

# 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

KA Davies<sup>1</sup>, H Ashwin<sup>1</sup>, CM Longshaw<sup>2</sup>, DA Burns<sup>2</sup>, GL Davis<sup>1</sup>, MH Wilcox<sup>1</sup>, on behalf of the EUCLID study group<sup>3</sup>

1. Leeds Institute for Biomedical and Clinical Sciences, University of Leeds, Leeds, United Kingdom

2. Astellas Pharma EMEA, Chertsey, United Kingdom

3. Members of the EUCLID study group are listed at the end of the article

Correspondence: Mark Harvey Wilcox (mark.wilcox@nhs.net)

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*Clostridium difficile* infection (CDI) is the major cause of infective diarrhoea in healthcare environments. As part of the European, multicentre, prospective, biannual, point-prevalence study of *Clostridium difficile* infection in hospitalised patients with diarrhoea (EUCLID), the largest *C. difficile* epidemiological study of its type, PCR ribotype distribution of *C. difficile* isolates in Europe was investigated. PCR ribotyping was performed on 1,196 *C. difficile* isolates from diarrhoeal samples sent to the European coordinating laboratory in 2012–13 and 2013 (from two sampling days) by 482 participating hospitals from 19 European countries. A total of 125 ribotypes were identified, of which ribotypes 027 (19%, n = 222), 001/072 (11%, n = 134) and 014/020 (10%, n = 119) were the most prevalent. Distinct regional patterns of ribotype distribution were noted. Of 596 isolates from patients with toxin-positive stools (CDI cases), ribotype 027 accounted for 22% (32/144) of infections in cases aged from 18 to less than 65 years, but the prevalence decreased in those aged ≥ 65 years (14% (59/412)) and further decreased in those aged ≥ 81 years (9% (18/195)). The prevalence of ribotype 027 and 176, but not other epidemic strains, was inversely proportional to overall ribotype diversity ( $R^2 = 0.717$ ). This study highlights an increased diversity of *C. difficile* ribotypes across Europe compared with previous studies, with considerable intercountry variation in ribotype distribution. Continuous surveillance programmes are necessary to monitor the changing epidemiology of *C. difficile*.

## Introduction

*Clostridium difficile* is the most common cause of infective diarrhoea in hospitalised patients, and is

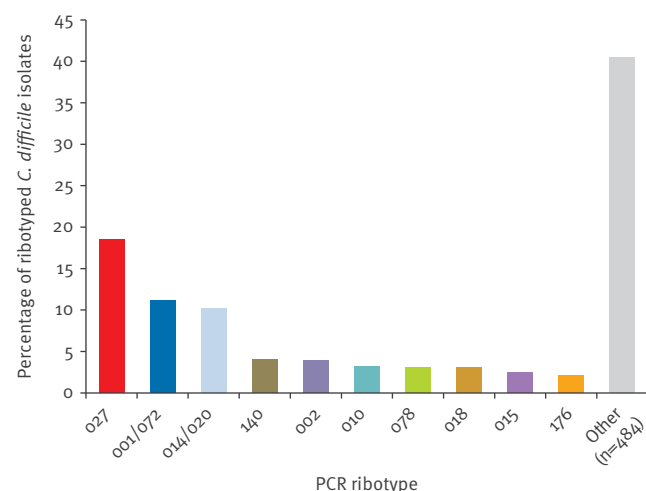
associated with substantial morbidity and mortality. Over the past decade, the burden of *C. difficile* infection (CDI) has increased in many European countries, with the annual incidence in Europe estimated at 124,000 cases in 2011–12 [1] with all-cause mortality rates of 3–30% [2–5]. CDI continues to be the focus of comprehensive national-level control and surveillance programmes in some countries, but the public health threat of CDI is not yet fully recognised across Europe.

*C. difficile* is an intensively typed pathogen, with a wide range of methods applied to understand its epidemiology. The emergence of so-called ‘hypervirulent’ *C. difficile* types has intensified the challenge of CDI. In the 1990s, strains belonging to PCR ribotype 027 (also referred to as restriction endonuclease type BI and North American pulsed-field type 1 (NAP-1)) were infrequently isolated from patients with CDI [6] but in the last decade this type has become highly represented among clinical isolates across Europe [7], with ribotype 027 often linked to outbreaks with increased disease severity [8–10]. In a 2008 study of *C. difficile* epidemiology in Europe, which consisted of a network of 106 laboratories in 34 countries, 65 different ribotypes were identified, of which ribotypes 014/020 (16%), 001 (9%) and 078 (8%) were the most prevalent [11]. Ribotype 027 accounted for 5% of all *C. difficile* isolates.

The European, multicentre, prospective, biannual, point-prevalence study of *Clostridium difficile* infection in hospitalised patients with diarrhoea (EUCLID) is the largest and most recent epidemiological study of *C. difficile*, encompassing 482 participating hospitals from 20 European countries (Austria, Belgium, Bulgaria,

**FIGURE 1**

Distribution of the 10 most commonly isolated *Clostridium difficile* PCR ribotypes from all *C. difficile* isolates in the participating countries, EUCLID, 2012–13 and 2013<sup>a</sup> (n = 1,196)



EUCLID: European, multicentre, prospective, biannual, point-prevalence study of *Clostridium difficile* infection in hospitalised patients with diarrhoea.

The percentages are the based on the total number of ribotyped *C. difficile* isolates.

Data from the following countries (n= 19) were included: Austria, Belgium, Bulgaria, Czech Republic, Finland, France, Germany, Greece, Hungary, Ireland, Italy, the Netherlands, Poland, Portugal, Romania, Slovakia, Spain, Sweden and United Kingdom. None of the faecal samples submitted from Slovenia during the two sampling days were found to be positive for *C. difficile* or its toxins.

<sup>a</sup> The countries submitted inpatient diarrhoeal samples on two sampling days (one day in winter, in December 2012 or January 2013, and one day in summer, in July or August 2013).

Czech Republic, Finland, France, Germany, Greece, Hungary, Ireland, Italy, the Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden and United Kingdom (UK)) [12]. The study measured the prevalence and underdiagnosis of CDI on two sampling days (one in winter and one in summer) in 2012 and 2013; participating hospitals forwarded inpatient diarrhoeal faecal samples to national coordinating laboratories for CDI testing by a study reference method. The mean measured rate of CDI was 7.0 cases (country range 0.7–28.7) per 10,000 patient-bed days and, across all hospitals on the two sampling days, 148/641 (23%) samples positive for CDI were not diagnosed by participating hospitals due to lack of clinical suspicion; a further 68 samples were not diagnosed due to sub-optimal laboratory diagnostic methods [12].

Here, we report the PCR ribotype distribution of *C. difficile* isolates in Europe from the 1,211 samples, including those from 595 patients with confirmed CDI, that were culture positive for *C. difficile* in EUCLID and

discuss the changing epidemiology of CDI from previous ribotype surveillance studies.

## Methods

### Study design

EUCLID followed the design of a previous point-prevalence study in Spain [13] and full methodology can be found in a previous EUCLID publication [12]. Briefly, the study was coordinated from the European coordinating laboratory in Leeds, UK. A national coordinating laboratory was selected for each of the 20 participating European countries and the national coordinators selected hospitals to cover all major geographical regions within each country. Hospitals were recruited at a rate of one per million population in all countries. All inpatient diarrhoeal samples submitted to the microbiology laboratory of the participating hospital on two sampling days (one day in winter, in December 2012 or January 2013, and one day in summer, in July or August 2013) were eligible for inclusion. Anonymised samples were sent from the participating hospital, within seven days, to the national coordinating laboratory for their country, where they were tested for CDI and cultured for *C. difficile*. Transport was refrigerated for six countries (Finland, France, Hungary, Portugal, Romania and Spain) in the winter sampling period and for all 20 countries in the summer.

Patients were defined as a CDI case if their faecal sample was positive according to a two-stage algorithm: membrane enzyme immunoassay for glutamate dehydrogenase and *C. difficile* toxins A and B (C DIFF QUIK CHEK COMPLETE, Techlab, United States). The incidence of CDI in children aged under 2 years, in whom diarrhoeal illness is common and *C. difficile* carriage rates are high [14], is unclear [15,16]. Patients under 2 years-old who tested positive for free toxin in the stool were therefore not included as cases of CDI.

*C. difficile* colonisation of patients was assumed for those whose faecal sample was positive for culture of *C. difficile* but negative for free *C. difficile* toxin.

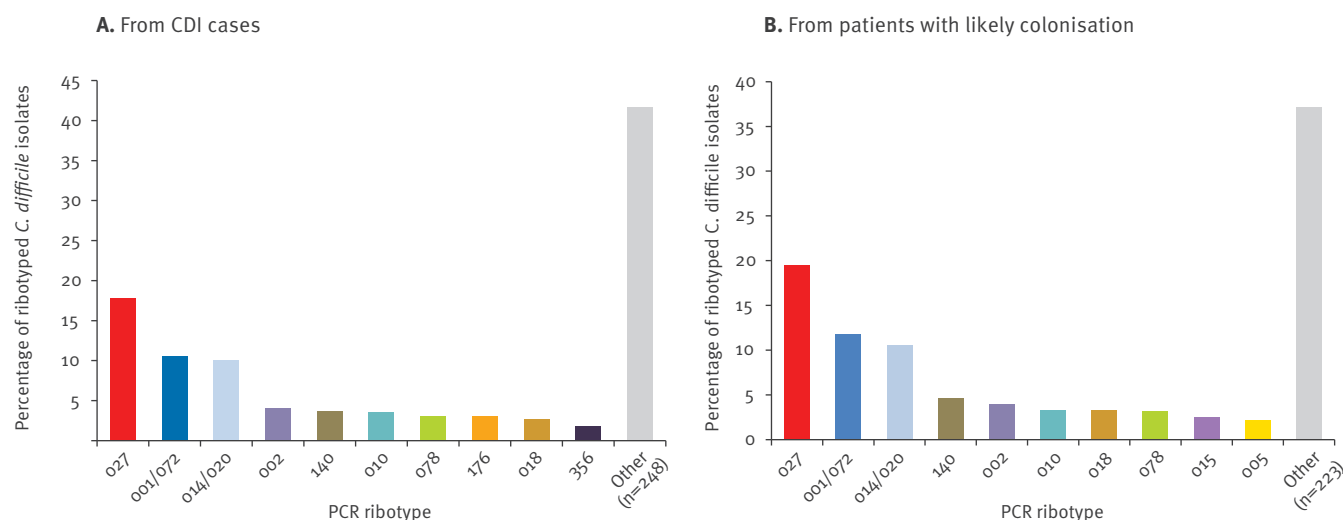
### PCR ribotyping analysis

Isolates of *C. difficile* were stored in brain-heart infusion broth supplemented with 10% glycerol at the national coordinating laboratories, before being frozen and transported to the European coordinating laboratory in Leeds, UK. All *C. difficile* isolates identified at national coordinating laboratories (regardless of whether or not the samples were positive for glutamate dehydrogenase and free toxin, indicating CDI) were sent to Leeds, to confirm pathogen identification and for PCR ribotyping analysis. PCR ribotyping was performed on all *C. difficile* isolates using the previously published capillary gel-based method [17].

Geographical distribution of ribotypes was based on the United Nations geoscheme for Europe [18]: Northern Europe (Finland, Ireland, Sweden and UK),

**FIGURE 2**

Distribution of the 10 most commonly isolated *Clostridium difficile* PCR ribotypes in isolates from (A) cases of *C. difficile* infection<sup>a</sup> (596 isolates) and (B) patients with likely colonisation<sup>b</sup> (600 isolates), EUCLID, 2012–13 and 2013<sup>c</sup>



CDI: *Clostridium difficile* infection; EUCLID: European, multicentre, prospective, biannual, point-prevalence study of *Clostridium difficile* infection in hospitalised patients with diarrhoea.

The percentages shown are the based on the total number of ribotyped *C. difficile* isolates.

Data from the following countries (n = 19) were included: Austria, Belgium, Bulgaria, Czech Republic, Finland, France, Germany, Greece, Hungary, Ireland, Italy, the Netherlands, Poland, Portugal, Romania, Slovakia, Spain, Sweden and United Kingdom. None of the faecal samples submitted from Slovenia during the two sampling days were found to be positive for *C. difficile* or its toxins.

<sup>a</sup> Positive for free *C. difficile* toxin, tested using a two-stage algorithm (membrane enzyme immunoassay for glutamate dehydrogenase and *C. difficile* toxins A and B).

<sup>b</sup> Positive for culture of *C. difficile* but negative for free *C. difficile* toxin.

<sup>c</sup> The countries submitted inpatient diarrhoeal samples on two sampling days (one day in winter, in December 2012 or January 2013, and one day in summer, in July or August 2013).

Western Europe (Austria, Belgium, France, Germany and the Netherlands), Southern Europe (Greece, Italy, Portugal and Spain) and Eastern Europe (Bulgaria, Czech Republic, Hungary, Poland, Romania and Slovakia). None of the faecal samples submitted from Slovenia during the two sampling days were found to be positive for *C. difficile* or its toxins.

### Statistical analyses

Simpson's index (D) was used to compare diversity in ribotype distribution among countries and patient age groups, and was calculated as follows:  $D = \frac{n(n-1)}{N(N-1)}$ , where n represents the total number of isolates of a particular ribotype and N represents the total combined number of isolates for all ribotypes. For ease of illustration, Simpson's reciprocal index (1/D) was plotted, where the lowest possible diversity is 1 (a population dominated by a single ribotype) and increasing values indicate increasing diversity.

Chi-squared test was used to compare the proportion of ribotypes from CDI cases among patient age groups.

## Results

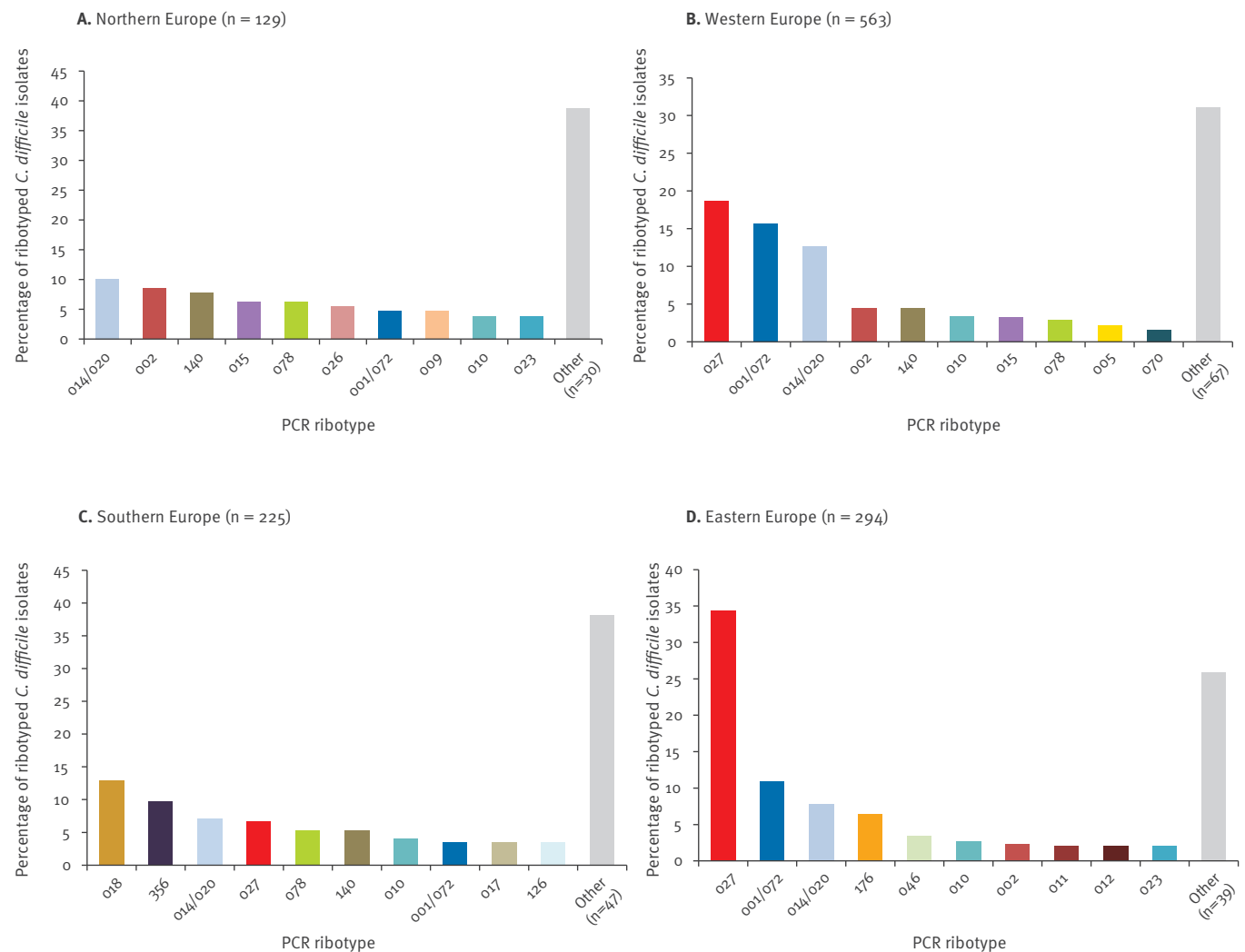
### Samples obtained for PCR ribotyping analysis

A total of 3,923 and 3,389 faecal samples were submitted during the winter and summer testing periods, respectively. A total of 15 samples were excluded due to incomplete data, giving a total of 7,297 samples for analysis.

A PCR ribotype was assigned to 1,194 of the 1,211 *C. difficile* isolates received by the European coordinating laboratory after removal of 17 sporadic isolates that could not be assigned to a ribotype (obtained from 19 countries). For two samples more than one ribotype was isolated, giving a total of 1,196 *C. difficile* isolates. The median age of patients for whom a *C. difficile* PCR ribotype was reported was 71 years (range: 1–99) and patient ward locations included medical (n=704), intensive therapy unit/high dependency unit (n=47), obstetrics and gynaecology (n=4), paediatric (n=138) and surgery (n=106).

**FIGURE 3**

PCR ribotype diversity of *Clostridium difficile* isolates by European region<sup>a</sup>, EUCLID, 2012–13 and 2013<sup>b</sup> (n = 1,196)



EUCLID: European, multicentre, prospective, biannual, point-prevalence study of *Clostridium difficile* infection in hospitalised patients with diarrhoea.

The charts show the proportion of the most common ribotypes per region; the percentages are based on the number of typed isolates in the region.

<sup>a</sup> The 19 participating countries were classified by European region as defined according to the United Nations geoscheme for Europe [18]: (A) Northern Europe: Finland, Ireland, Sweden and United Kingdom; (B) Western Europe: Austria, Belgium, France, Germany and the Netherlands; (C) Southern Europe: Greece, Italy, Portugal and Spain; and (D) Eastern Europe: Bulgaria, Czech Republic, Hungary, Poland, Romania and Slovakia. None of the faecal samples submitted from Slovenia during the two sampling days were found to be positive for *C. difficile* or its toxins.

<sup>b</sup> The countries submitted inpatient diarrhoeal samples on two sampling days (one day in winter, in December 2012 or January 2013, and one day in summer, in July or August 2013).

### *C. difficile* PCR ribotype diversity in Europe

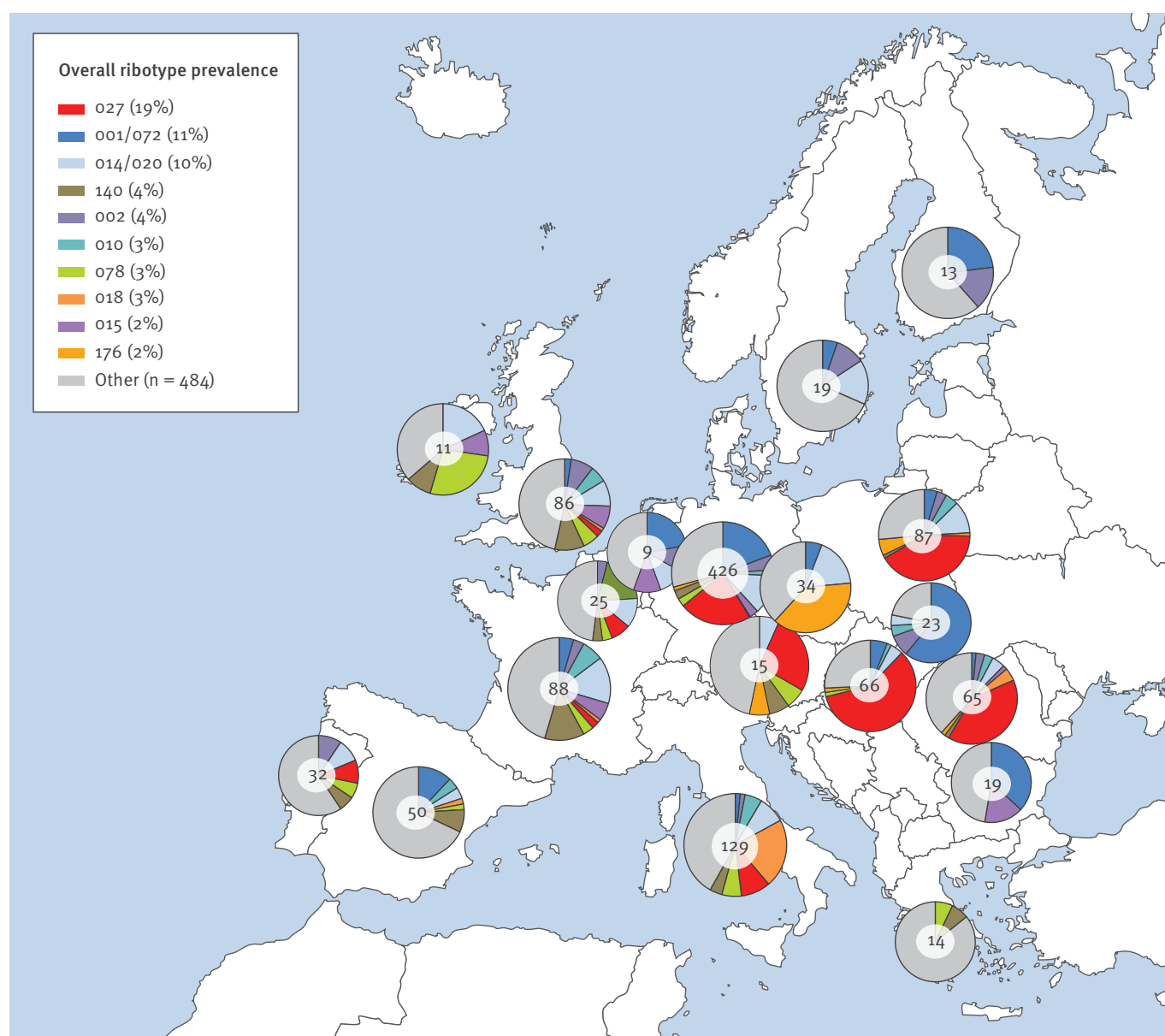
We identified *C. difficile* isolates belonging to 125 different ribotypes across 19 countries; the 10 most commonly isolated ribotypes received by the European coordinating laboratory are shown in Figure 1. *C. difficile* ribotype 027 was the most prevalent (19%, n = 222); ribotypes 001/072 (11%, n = 134) and 014/020 (10%, n = 119) were the second and third most prevalent, respectively. Ribotype 078, the third most prevalent

ribotype in a previous study in 2008 [11], accounted for 3% (n = 37) of isolates in our study.

Of the 1,196 *C. difficile* isolates where a PCR ribotype was identified, 596 were isolated from stool samples of 595 CDI cases (positive for free *C. difficile* toxin), while 600 were from 599 patients who were likely to be colonised (positive for culture of *C. difficile* but negative for free *C. difficile* toxin). The 10 most commonly

**FIGURE 4**

Geographical distribution of *Clostridium difficile* PCR ribotypes, by participating European country<sup>a</sup>, EUCLID, 2012–13 and 2013<sup>b</sup> (n = 1,196)



EUCLID: European, multicentre, prospective, biannual, point-prevalence study of *Clostridium difficile* infection in hospitalised patients with diarrhoea.

Pie charts show the proportion of the most common ribotypes per country and the number in the centre of the charts is the number of typed isolates in the country.

<sup>a</sup> Austria, Belgium, Bulgaria, Czech Republic, Finland, France, Germany, Greece, Hungary, Ireland, Italy, the Netherlands, Poland, Portugal, Romania, Slovakia, Spain, Sweden and United Kingdom. None of the faecal samples submitted from Slovenia during the two sampling days were found to be positive for *C. difficile* or its toxins.

<sup>b</sup> The countries submitted inpatient diarrhoeal samples on two sampling days (one day in winter, in December 2012 or January 2013, and one day in summer, in July or August 2013).

isolated ribotypes from samples from CDI cases (Figure 2A) and those from patients with likely *C. difficile* colonisation (Figure 2B) were compared. The ribotype distribution was found to be largely similar between CDI cases and patients with likely colonisation, suggesting

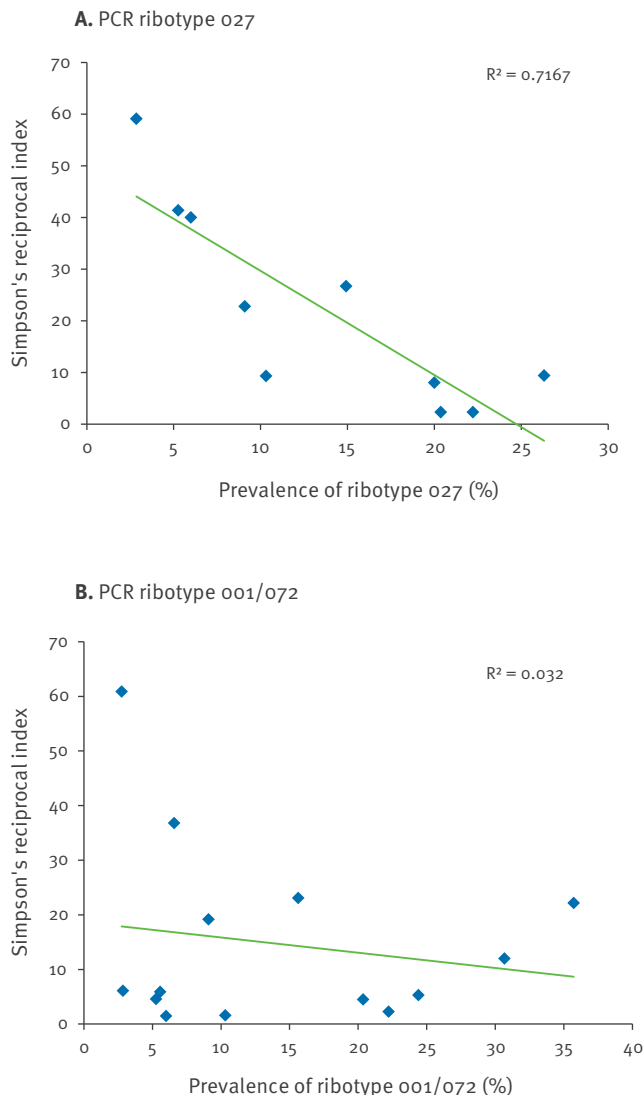
no obvious over-representation of *C. difficile* isolates associated with colonisation or infection.

The geographical distribution of all *C. difficile* ribotypes isolated in this study is summarised in Figures 3 and 4. Many of the most commonly isolated ribotypes were



**FIGURE 5**

Relationship between Simpson's reciprocal index of *Clostridium difficile* PCR ribotype diversity and EUCLID-measured prevalence<sup>a</sup> of (A) ribotype 027<sup>b</sup> (n = 222) and (B) ribotype 001/072<sup>c</sup> (n = 134), EUCLID, 2012–13 and 2013<sup>d</sup>



EUCLID: European, multicentre, prospective, biannual, point-prevalence study of *Clostridium difficile* infection in hospitalised patients with diarrhoea.

<sup>a</sup> As reported in [12].

<sup>b</sup> The 10 countries where ribotype 027 was isolated were Austria, Belgium, France, Germany, Hungary, Italy, Poland, Portugal, Romania, United Kingdom.

<sup>c</sup> The 14 countries where ribotype 001/072 was isolated were Bulgaria, Czech Republic, Finland, France, Germany, Hungary, Italy, Netherlands, Poland, Romania, Slovakia, Spain, Sweden, United Kingdom.

<sup>d</sup> The countries submitted inpatient diarrhoeal samples on two sampling days (one day in winter, in December 2012 or January 2013, and one day in summer, in July or August 2013).

found across each region (Figure 3). Among these were ribotype 001/072 (in 14 countries), 014/020 (in 16 countries), 002 (in 12 countries), 078 (in 11 countries) and 027 (in 10 countries). However, we also observed substantial variation in ribotype distribution among the study countries (Figure 4). For example, ribotypes 018 and 356 were commonly isolated in Italy (22% (28/129) and 17% (22/129) prevalence, respectively) but were rarely isolated in other countries. Similarly, ribotype 176, which is closely related to ribotype 027, represented 38% (13/34) of all isolates in the Czech Republic, but only 2% (26/1,196) of total isolates. Distinct regional patterns in the distribution of *C. difficile* ribotypes were noted (Figure 4).

The proportion of samples that were positive and negative for free *C. difficile* toxin received from each participating country was similar, suggesting that the geographical distribution of ribotypes was not influenced by over- or under-representation of isolates associated with either CDI cases or likely colonisation.

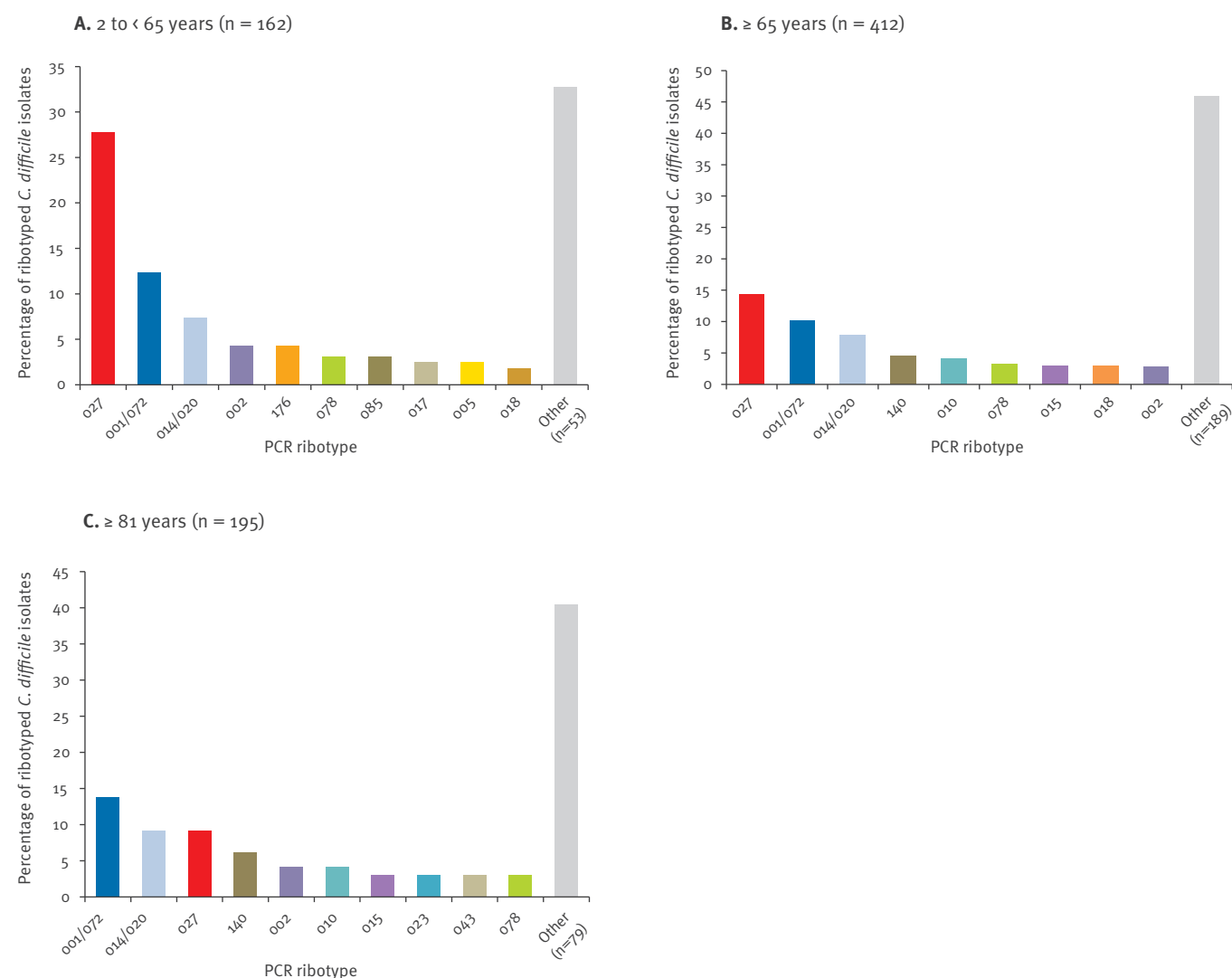
### Relationship between ribotype diversity and prevalence of ribotype 027

Given that most ribotype 027 strains were found to be localised mainly to four countries (Germany, Hungary, Poland and Romania) [12], we investigated the relationship between prevalence of ribotype 027 in the 10 countries in which it was identified in EUCLID and the overall ribotype diversity among all *C. difficile* isolates (from CDI cases and those with likely colonisation) received during the study from those countries. Using Simpson's reciprocal index of diversity, we found that ribotype diversity decreased as the prevalence of ribotype 027 increased in the 10 countries where ribotype 027 was isolated ( $R^2 = 0.717$ ; Figure 5A). To determine whether this was a common feature of epidemic *C. difficile* types, we performed the same analysis on ribotype 001/072 from the 14 countries where this type was isolated, but noted no obvious linear relationship between country ribotype diversity and prevalence of 001/072 ( $R^2 = 0.032$ ; Figure 5B). Taken together, these data suggest that countries with a high prevalence of ribotype 027 strains have a lower overall ribotype diversity than countries with a low prevalence of ribotype 027.

A similar level of endemicity was observed in EUCLID for ribotype 176 in the Czech Republic (38% (13/34) of all ribotypes in the Czech Republic). *C. difficile* ribotype 176 is thought to share many similarities to ribotype 027 [19] and it has been suggested that this type may often be misdiagnosed as a ribotype 027 infection [20]. Therefore, we repeated this analysis to include both ribotype 027 and ribotype 176 and the findings were similar, with ribotype diversity decreasing as the prevalence of ribotypes 027 and 176 increased ( $R^2 = 0.722$ ; data not shown). This suggests that our observation is not limited to ribotype 027 and may extend to other closely related ribotypes with epidemic potential.

**FIGURE 6**

*Clostridium difficile* PCR ribotype diversity among patients with a confirmed diagnosis of *C. difficile* infection in the study, by age group (596 isolates from 595 CDI cases), EUCLID, 2012–13 and 2013<sup>a</sup>



EUCLID: European, multicentre, prospective, biannual, point-prevalence study of *Clostridium difficile* infection in hospitalised patients with diarrhoea.

The charts show the proportion of most common ribotypes per age group; the percentages are based on the number of typed isolates.

Data from the following countries (n = 19) were included: Austria, Belgium, Bulgaria, Czech Republic, Finland, France, Germany, Greece, Hungary, Ireland, Italy, the Netherlands, Poland, Portugal, Romania, Slovakia, Spain, Sweden and United Kingdom. None of the faecal samples submitted from Slovenia during the two sampling days were found to be positive for *C. difficile* or its toxins.

<sup>a</sup> The countries submitted inpatient diarrhoeal samples on two sampling days (one day in winter, in December 2012 or January 2013, and one day in summer, in July or August 2013).

### Effect of patient age on *C. difficile* PCR ribotype distribution

A total of 596 *C. difficile* PCR ribotypes were identified from the faecal samples of 595 CDI-positive patients, aged 1–99 years, in the study. To investigate if ribotype diversity and distribution varied according to patient age, we analysed the ribotype distribution in four patient age groups: 2 to <18 years (n=18),

18 to <65 years (n=144), ≥65 years (n=412) and ≥81 years (n=195). As the number of patients aged 2 to <18 years was small, the combined age group of 2 to <65 years is shown (Figure 6). The ribotypes of isolates from samples of patients aged under 2 years were not included in the analysis, as the role of *C. difficile* in infants is uncertain. We found that the number of unique ribotypes identified increased with patient age.

When comparing two age groups with similar patient numbers, 39 individual ribotypes were isolated in patients aged 18 to <65 years, while 59 were identified in patients  $\geq 81$  years. Analysis of Simpson's reciprocal index of diversity showed that overall ribotype diversity was higher in patients aged  $\geq 81$  years (Simpson's reciprocal index: 21.16) than in those aged 18 to <65 years (Simpson's reciprocal index: 10.1).

Ribotype 001/072 was commonly found in all age groups, with no obvious differences in distribution according to patient age (13% ( $n = 19/144$ ) in CDI cases aged 18 to <65 years, 10% ( $n = 42/412$ ) in  $\geq 65$  year-olds and 14% ( $n = 27/195$ ) in  $\geq 81$  year-olds). Other commonly isolated ribotypes, such as 014/020 (11% ( $n = 16/144$ ), 8% ( $n = 32/412$ ) and 9% ( $n = 18/195$ ), respectively) and 078 (3% ( $n = 5/144$ ), 3% ( $n = 13/412$ ) and 3% ( $n = 6/195$ ), respectively) were also consistently found in all patient age groups, with no noticeable age-associated variation in prevalence (patients aged 2 to <18 years were excluded from this analysis due to the small sample size).

Ribotype 027 occurred in all patient age groups but we observed considerable variation in prevalence. While this ribotype accounted for 22% ( $n = 32/144$ ) of CDI cases aged 18 to <65 years, the prevalence was significantly lower in those aged  $\geq 65$  years (14%,  $n = 59/412$ ) and was significantly further decreased in those aged  $\geq 81$  years (9%,  $n = 18/195$ ) (chi-squared test  $p = 0.001$ ).

To exclude any bias from country-specific variation in our analysis, we investigated the differences in ribotype distribution in CDI cases aged 18 to <65 years ( $n = 51$ ) and  $\geq 65$  years ( $n = 172$ ) from participating hospitals in Germany (the largest country in the study). We observed a similar trend, with the prevalence of ribotype 027 in patients aged 18 to <65 years (33%,  $n = 17/51$ ) double that of the prevalence in those aged  $\geq 65$  years (15%,  $n = 26/172$ ).

We observed that of 117 isolated *C. difficile* ribotypes from patients aged under 2 years, only 22 (18.8%) were associated with a positive test result for detection of free *C. difficile* toxin in the stool sample. By contrast, 26.5% (18/68) of ribotypes isolated from patients aged 2 to <18 years, 48.6% (144/296) from those aged 18 to <65 years, 57.6% (412/712) from those aged  $\geq 65$  years and 60.6% (195/322) from those aged  $\geq 81$  years were associated with positive test results; the differences in the rates of toxin-positive test results among these age groups were statistically significant ( $p < 0.001$ ). This finding supports the view that detection of *C. difficile* in infants and neonates often reflects asymptomatic colonisation.

Interestingly, ribotype 356 was commonly isolated in those aged under 2 years (4/22) but was rarely seen in those  $\geq 65$  years (2%, 7/412) and was not found at all in patients aged 2 to <65 or  $\geq 81$  years. This suggests

that ribotype 356 may be more commonly found in infants and neonates than older patients, although more data from a larger sample are required to verify this observation.

## Discussion

The findings of this EUCLID analysis highlight the changing epidemiology of *C. difficile* in Europe. We found an increase in overall ribotype diversity, with more than double the number of ribotypes identified in this study compared with data from 2008 [11]. It is important to note, however, that the possible suboptimal testing methodology and selection of cases for isolate ribotyping in the previous study, in addition to the smaller sample size, would likely have led to an under-representation of the recorded strain diversity. Nevertheless, notably, the prevalence of ribotype 027 had increased more than threefold (from 5% to 18.6%) since 2008 [11] and 027 was the most commonly isolated ribotype in the participating European countries in our study (on two sampling days in 2012–13 and 2013).

No clear difference in ribotype distribution was observed when samples that tested positive for free *C. difficile* toxin were compared with those that tested negative. This suggests that there is no discernible difference in those ribotypes causing *C. difficile* disease and those involved with colonisation, at least in inpatients with diarrhoea. The toxin component of the testing algorithm used in our study has been reported to have a sensitivity of 67.3% in the combined test and 84.3% as a single assay [21,22]. Thus, some patients classified here as likely colonised will in fact have been missed CDI cases.

Some similarities with the 2008 study [11] were observed, with ribotype 001/072 and ribotype 014 remaining highly prevalent among *C. difficile* clinical isolates across many European countries. However, we observed an almost threefold reduction in the prevalence of ribotype 078 in this study compared with that in 2008 [11]. Also, ribotype 106, which was associated with 26% and 20% of CDI cases in England in 2005 [23] and 2007–08 [24], respectively, was not found at all in the UK study hospitals in our study and accounted for only 0.6% of all *C. difficile* isolates in Europe. Ribotype 027 previously accounted for 55% of isolates in England in 2007–08 [25], but only represented 2.3% of UK isolates in the present study.

Our previous analysis showed a clear shift in ribotype 027 endemicity, from the UK and Ireland in 2008 to Germany, Hungary, Poland and Romania on the two sampling days in 2012–13 and 2013 [12]. The reason for a shift in ribotype 027 prevalence towards Germany and Eastern Europe is not clear, but may have been influenced by national CDI testing policies. Our earlier analysis identified an inverse correlation between the rate of CDI testing and prevalence of ribotype 027 across Europe [12]. Thus, an increased awareness of



CDI, via the use of optimum diagnostic tests, may have permitted the implementation of more timely infection prevention and antimicrobial prescribing interventions in Northern Europe, and so better control of epidemic strains such as ribotype 027 in this region since 2008 [12].

The substantial variation in ribotype distribution observed among study countries and regions is in keeping with the results of the 2008 study, in which the most commonly isolated *C. difficile* ribotypes were found in many countries across Europe and the geographical distribution of some ribotypes suggested regional spread [11]. Our findings highlight the diverse epidemiology of *C. difficile* across Europe. We observed that a high prevalence of ribotypes 027 and 176 was associated with low overall country-specific ribotype diversity, which is perhaps unsurprising. Countries with CDI outbreaks caused by epidemic strains such as ribotypes 027 and 176 would likely have high incidence rates but lower overall diversity due to more health-care-associated transmission of dominant ribotypes. This scenario may have been seen first-hand in the UK, where CDI incidence rapidly increased in 2006 with the emergence of ribotype 027 [23-25]. Subsequent infection control measures and antibiotic stewardship may have since shifted the UK into an endemic scenario with high ribotype diversity and a low prevalence of ribotype 027 [26].

We found no correlation between ribotype 001/072 prevalence and overall ribotype diversity, suggesting that ribotypes 027 and 176 may be more successful at outcompeting such other ribotypes with epidemic potential. The drivers for dominant ribotypes in particular countries, for example ribotype 176 in the Czech Republic and ribotype 018 in Italy, are not yet known. In the UK, the practice of restricting prescriptions of cephalosporins and fluoroquinolones since 2009 has been associated with falling prevalence of ribotype 027, which suggests that reduced selection of antibiotic-resistant (in this case, fluoroquinolones) *C. difficile* clones could be a key control measure [25,27]. Future studies of comparative fitness among different *C. difficile* ribotypes would be of particular interest.

The observed differences in ribotype distribution for CDI cases among patient age groups also suggest that some ribotypes may be more likely to cause CDI. Of note was the significant reduction in ribotype 027 prevalence with increasing patient age, which is perhaps at odds with the known poor clinical outcomes associated with this strain type in elderly patients [28]. It is possible that our data reflect differences in *C. difficile* selection pressures according to age; for example, less frequent use of high CDI-risk antibiotics in elderly patients [29,30]. Overall ribotype diversity appeared to increase with age, which may be related to the observed inverse correlation between ribotype diversity and 027 prevalence.

We noted the presence of some known non-toxigenic *C. difficile* types among isolates associated with CDI positive tests, with ribotypes 140 (3.7%) and 010 (3.5%) the fourth and sixth most commonly isolated in the participating countries in Europe, respectively. A likely explanation for this finding is that the submitted faecal sample contained more than one ribotype (including toxigenic ribotypes responsible for a positive toxin test) but, when *C. difficile* was cultured, the predominant strain was ribotype 140 or 010. The rate of mixed *C. difficile* genotypes in faecal samples of patients with CDI has ranged from 7% to 13% in previous studies [31-34] and the coexistence of multiple PCR ribotypes has been previously reported as a potential limitation of *C. difficile* epidemiological studies [35]. In our study, several single *C. difficile* colonies were pooled before DNA extraction and, while this method allows accurate identification of the predominant ribotype, not every ribotype present within the sample can be identified. Therefore, in some cases the relative abundance of the disease-causing ribotype may have been too low for identification.

The findings of this analysis from EUCLID emphasise the importance of continuous national and European surveillance programmes to monitor the dynamic epidemiology of *C. difficile*, including use of optimal diagnostic methods to identify CDI cases. Further studies are also necessary to better understand how *C. difficile* ribotype distribution varies among patient populations, and factors contributing to an observed shift of ribotype 027 to Germany and Eastern Europe.

#### EUCLID study group

European coordinators: Kerrie A. Davies (Leeds Teaching Hospitals NHS Trust, UK); Mark H. Wilcox (University of Leeds, UK); Georgina Davis (University of Leeds, UK). European coordinator and representative of sponsor: Christopher M. Longshaw (Astellas Pharma EMEA). National coordinators: Ed Kuijper (Leiden University Medical Centre, Netherlands); Lutz von Muller (Saarland University Medical Centre, Germany); Outi Lyytikäinen, Silja Mentula (National Institute of Health and Welfare, Finland); Fidelma Fitzpatrick (Health Protection Surveillance Centre and Beaumont Hospital, Ireland); Emilio Bouza (Catedrático-Jefe de Servicio, Microbiología Clínica e Infecciosas, Hospital General Universitario 'Gregorio Marañón', Spain); Frederic Barbut (Saint-Antoine Hospital, France); Monica Oleastro (National Institute of Health, Portugal); Michel Delmee (University of Louvain, Belgium); Paola Mastrantonio (Istituto Superiore di Sanità, Italy); Torbjorn Noren (Örebro University Hospital, Sweden); Franz Allerberger (Austrian Agency for Health and Food Safety, Austria); Hanna Pituch (Department of Medical Microbiology, Medical University of Warsaw, Poland); Maja Rupnik (National Laboratory for Health, Environment and Food, Slovenia); Zsuzsanna Barna (National Centre for Epidemiology, Hungary); Efthymia Petinaki (University Hospital of Larissa, Greece); Otakar Nyč (University Hospital FN Motol, Czech Republic); Daniela Lemeni (Cantacuzino Institute, Romania); Kate Ivanova (National Centre of Infectious and Parasitic Diseases, Bulgaria); Elena Novakova (Jessenius Faculty of Medicine in Martin Comenius University in Bratislava, Slovakia).

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## Conflict of interest

HA and GD: none declared.

MHW has received grant support/consultancy fees/honorarium from Astellas. KAD has received honoraria from Astellas. CML and DAB are employees of Astellas Pharma EMEA.

## Authors' contributions

The EUCLID study was designed by KAD, MHW and CML with support from the EUCLID core group and on behalf of the EUCLID study group. GLD was responsible for project management and sample logistics. HA performed PCR ribotyping analyses. KAD, DAB, CML and MHW analysed data and wrote the report. All authors reviewed drafts of the report.

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