis of Antibiotics and

611   the Risk of Community-Associated *Clostridium difficile* Infection. *Antimicrob Agents*

612   *Chemother* 2013; **57**: 2326–32.

613   34.   Talpaert MJ, Rao GG, Cooper BS, Wade P. Impact of guidelines and enhanced

614   antibiotic stewardship on reducing broad-spectrum antibiotic usage and its effect on

615   incidence of *Clostridium difficile* infection. *J Antimicrob Chemother* 2011; **66**: 2168–74.

616   35.   Sarma JB, Marshall B, Cleeve V, Tate D, Oswald T, Woolfrey S. Effects of

617   fluoroquinolone restriction (from 2007 to 2012) on *Clostridium difficile* infections:

618   interrupted time-series analysis. *J Hosp Infect* 2015; **91**: 74–80.

619 36. Feazel LM, Malhotra A, Perencevich EN, Kaboli P, Diekema DJ, Schweizer ML.

620 Effect of antibiotic stewardship programmes on *Clostridium difficile* incidence: a systematic

621 review and meta-analysis. *J Antimicrob Chemother* 2014; **69**: 1748–54.

622 37. Belmares J, Johnson S, Parada JP, et al. Molecular epidemiology of *Clostridium*

623 *difficile* over the course of 10 years in a tertiary care hospital. *Clin Infect Dis* 2009; **49**: 1141–

624 7.

625 38. Valiquette L, Cossette B, Garant MP, Diab H, Pépin J. Impact of a reduction in the

626 use of high-risk antibiotics on the course of an epidemic of *Clostridium difficile*-associated

627 disease caused by the hypervirulent NAP1/027 strain. *Clin Infect Dis* 2007; **45** Suppl 2:

628 S112–21.

629 39. Hensgens MP, Goorhuis A, van Kinschot CM, Crobach MJ, Harmanus C, Kuijper EJ.

630 *Clostridium difficile* infection in an endemic setting in the Netherlands. *Eur J Clin Microbiol*

631 *Infect Dis* 2011; **30**: 587–93.

632 40. Loo VG, Bourgault AM, Poirier L, et al. Host and pathogen factors for *Clostridium*

633 *difficile* infection and colonization. *N Engl J Med* 2011; **365**: 1693–703.

634 41. English surveillance programme for antimicrobial utilisation and resistance

635 (ESPAUR) 2010 to 2014, Report 2015.

636 https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/477962/ESPA

637 UR_Report_2015.pdf Accessed February 16, 2016.

31

DECLARATION OF INTERESTS

33

CONTRIBUTORS

KED, XD, TPQ, MHW, TEAP, ASW and DWC designed the study with input from DWE, NS, TG, RMH and DJW.

KED, DWE, NS, DG, AV, SJO, WNF, JF, KM and JM collected specimens from Oxfordshire and Leeds, cultured *C. difficile* and, or extracted chromosomal *C. difficile* DNA for WGS. SG, EJCG and DMC contributed the 'Fidaxomicin clinical trial' isolate collection. EJCG and DMC performed fluoroquinolone susceptibility testing.

The Modernising Microbiology Informatics Group, JMF, TG and DHW optimised or performed the assembly of short DNA sequence reads.

KED derived genotype data from WGS and identified genomes for the construction of CFML dual-scaled phylogenies. All phylogenies were constructed 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 CDI.

Biostatistical analysis: TPQ, ASW and TEAP performed the analysis of Oxfordshire and national incidence and antimicrobial prescribing data. WNF, TPQ, ASW and MHW performed the analysis of Leeds incidence and antimicrobial prescribing data. DWE, TPQ, ASW and TEAP performed SNV analysis (Figure 5, S8).

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.

KED, XD, TPQ contributed equally to the work. MHW, TEAP, ASW and DWC contributed equally to the work.