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The *Clos*ER Study: results from a three-year pan-European longitudinal surveillance of antibiotic resistance among prevalent *Clostridium difficile* ribotypes, 2011–2014

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ABSTRACT

Objectives: Until the introduction of fidaxomicin, antimicrobial treatment for *Clostridium difficile* infection (CDI) was limited to metronidazole and vancomycin. The changing epidemiology of CDI and emergence of epidemic *C. difficile* PCR ribotype 027 necessitates continued surveillance to identify shifts in antibiotic susceptibility. *Clos*ER, currently the largest pan-European epidemiological study of *C. difficile* ribotype distribution and antibiotic susceptibility, aimed to undertake antimicrobial resistance surveillance pre- and post-introduction of fidaxomicin.

Methods: Between July 2011 and July 2014, 39 sites across 22 European countries submitted 2830 *C. difficile* isolates for ribotyping, toxin testing and susceptibility testing to metronidazole, vancomycin, fidaxomicin, rifampicin, moxifloxacin, clindamycin, imipenem, chloramphenicol and tigecycline.

Results: Ribotypes 027, 014, 001, 078, 020, 002, 126, 015 and 005 were most frequently isolated, while emergent ribotypes 198 and 356 were identified in Hungary and Italy, respectively. All isolates were susceptible to fidaxomicin, with scarce resistance to metronidazole (0.2% [*n*=6/2694]), vancomycin (0.1% [*n*=2/2694]) and tigecycline (0%). Rifampicin, moxifloxacin and clindamycin resistance was evident in multiple ribotypes. Lack of ribotype diversity correlated with greater antimicrobial resistance. Epidemic ribotypes (027/001) were associated with multiple antimicrobial resistance, and ribotypes 017, 018 and 356 with high-level resistance. Additional factors may also influence local ribotype prevalence.

Conclusions: Fidaxomicin susceptibility was retained post-introduction, and resistance to metronidazole and vancomycin was rare. Continued surveillance is needed, with more accurate classification and clarification of ribotype subtypes to further understand their role in the spread of resistance. Other factors may also influence changes in prevalence of *C. difficile* ribotypes with reduced antibiotic susceptibility.

Introduction

Clostridium difficile infection (CDI) is a major cause of nosocomial diarrhoea and a problem in healthcare environments and the community, causing significant morbidity and mortality worldwide [1]. So-called 'hypervirulent' strains, such as those belonging to polymerase chain reaction (PCR) ribotype (RT) 027 [2,3], are increasingly represented among clinical isolates, and the incidence and severity of CDI is rising [4]. A recent report on antimicrobial resistance cites *C. difficile* as a microorganism with an urgent threat level [5].

Vancomycin and metronidazole are commonly used treatments for CDI. Fidaxomicin, a macrocyclic antimicrobial with potent anti-*C. difficile* activity, was introduced to the European market in 2011 for the treatment of CDI. European marketing authorisation for fidaxomicin included a commitment to undertake antimicrobial resistance surveillance pre- and post-introduction. The three-year *Clos*ER (*Clostridium difficile* European Resistance) *in vitro* surveillance study aimed to: identify and monitor longitudinal susceptibility of contemporaneous *C. difficile* clinical isolates to antibiotics used for CDI treatment and those implicated in selection pressure; establish a comprehensive susceptibility database baseline for ongoing surveillance; and provide data on geographical distribution of clinical *C. difficile* strain types with analysis by region across Europe [6].

Presented here are final epidemiological and antimicrobial susceptibility data for *C. difficile* isolates collected over the three-year study period: pre-fidaxomicin introduction (July 2011–June 2012), and post-fidaxomicin introduction (July 2012–July 2014). Preliminary first-year data (pre-fidaxomicin introduction) are described elsewhere [6], but are updated here to include further results from sites/submissions for that period which were previously unavailable.

Methods

Study design

The study design of *Clos*ER has been described in full elsewhere [6]. Briefly, 41 sites from 28 European countries participated and were asked to submit 25 *C. difficile* isolates or toxin-positive faecal samples from de-duplicated CDI cases each year. No further stipulations were made regarding selection of isolates/samples. Centres were mainly national or regional *C. difficile* referral laboratories selected using the European Study Group on *C. difficile* (ESGCD) network, and with ESGCD approval.

Isolates or faecal samples were submitted to a central laboratory (in Leeds, UK) for *C. difficile* culture, PCR ribotyping, determination of toxin status by cell culture cytotoxicity assay, and susceptibility to metronidazole, vancomycin, rifampicin, fidaxomicin, imipenem, moxifloxacin, clindamycin, chloramphenicol and tigecycline.

Culture and toxin testing

Alcohol-shocked faecal specimens/*C. difficile* isolates were inoculated on to cycloserine-cefoxitin-egg-yolk agar (Lab M, Heywood, Lancashire, UK) with lysozyme and cultured anaerobically for 48 hours at 37℃. Forty-eight-hou r anaerobic brain–heart infusion broth culture supernatants of each test isolate were added to a Vero cell culture cytotoxicity assay with *Clostridium sordellii* antitoxin (Pro-Lab Diagnostics, Bromborough, UK) neutralisation.

Ribotyping

PCR ribotyping, using capillary electrophoresis, was performed on each isolate by the *C. difficile* Ribotyping Network Reference Laboratory at Leeds Teaching Hospitals Trust, Leeds, UK [7]. Ribotypes were assigned against the UK *C. difficile* reference library at Leeds.

Antimicrobial susceptibility testing

Susceptibility of isolates (minimum inhibitory concentrations [MICs]) to metronidazole, vancomycin, rifampicin, chloramphenicol (Sigma), moxifloxacin (Bayer), clindamycin, tigecycline (Pfizer), imipenem (MSD) and fidaxomicin (Astellas) were determined using a Clinical and Laboratory Standards Institute agar incorporation method with Wilkins–Chalgren agar [6,8,9] and breakpoints, as previously described [6].

Cumulative resistance scores

MIC results for each isolate were designated susceptible (S), intermediately resistant (I) or fully resistant (R), according to breakpoints, and assigned a score (S=0, I=1 and R=2). A cumulative resistance score (CRS) based on susceptibility to each of the nine antimicrobials tested was then generated for each isolate. Thus, an isolate that was fully susceptible to four, intermediately resistant to two, and resistant to three antimicrobials would generate a CRS of 8 (0 + 0 + 0 + 0 + 1 + 1 + 2 + 2 + 2). Isolates were grouped by country, and mean CRSs were generated for each country over the three years.

Results

Over three years, 2830 samples/isolates (from 39 sites in 22 countries) were submitted to the central laboratory for testing, yielding 2694 *C. difficile* isolates (95.3%). Of these, 95.6% (*n*=2577) were toxin-positive.

PCR ribotyping

In Year 1, 114 known PCR RTs were isolated from samples; in Year 2, 144; and in Year 3, 120. The most commonly isolated European RT was RT027, which accounted for: 12.2% (*n*=115/943) in Year 1; 11.8% (*n*=112/948) in Year 2; and 12.6% (*n*=101/804) in Year 3. The prevalence of other RTs is presented in Table 1.

RT prevalence differed by region, with certain countries exhibiting highly predominant RTs, and others showing a diverse range of RTs (Fig. 1a–c). RT027 was particularly associated with Denmark, Hungary, Italy and Poland; RTs 018 and 078 with Italy; RTs 176 and 017 with the Czech Republic; and a high prevalence of RT001 with Latvia and Slovakia.

Emergent RTs 198 and 356 were also evident, and exclusive to Hungary and Italy, respectively (Fig. 1a–c).

RT diversity scores were calculated for each country (total number of RTs detected in that country divided by the number of isolates tested from that country). Scores closest to 1 and 0 indicated the greatest and least diversity of RTs, respectively. Belgium submitted the greatest diversity of RTs in Years 1 and 2, with scores of 0.96 and 0.80, respectively.

Antimicrobial susceptibility

All isolates were susceptible to fidaxomicin, with geometric mean MICs of 0.04–0.05 mg/L (Table 2a), and a normal unimodal distribution for all study years (Fig. 2). Among prevalent RTs, only RT027 and RT198 showed evidence of increased geometric mean MICs: in Years 1–3, from 0.04 to 0.08 mg/L for RT027; and from 0.04 to 0.10 mg/L for RT198 (Table 3).

Metronidazole, vancomycin and tigecycline were active against 97.9% (*n*=2692/2698), 98.6% (*n*=2659/2698) and 100.0%, respectively, of isolates tested. There was little variation in sensitive, intermediate and resistant isolates collected across Europe during Years 1–3 (Table 2b). Reduced metronidazole susceptibility was mainly observed for RT027 and RT198, and is reflected in higher geometric mean metronidazole MICs (Table 3).

Higher geometric mean vancomycin MICs were observed for RT018 (2.00 mg/L) and RT356 (2.28 mg/L, Year 1 only) (Table 3). No particular RTs were associated with vancomycin resistance.

The proportion of isolates showing rifampicin resistance was similar in Years 1 and 2, with a slight reduction in Year 3 (Table 2b). Rifampicin resistance was notable in Hungary (38.7–56.6% [*n* =29/75–43/76]), Italy (36.6–47.0% [*n*=34/93–39/83]) and the Czech Republic (40.0–64.0% [*n*=10/25–14/22]). Rifampicin resistance in Denmark decreased from 40.9% (*n*=9/22) in Year 1, to 18.2% (*n*=4/22) by Year 3, due to a decrease in the number of RT027 isolates. In Poland, rifampicin resistance was low in Year 1 (5.0% [*n*=1/20]), but rates increased in Years 2 and 3 (37.9% [*n*=11/29] and 44.0% [*n*=11/25], respectively). Rifampicin resistance was evident in multiple RTs, notably in RT027, RT001, RT018, RT356, RT017, RT176 and RT198 (Table 3).

Moxifloxacin and clindamycin resistance was widespread, particularly among some of the more prevalent RTs (Table 3). Moxifloxacin resistance decreased over Years 1–3 from 39.5% (*n*=372/943) to 33.5% (*n*=269/803). Clindamycin resistance increased from 49.8% (*n*=470/943) to 64.3% (*n*=516/803) (Table 2b), but showed variations in the proportion of resistant isolates from the same country, possibly due to fluctuations in individual RT prevalence.

The majority of isolates were sensitive to imipenem (Table 2b). Geometric mean MICs were marginally higher for RT027 and RT198 than for most other RTs during the study period (Table 3). During Years 2 and 3, there was evidence of decreasing imipenem susceptibility to RT017 (geometric mean MIC: 9.8 mg/L and 10.6 mg/L, respectively) (Table 3). Most isolates were susceptible to chloramphenicol (Table 2b). For RT017, geometric mean MICs increased from 7.66 mg/L (Year 1) to 14.59 mg/L (Year 3) (Table 3). Reduced susceptibility to tigecycline (>0.25 mg/L) was scarce; geometric mean MICs were marginally raised for RT012 in Years 1 and 2 (Table 3).

193

194 *Multiple antimicrobial resistance*

195 During the study, antimicrobial resistance to three or more antibiotics was evident for RTs
196 001, 106, 018, 356 and 012. RTs 027, 017 and 198 showed resistance to five or more
197 antibiotics, including metronidazole, rifampicin, moxifloxacin, clindamycin, imipenem and
198 chloramphenicol (Table 3).

199

200 *Antimicrobial susceptibility by country (*Fig. S1a,b,c)

201 Poland, Latvia, the Czech Republic and Hungary had consistently high CRSs in all three
202 years. Cyprus also showed a high CRS in Year 1, associated with a high prevalence of
203 RT027; however, scores decreased consistently in subsequent years despite the continued
204 high prevalence of RT027. This was due to decreasing resistance of RT027 isolates over the
205 three years. Sweden consistently had the lowest CRSs in all years (0.88, 0.52 and 1.16).

206 There was a significant inverse correlation between the number of RTs identified in a locality
207 and the mean CRS (Pearson correlation Year 1, r=−0.55, p=0.0095; Year 2, r=−0.59,
208 p=0.003; Year 3, r=0.47, p=0.03). This indicated lower antimicrobial resistance levels in
209 countries with a greater diversity of *C. difficile* RTs.

210

211 **Discussion**

212 This is the largest pan-European study of *C. difficile* RT and antibiotic susceptibility
213 epidemiology to date. Surveillance of over 2800 isolates from 22 European countries
214 showed a diverse array of 144 RTs. The most commonly isolated RTs were broadly similar
215 to those already reported [4,10]. Previously described epidemic or highly prevalent RTs
216 (014/20, 027, 001/072 and 078) [6,10,11] were similar, but inter-country variations were

apparent in the relative prevalence of particular strains. This is consistent with the endemic and epidemic spread of *C. difficile* previously documented [10–12].

Compared with the most recent surveillance results from EUCLID (a European, multicentre, prospective, biannual, point-prevalence study of CDI in hospitalised patients with diarrhoea), in which the incidence of RT027 was 19% among 1196 *C. difficile* isolates tested [4], our study found a lower incidence (11.8–12.6%) for this RT. This variability may result from the greater number of isolates tested in our study, leading to different levels of prevalence for each RT. Our study also demonstrated that RTs reported previously as highly prevalent (RT001 in Latvia and Slovakia, RT027 in Poland and Hungary, and RT017 and RT176 in the Czech Republic) remained so in those countries.

The emergence of RT356 in Italy in Year 1, with its accompanying high levels of antimicrobial resistance, has been described [6,13] (24th European Congress of Clinical Microbiology and Infectious Diseases [ECCMID], abstract R483). PCR banding profiles of RT018 and RT356 are closely related (94% similarity) and genome sequencing of these isolates may inform both strain provenance and resistance development [6]. As reported previously [6], methodological advances have allowed greater RT discrimination, which improves understanding of local fluctuations and highlights the need for a consensus on RT nomenclature.

Consistent with previously reported good activity (range 0.007–1.000 mg/L) [14]**,** fidaxomicin was the most widely active CDI treatment in this study, with no clear evidence of reduced susceptibility or resistance. There was scant evidence of decreased susceptibility to fidaxomicin from Year 1 (pre-fidaxomicin introduction) through Years 2 and 3 (post-fidaxomicin introduction). Similarly, a recent US National Sentinel Surveillance Study reported no change in fidaxomicin activity against *C. difficile* (*n*=925 isolates) over 12 months following fidaxomicin introduction [15].

It has been suggested that so-called 'hypervirulent' *C. difficile* strains (including RT027) may be less susceptible to fidaxomicin than other RTs [14]. Only small differences in fidaxomicin susceptibility were observed between RTs in our study. Notably, the increase in geometric mean fidaxomicin MICs for RT027 was merely 0.04 mg/L over three years. The significance of this observation is questionable, particularly in the context of high faecal fidaxomicin concentrations during therapy (>1000 µg/g), which are >5000-fold higher than the MIC_{90} [16].

Reduced metronidazole susceptibility was uncommon across all years, and most evident among RT027 and RT198, as previously described [17] (25^{th} European Congress of Clinical Microbiology and Infectious Diseases [ECCMID], abstract EV0260). RT198 and RT027 are closely related [18]; it is therefore possible that a mechanistic similarity between the two RT subtype influences reduced metronidazole susceptibility. Continued surveillance is necessary to increase knowledge about emerging RTs with genomic similarities to highly virulent and dynamic strains.

Vancomycin resistance was scarce and not consistently RT-associated. Again, the clinical significance of higher vancomycin MICs is unclear in the light of high gut vancomycin concentrations *in vivo* [19].

Our study confirms previously reported associations between prevalent RTs (e.g. RT027 and RT001) and resistance to moxifloxacin, clindamycin and chloramphenicol [12,20], with some evidence of location clustering (chloramphenicol-resistant RT001 in Germany, The Netherlands and Latvia). To date, imipenem resistance has not been well-documented in *C. difficile*, but intermediate and complete resistance was evident throughout our study, particularly in RTs 027,198 and 017.

This study underlines the association of well-known epidemic RTs 027 and 001 with multiple antimicrobial resistance, and highlights the association and emergence of other RTs (017, 018, 198, 356) with high levels of resistance, as previously described [6]. Increasing multiple

antimicrobial resistance was observed in RT198 in Hungary, RT027 in Italy, and RT017 in the Czech Republic, indicating a potential role for local antimicrobial prescribing as a selection pressure.

RT027 remained prevalent in Cyprus and became progressively less resistant to some antimicrobials, but retained high levels of fluoroquinolone resistance. Recent evidence suggests that resistance to macrolide–lincosamide–streptogramin antibiotics may confer a fitness cost to *C. difficile*, while others, e.g. fluoroquinolone resistance, do not [21,22]. Fluoroquinolone resistance may therefore be maintained even in the absence of fluoroquinolone pressure. European Centre for Disease Prevention and Control data show that both fluoroquinolone and macrolide–lincosamide–streptogramin antibiotic consumption in Cyprus decreased to their lowest levels in eight years [23]. This may indicate that antimicrobial pressure is only part of the picture and other factors influence the emergence and decline in prevalence of *C. difficile* RTs.

There was a consistent, significant inverse correlation between the number of RTs identified in a locality and the mean CRSs, indicating lower antimicrobial resistance levels among countries with a greater diversity of *C. difficile* RTs. This may be because of the introduction of mandatory reporting programmes, with consequent increased awareness, antimicrobial stewardship and infection control interventions decreasing the rates of endemic RTs. Interestingly, the UK had a low mean CRS and a low RT diversity score, possibly due to the large denominator sample size (n=123–150 isolates), which also included the greatest number of RTs from a single country.

Selection bias may also be possible, as we requested only 25 patient de-duplicated, toxin-positive faecal samples (or *C. difficile* isolates) collected during each study period, but made no further stipulations. Participating centres were predominantly national or regional *C. difficile* reference facilities and therefore some submissions likely included outbreak strains, possibly influencing the data. In addition, there is inherent sensitivity loss in gathering

international epidemiological surveillance data. However, the similarity of the predominant RTs between this study and that of Bauer et al. [10], plus the continued presence of antimicrobial resistance phenotypes among certain prevalent RTs, indicate a degree of confidence [3].

In conclusion, our study highlights the epidemiological and antimicrobial findings for *C. difficile* isolates collected pre- and post-fidaxomicin introduction across Europe. It highlights the high level of retained susceptibility to fidaxomicin across Europe two years post-introduction, and the rarity of resistance to metronidazole and vancomycin. It also reinforces the potential impact of 'hypervirulent' strains of *C. difficile*, such as RT027 and RT001, and emerging RTs (198, 356) on geographical resistance patterns, supporting the hypothesis that increased awareness, infection control and antimicrobial stewardship may result in increased RT diversity and reduced antimicrobial resistance. Ultimately, this study highlights the need for continued surveillance to further understand how the epidemiological landscape may be affected by the introduction of novel antimicrobial agents against CDI.

Authors' contributions and declaration

The material is original and has not been submitted elsewhere.

All primary authors have made substantive intellectual contributions to the manuscript, approved the final version for submission, and are able to account for its content.

The participants of the Study Group submitted *C. difficile* isolates or toxin-positive faecal samples for testing.

All applicable parts of the STROBE guidelines were followed in the reporting of this study.

Transparency declaration

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References

[1] Martin J, Monaghan T, Wilcox MH. *Clostridium difficile* infection: advances in epidemiology, diagnosis and understanding of transmission. Nat Rev Gastro Hep 2016;13:206–16. doi:10.1038/nrgastro.2016.25.

[2] Finegold SM, Molitoris D, Vaisanen ML, Song Y, Bolanos M. In vitro activities of OPT-80 and comparator drugs against intestinal bacteria. Antimicrob Agents Chemother 2004;48:4898–902. doi:10.1128/AAC.48.12.4898.

[3] van Dorp SM, Kinross P, Gastmeier P, Behnke M, Kola A, Delmée M, et al. Standardised surveillance of *Clostridium difficile* infection in European acute care hospitals: a pilot study, 2013. Euro Surveill 2016;21:pii=30293. doi:10.2807/1560-7917.ES.2016.21.29.30293.

[4] Davies KA, Ashwin H, Longshaw CM, Burns DA, Davis GL, Wilcox MH. Diversity of *Clostridium difficile* PCR ribotypes in Europe: results from the European, multicentre, prospective, biannual, point-prevalence study of *Clostridium difficile* infection in hospitalised patients with diarrhoea (EUCLID), 2012 and 2013. Euro Surveill 2016;21:pii=30294. doi:10.2807/1560-7917.ES.2016.21.29.30294.

[5] Centers for Disease Control and Prevention. Antibiotic resistance threats in the United States 2013. https://www.cdc.gov/drugresistance/threat-report-2013/ (accessed September 17, 2017).

[6] Freeman J, Vernon J, Morris K, Nicholson S, Todhunter S, Longshaw C, et al. Pan-European longitudinal surveillance of antibiotic resistance among prevalent *Clostridium difficile* ribotypes. Clin Microbiol Infect 2015;21:e9–16. doi:10.1016/j.cmi.2014.09.017.

[7] Indra A, Huhulescu S, Schneeweis M, Hasenberger P, Kembichler S, Fiedler A, et al. Characterization of *Clostridium difficile* isolates using capillary gel electrophoresis-

based PCR ribotyping. J Med Microbiol 2008;57:1377–82.

[8] Baines SD, O'Connor R, Freeman J, Fawley WN, Harmanus C, Mastrantonio P, et al. Emergence of reduced susceptibility to metronidazole in *Clostridium difficile*. J Antimicrob Chemother 2008;62:1046–52.

[9] Clinical Laboratory Standards Institute. Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria: Approved Standard—Eighth Edition. CLSI document M11-A8. Wayne, PA, USA: CLSI; 2012.

[10] Bauer MP, Notermans DW, van Benthem BH, Brazier JS, Wilcox MH, Rupnik DL, et al. *Clostridium difficile* infection in Europe: a hospital-based survey. Lancet 2011;377:63–73. doi:http://dx.doi.org/10.1016/S0140-6736(10)61266-4.

[11] Freeman J, Bauer MP, Baines SD, Corver J, Fawley WN, Goorhuis B, et al. The changing epidemiology of *Clostridium difficile* infections. Clin Microbiol Infect 2010;23:529–49.

[12] Spigaglia P, Barbanti F, Dionisi AM, Mastrantonio P. *Clostridium difficile* isolates resistant to fluoroquinolones in Italy: emergence of PCR ribotype 018. J Clin Microbiol 2010;48:2892–6. doi:10.1128/JCM.02482-09.

[13] Spigaglia P, Barbanti F, Morandi M, Moro ML, Mastrantonio P. Diagnostic testing for *Clostridium difficile* in Italian microbiological laboratories. Anaerobe 2016;37:29–33. doi:10.1016/j.anaerobe.2015.11.002.

[14] Goldstein EJC, Citron DM, Sears P, Babakhani F, Sambol SP, Gerding DN. Comparative susceptibilities to fidaxomicin (OPT-80) of isolates collected at baseline, recurrence, and failure from patients in two Phase III trials of fidaxomicin against *Clostridium difficile* infection. Antimicrob Agents Chemother 2011;55:5194–9. doi:10.1128/AAC.00625-11.

[15] Snydman DR, McDermott LA, Jacobus N V, Thorpe C, Stone S, Jenkins SG, et al.

U.S.-based national sentinel surveillance study for the epidemiology of *Clostridium difficile*-associated diarrheal isolates and their susceptibility to fidaxomicin. Antimicrob Agents Chemother 2015;59:6437–43. doi:10.1128/AAC.00845-15.

[16] Sears P, Crook DW, Louie TJ, Miller MA, Weiss K. Fidaxomicin attains high fecal concentrations with minimal plasma concentrations following oral administration in patients with *Clostridium difficile* infection. Clin Infect Dis 2012;55 Suppl 2:S116–20. doi:10.1093/cid/cis337.

[17] Debast SB, Bauer MP, Sanders IMJG, Wilcox MH, Kuijper EJ. Antimicrobial activity of lff571 and three treatment agents against *Clostridium difficile* isolates collected for a pan-European survey in 2008: clinical and therapeutic implications. J Antimicrob Chemother 2013;68:1305–11. doi:10.1093/jac/dkt013.

[18] Valiente E, Cairns MD, Wren BW. The *Clostridium difficile* PCR ribotype 027 lineage: a pathogen on the move. Clin Microbiol Infect 2014;20:396–404. doi:10.1111/1469-0691.12619.

[19] Gonzales M, Pepin J, Frost EH, Carrier JC, Sirard S, Fortier L-C, et al. Faecal pharmacokinetics of orally administered vancomycin in patients with suspected *Clostridium difficile* infection. BMC Infect Dis 2010;10:363. doi:10.1186/1471-2334-10-363.

[20] Spigaglia P, Barbanti F, Mastrantonio P, Ackermann G, Balmelli C, Barbut F, et al. Multidrug resistance in European *Clostridium difficile* clinical isolates. J Antimicrob Chemother 2011;66:2227–34. doi:10.1093/jac/dkr292.

[21] Wasels F, Kuehne SA, Cartman ST, Spigaglia P, Barbanti F, Minton NP, et al. Fluoroquinolone resistance does not impose a cost on the fitness of *Clostridium difficile in vitro*. Antimicrob Agents Chemother 2015;59:1794–6. doi:10.1128/AAC.04503-14.

[22] Wasels F, Spigaglia P, Barbanti F, Mastrantonio P. *Clostridium difficile* erm(B)-containing elements and the burden on the *in vitro* fitness. J Med Microbiol 2013;62:1461–7. doi:10.1099/jmm.0.057117-0.

[23] European Centre for Disease Prevention and Control (ECDC). Trend of antimicrobial consumption by country. Antimicrob Consum Database 2017. https://ecdc.europa.eu/en/antimicrobial-consumption/database/trend-country (accessed September 26, 2017).

438 **Tables**

439

440 **Table 1**

441 Percentage prevalence (≥1%) of *Clostridium difficile* PCR ribotypes in the 3 years from July

442 2011 to July 2014 of the *Clos*ER study

443

Year 1 (*n*=943)			**Year 2 (*n*=948)**			**Year 3 (*n*=804)**		
Ribotype	***n***	**% prevalence**	**Ribotype**	***n***	**% prevalence**	**Ribotype**	***n***	**% prevalence**
027	115	12.2	**027**	112	11.8	**027**	101	12.6
001	86	9.1	**014**	89	9.4	**014**	85	10.6
078	76	8.1	**001**	77	8.1	**001**	63	7.8
014	74	7.8	**002**	53	5.6	**020**	44	5.5
020	38	4.0	**078**	53	5.6	**078**	43	5.4
126	35	3.7	**020**	49	5.2	**126**	41	5.1
002	34	3.6	**005**	31	3.3	**002**	35	4.4
015	32	3.4	**015**	31	3.3	**005**	31	3.9
005	31	3.3	**126**	31	3.3	**015**	22	2.7
106	24	2.5	018	28	3.0	046	19	2.4
023	23	2.4	023	20	2.1	106	18	2.2
018	21	2.2	017	18	1.9	017	15	1.9
356	21	2.2	046	17	1.8	176	13	1.6
012	19	2.0	012	14	1.5	018	12	1.5
011	16	1.7	198	14	1.5	003	11	1.4

017	16	1.7	106	13	1.4	010	11	1.4
046	15	1.6	056	11	1.2	081	11	1.4
087	15	1.6	029	10	1.1	011	10	1.2
056	11	1.2	039	10	1.1	023	10	1.2
			081	10	1.1	070	10	1.2
						012	9	1.1
						198	8	1.0

444 PCR, polymerase chain reaction.

445 Bold type indicates the nine most commonly isolated ribotypes across Years 1–3.

446

447

448 **Table 2a**

449 MIC_{50}, MIC_{90} and geometric mean MICs of *Clostridium difficile* isolates in the three years of

450 the *Clos*ER study (July 2011–July 2014)

mg/L	Years	M	V	FDX	RIF	MXF	CLINDA	IMI	CHLOR	TIG
MIC_{50}	Y1	0.25	1	0.06	0.002	2	4	4	8	0.06
MIC_{50}	Y2	0.25	0.5	0.06	0.002	2	8	4	4	0.03
MIC_{50}	Y3	0.25	0.5	0.06	0.002	2	8	4	4	0.03
MIC_{50}	**All years**	**0.25**	**0.5**	**0.06**	**0.002**	**2**	**8**	**4**	**4**	**0.03**
MIC_{90}	Y1	2	2	0.125	32	32	128	8	8	0.06
MIC_{90}	Y2	1	1	0.125	32	32	128	8	8	0.06
MIC_{90}	Y3	1	1	0.125	32	32	128	8	8	0.06
MIC_{90}	**All years**	**1**	**1**	**0.125**	**32**	**32**	**128**	**8**	**8**	**0.06**
Geometric mean MIC	Y1	0.37	0.76	0.04	0.01	4.79	5.72	4.75	5.82	0.05
Geometric mean MIC	Y2	0.33	0.70	0.05	0.01	3.90	7.61	3.88	5.39	0.04
Geometric mean MIC	Y3	0.29	0.70	0.05	0.01	3.73	9.18	4.17	6.06	0.04
Geometric mean MIC	**All years**	**0.56**	**1.22**	**0.05**	**0.01**	**4.14**	**7.26**	**4.26**	**5.73**	**0.04**

451 CHLOR, chloramphenicol; CLINDA, clindamycin; FDX, fidaxomicin; IMI, imipenem; M,

452 metronidazole; MIC, minimum inhibitory concentrations; MXF, moxifloxacin; RIF, rifampicin;

453 TIG, tigecycline; V, vancomycin.

454 MIC_{50}/MIC_{90}, minimum inhibitory concentration at which 50%/90% of isolates are inhibited.

455

Table 2b

Proportions of sensitive, intermediately sensitive and resistant *Clostridium difficile* isolates in the three years of the *Clos*ER study (July 2011–July 2014)

	Years	M	V	FDX	RIF	MXF	CLINDA	IMI	CHLOR	TIG
Sensitive (%)	Y1	97.9	96.7	100.0	80.5	58.7	37.6	62.7	92.9	100.0
	Y2	98.1	98.8	100.0	82.1	64.5	29.1	77.3	93.1	100.0
	Y3	96.9	99.8	100.0	86.8	66.0	18.3	78.1	91.5	100.0
	All years	**97.9**	**98.6**	**100.0**	**83.2**	**63.1**	**29.0**	**72.6**	**92.8**	**100.0**
Intermediately sensitive (%)	Y1	2.0	2.4		6.0	1.8	12.4	30.1	3.5	
	Y2	1.3	0.6		3.7	1.0	13.7	19.7	2.6	
	Y3	2.6	0.1		1.5	0.5	17.4	19.7	5.1	
	All years	**1.9**	**1.1**		**3.9**	**1.1**	**14.4**	**23.3**	**3.7**	
Resistant (%)	Y1	0.1	0.9		13.5	39.5	49.8	7.2	3.6	
	Y2	0.1			13.7	34.1	56.7	2.3	3.7	
	Y3	0.5	0.1		11.6	33.5	64.3	2.2	3.4	
	All years	**0.2**	**0.1**		**13.0**	**35.8**	**56.6**	**4.0**	**3.6**	

CHLOR, chloramphenicol; CLINDA, clindamycin; FDX, fidaxomicin; IMI, imipenem; M, metronidazole; MXF, moxifloxacin; RIF, rifampicin; TIG, tigecycline; V, vancomycin.

462 **Table 3**

463 Geometric mean MICs of prevalent *Clostridium difficile* PCR ribotypes in the three years of

464 the *Clos*ER study (July 2011–July 2014)

	Ribotype	M	V	FDX	RIF	MXF	CLINDA	IMI	CHLOR	TIG
Y1	027	**1.41**	0.70	0.04	**0.472**	**22.16**	3.55	**7.13**	4.91	0.04
Y2	027	**1.47**	0.81	**0.07**	**0.303**	**22.71**	4.82	**6.94**	5.15	0.04
Y3	027	**1.24**	0.71	**0.08**	**0.351**	**18.35**	6.34	**6.69**	5.72	0.03
Y1	001	0.47	0.83	0.01	0.007	**12.18**	**48.63**	5.18	**8.74**	0.04
Y2	001	0.33	0.64	0.01	0.006	**14.06**	**41.84**	3.48	6.84	0.04
Y3	001	0.38	0.66	0.02	0.006	**13.45**	**54.40**	5.08	**7.91**	0.03
Y1	078	0.27	0.63	0.04	0.003	3.39	2.85	3.18	5.07	0.05
Y2	078	0.21	0.69	0.05	0.003	2.63	4.93	3.04	4.21	0.04
Y3	078	0.19	0.62	0.05	0.003	2.51	6.92	3.09	4.93	0.04
Y1	014	0.27	0.65	0.06	0.002	2.41	5.15	4.31	5.87	0.05
Y2	014	0.25	0.60	0.06	0.003	1.95	4.83	3.16	4.90	0.04
Y3	014	0.22	0.63	0.06	0.003	2.00	8.20	3.77	5.97	0.04
Y1	020	0.31	0.73	0.07	0.002	2.63	6.43	4.80	5.66	0.05
Y2	020	0.27	0.64	0.06	0.002	2.07	4.06	2.96	5.09	0.05
Y3	020	0.22	0.65	0.06	0.002	1.68	6.94	3.42	5.75	0.04
Y1	126	0.23	0.66	0.04	0.003	**9.37**	**15.69**	3.62	5.38	0.06
Y2	126	0.23	0.60	0.05	0.003	5.79	**25.40**	3.10	4.59	0.05
Y3	126	0.17	0.62	0.04	0.003	4.58	**15.47**	3.05	5.52	0.04
Y1	002	0.24	0.65	0.05	0.002	2.13	6.80	4.00	5.89	0.04
Y2	002	0.25	0.74	0.06	0.003	1.86	5.40	3.04	4.74	0.03
Y3	002	0.19	0.74	0.07	0.002	2.25	7.54	3.55	5.17	0.03
Y1	015	0.20	0.62	0.04	0.001	1.58	3.15	5.30	5.30	0.05
Y2	015	0.23	0.70	0.04	0.003	1.54	1.65	3.23	4.00	0.04

Y3	015	0.19	0.60	0.04	0.002	1.21	3.31	4.13	5.15	0.03
Y1	005	0.26	0.82	0.05	0.003	2.50	4.28	5.12	5.00	0.05
Y2	005	0.23	0.83	0.05	0.002	1.62	4.70	3.10	4.19	0.05
Y3	005	0.22	0.87	0.06	0.003	1.60	5.59	3.42	5.47	0.04
Y1	106	0.65	0.82	0.07	0.002	**8.48**	6.17	6.54	6.92	0.04
Y2	106	0.40	0.90	0.04	0.004	5.22	**14.38**	4.95	**7.58**	0.05
Y3	106	0.37	0.71	0.08	0.003	5.44	8.00	4.67	4.67	0.04
Y1	023	0.19	0.72	0.06	0.003	1.83	0.83	3.65	6.48	0.05
Y2	023	0.25	0.75	0.07	0.002	1.39	1.34	3.59	4.46	0.04
Y3	023	0.22	0.76	0.07	0.002	1.74	1.00	3.25	5.66	0.04
Y1	018	0.41	**2.00**	0.04	**2.072**	**35.33**	4.42	5.56	5.04	0.04
Y2	018	0.31	0.69	0.05	**1.111**	**11.89**	7.43	5.80	4.76	0.04
Y3	018	0.20	0.67	0.03	**1.425**	**11.31**	8.00	5.34	5.04	0.04
Y1	356	0.61	**2.28**	0.04	**18.871**	**50.80**	8.55	5.86	4.88	0.04
Y2	356	0.57	0.57	0.05	**32.000**	**32.00**	**13.93**	6.06	4.59	0.05
Y3	356	0.13	**1.00**	0.03	**32.000**	**16.00**	**32.00**	4.00	4.00	0.03
Y1	012	0.27	0.72	0.05	0.005	2.40	**51.42**	5.36	**9.60**	**0.08**
Y2	012	0.39	0.91	0.05	0.002	1.81	**55.17**	3.81	6.56	**0.08**
Y3	012	0.29	0.79	0.06	0.002	2.52	**69.12**	4.00	**11.76**	**0.06**
Y1	017	0.27	0.65	0.02	**0.925**	**18.22**	**43.34**	5.91	**7.66**	0.04
Y2	017	0.22	0.61	0.02	**1.187**	**13.59**	**39.24**	**9.81**	**14.75**	0.05
Y3	017	0.22	0.72	0.05	**0.420**	**9.19**	**50.80**	**10.56**	**14.59**	0.05
Y1	198	**1.19**	0.39	0.04	0.006	5.66	0.77	**7.34**	4.00	0.03
Y2	198	**1.64**	0.55	**0.08**	**0.015**	**23.78**	**12.49**	**7.61**	4.88	0.03
Y3	198	**1.68**	0.50	**0.10**	0.004	**24.68**	4.00	**8.00**	8.00	0.04
Y1	**All isolates**	**0.37**	**0.76**	**0.04**	**0.008**	**4.79**	**5.72**	**4.75**	**5.82**	**0.05**
Y2	**All isolates**	**0.33**	**0.70**	**0.05**	**0.009**	**3.90**	**7.61**	**3.88**	**5.39**	**0.04**

Y3	**All isolates**	**0.29**	**0.70**	**0.05**	**0.007**	**3.73**	**9.18**	**4.17**	**6.06**	**0.04**

CHLOR, chloramphenicol; CLINDA, clindamycin; FDX, fidaxomicin; IMI, imipenem; M, metronidazole; MICs, minimum inhibitory concentrations; MXF, moxifloxacin; PCR, polymerase chain reaction; RIF, rifampicin; TIG, tigecycline; V, vancomycin.

Bold type indicates higher geometric mean MICs of prevalent *Clostridium difficile* PCR ribotypes.

471 **Figure legends**

472

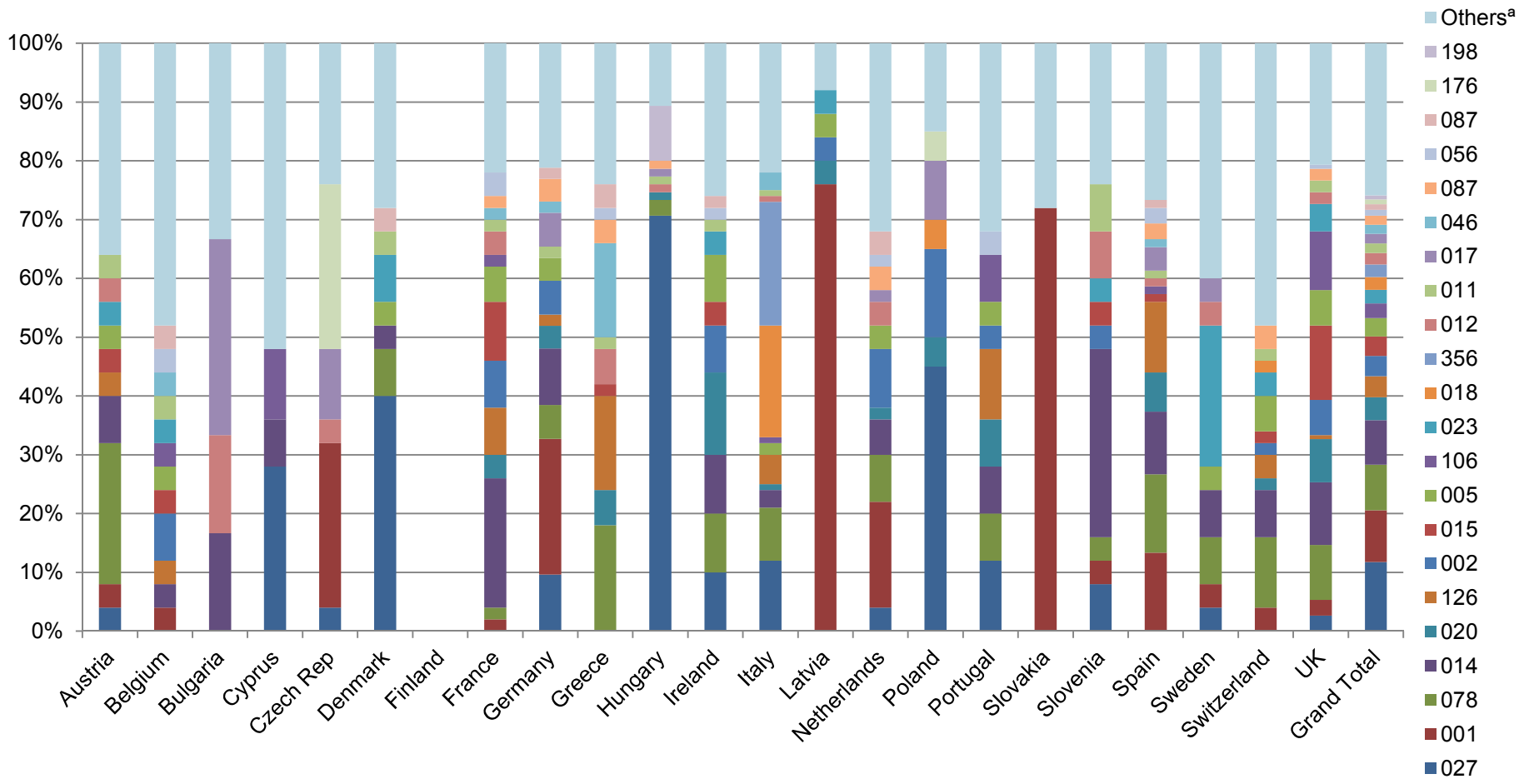
473 **Fig. 1.** Percentage prevalence of *Clostridium difficile* PCR ribotypes in 22 European
474 countries. (a) Year 1 of the *Clos*ER study. There were no submissions from Finland in Year
475 1. (b) Year 2 of the *Clos*ER study. (c) Year 3 of the *Clos*ER study. There were no
476 submissions from Finland or Slovakia in Year 3.
477 [a]*C. difficile* ribotypes with prevalence <1% per given year. PCR, polymerase chain reaction.

478

479 **Fig. 2.** Fidaxomicin MIC distribution for all isolates in Years 1, 2 and 3 of the *Clos*ER study.
480 MIC, minimum inhibitory concentration.

481

482 **Supplementary Fig. S1**. Distribution of cumulative antimicrobial resistance of *Clostridium*
483 *difficile* in 22 European countries: mean cumulative resistance scores. (a) Year 1 of the
484 *Clos*ER study. (b) Year 2 of the *Clos*ER study. (c) Year 3 of the *Clos*ER study.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Cyprus
Czech Rep
Denmark
Finland
France
Germany
Greece
Hungary
Ireland
Italy
Latvia
Netherlands
Poland
Portugal
Slovakia
Slovenia
Spain
Sweden
Switzerland
UK
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015
002
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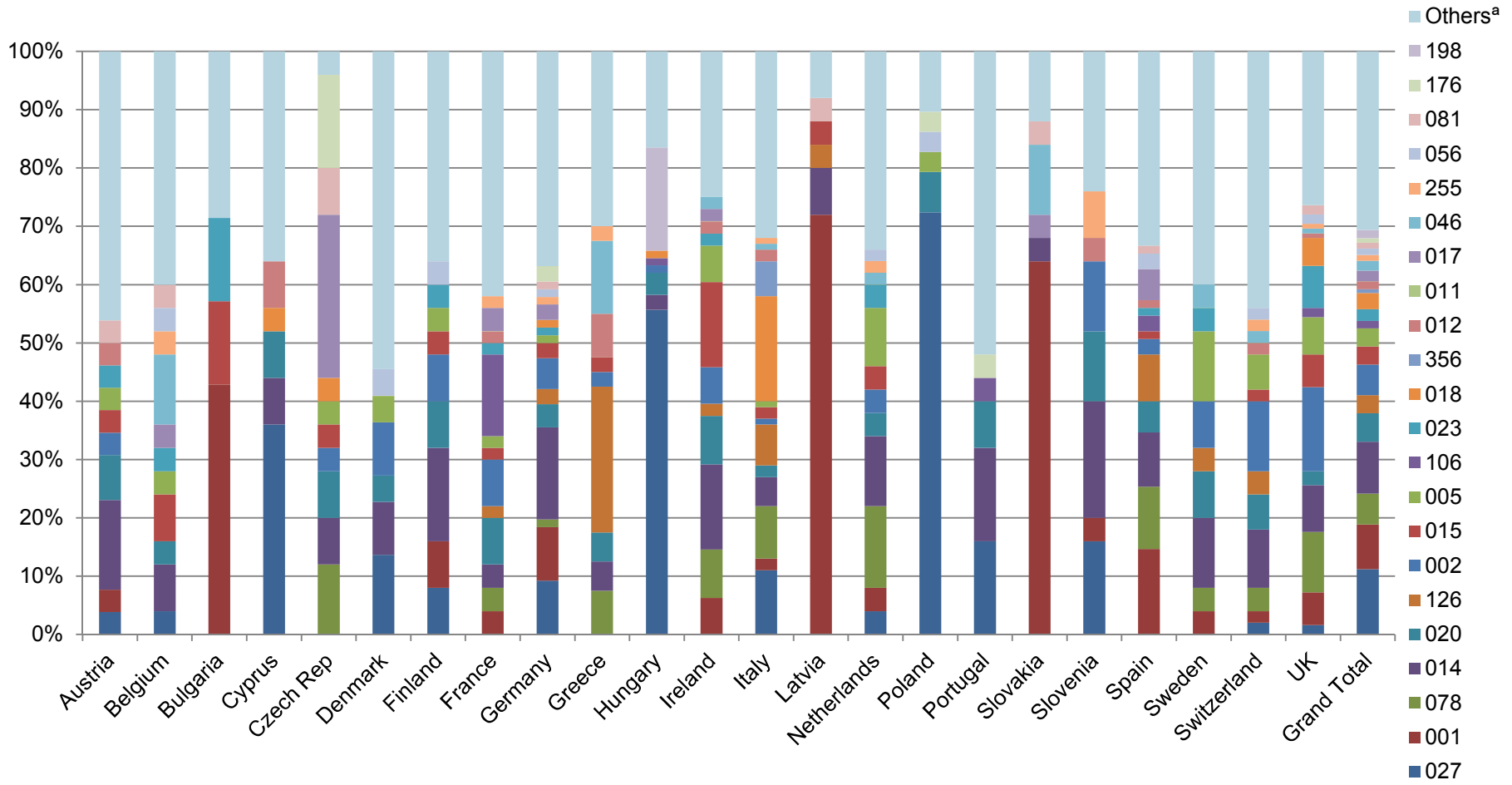
020
014
078
001
027

Figure 1a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Finland
France
Germany
Greece
Hungary
Ireland
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Latvia
Netherlands
Poland
Portugal
Slovakia
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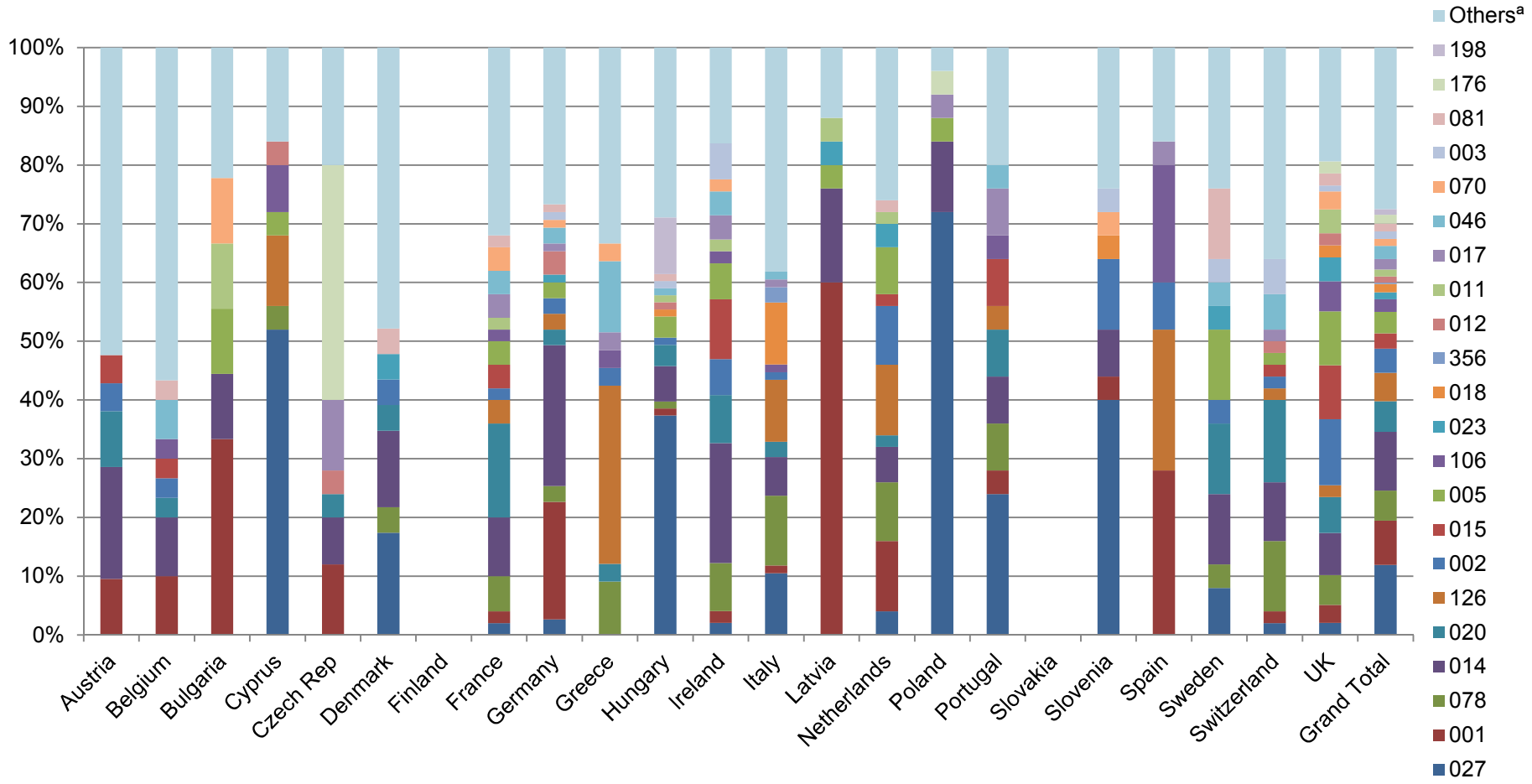
078
001
027

Figure 1b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Finland
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Germany
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Hungary
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Latvia
Netherlands
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Portugal
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Slovenia
Spain
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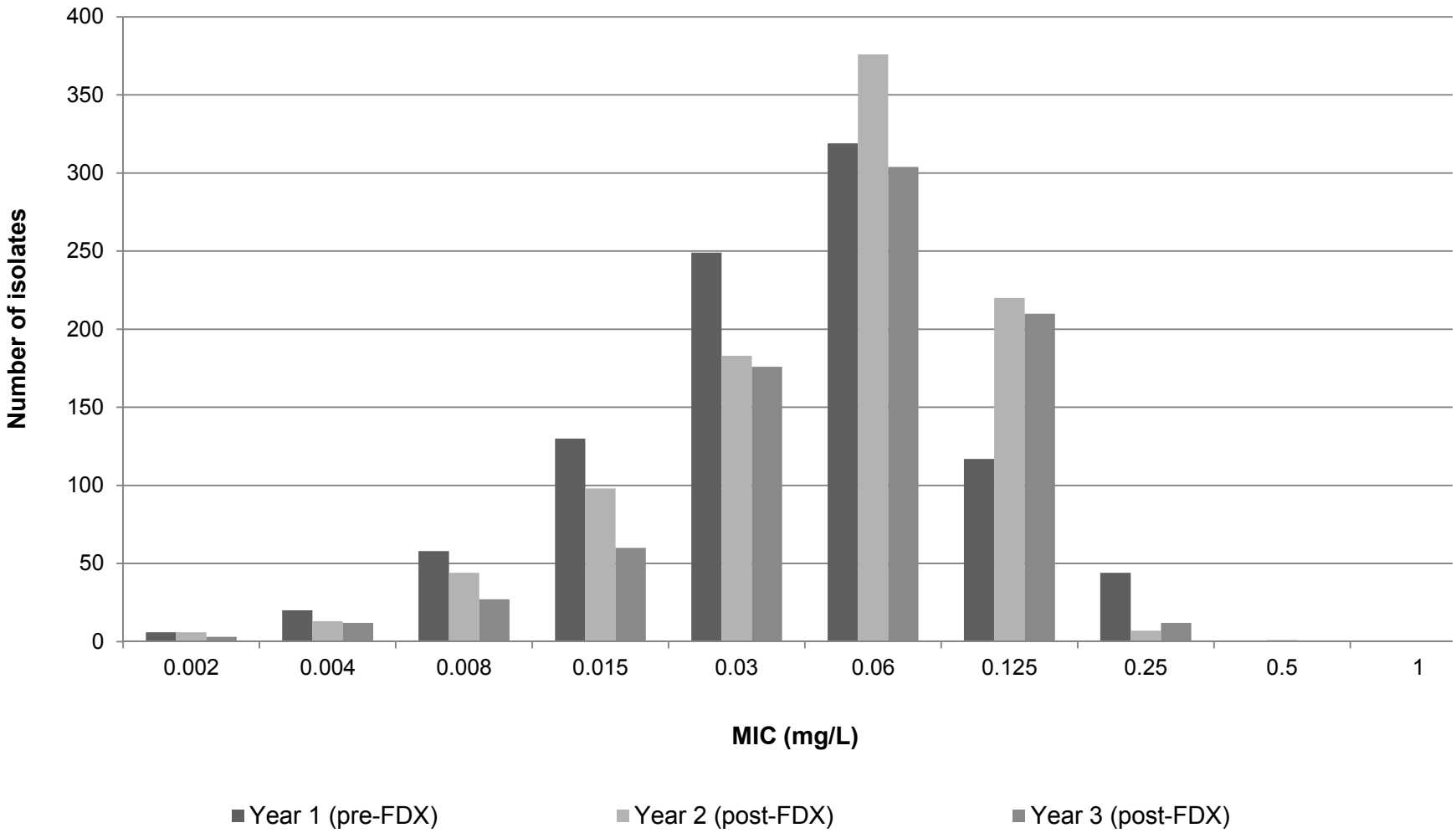
078
001
027

Figure 1c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300
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200
150
100
50
0
Number of isolates
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0.004
0.008
0.015
0.03
0.06
0.125
0.25
0.5
1
MIC (mg/L)
Year 1 (pre-FDX)
Year 2 (post-FDX)
Year 3 (post-FDX)

Figure 2