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Ribotypes and New Virulent Strains Across Europe

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Abstract

Clostridium difficile is a major bacterial cause of post-antibiotic diarrhoea. The epidemiology of *C. difficile* infections (CDI) has dramatically changed since the early 2000s, with an increasing incidence and severity across Europe. This trend is partly due to the emergence and rapid worldwide spread of the hypervirulent and epidemic PCR ribotype 027. Profiles of patients with CDI have also evolved, with description of community-acquired (CA) infections in patients with no traditional risk factors for CDI. However, recent epidemiological studies indicated that some European countries have successfully controlled the dissemination of the 027 clone whereas other countries recently reported the emergence of other virulent or unusual strains. The aims of this review are to summarize the current European CDI epidemiology and to describe the new virulent *C. difficile* strains circulating in Europe, as well as other potential emerging strains described elsewhere. Standardized typing methods and surveillance programmes are mandatory for a better understanding and monitoring of CDI in Europe.

Keywords

C. difficile · PCR ribotypes · Emerging strains · European epidemiology · Binary toxin

1 Introduction

Clostridium difficile is the main bacterial cause of hospital-acquired diarrhoea; it is responsible for 15–25% of post-antibiotic diarrhoea and for virtually all cases of pseudomembranous colitis (Bartlett and Gerding 2008). *C. difficile* infection

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(CDI) epidemiology has dramatically changed in Europe since the beginning of the 2000s. The incidence has increased over the last 10 years from 2.45 cases per 10,000 patient-days in 2005 (Barbut et al. 2007), to 4.1 in 2008 (Bauer et al. 2011) and 7.0 in 2012–2013 (Davies et al. 2014). Nevertheless, the incidence of CDI varies widely across European countries from 0.7 to 28.7 per 10,000 patient-bed days per hospital. This trend is likely to result from a combination of several factors, including the level of awareness of CDI among physicians, the type of methods/algorithm for CDI diagnosis implemented in each country, and the global spread of the PCR ribotype (RT) 027 clone. A European study showed that there is still a substantial underdiagnosis of CDI coupled with large variations in testing policies among European countries (Davies et al. 2014).

In Europe, the hypervirulent epidemic RT 027 strain (or REA type BI/NAP1/toxinotype III) was first reported in England in 2005 (Smith 2005) and has since rapidly spread in other European countries. RT 027 is characterized by an 18 bp deletion and a deletion at position 117 in *tcdC* gene, resulting in the inactivation of the toxin repressor TcdC and higher amounts of toxin production (Warny et al. 2005), although the role of *tcdC* mutation in toxin overproduction is currently debated (Murray et al. 2009; Cartman et al. 2012). Moreover, epidemic 027 strains also produce an additional toxin, the binary toxin, and are resistant to erythromycin and moxifloxacin, which may have conferred a selective advantage. The same combination of genetic and phenotypic features can be found in other rare RT, such as RT 176 (Krutova et al. 2015; Drabek et al. 2015). RT 027-related CDI are associated with a higher rate of complications and recurrences (Sundram et al. 2009). The RT 027 has disseminated throughout Europe, with a clear shift in its regional repartition from United Kingdom and Ireland in 2008 (Bauer et al. 2011) to Eastern Europe in 2012–2013 (Davies et al. 2016b). Some countries have successfully controlled its spread and decreased its prevalence (Hensgens et al. 2009; Fawley et al. 2016), while other were recently hit by large outbreaks (Bouza et al. 2017). In addition, other virulent or unusual PCR ribotypes are emerging.

2 *C. difficile* Typing Methods

2.1 PCR Ribotyping

PCR ribotyping is the reference method for *C. difficile* typing in Europe. It relies on the presence of several alleles of the rRNA operon in the *C. difficile* genome. The length polymorphism of the intergenic spacer region between 16S and 23S rRNA genes results in RT-specific patterns after genomic amplification and migration (Bidet et al. 1999). PCR ribotyping was first developed using agarose gel electrophoresis, but the capillary gel-based electrophoresis method has now been widely adopted. The latter enables better standardization and easier comparison between laboratories and is recommended as the reference technique in Europe (Fawley et al. 2015).

Most European countries use a common nomenclature, but some laboratories developed their own local databases. An online database containing internationally recognised capillary electrophoresis RT profiles is available (WEBRIBO, https://webribo.ages.at/, Indra et al. 2008). However, there is no standardized protocol since several primer sets were published (Stubbs et al. 1999; Bidet et al. 1999), some of them enabling direct PCR ribotyping from stool samples (Janezic et al. 2011). Harmonization of the PCR ribotyping method and nomenclature is therefore essential and needs to be improved in Europe, in order to detect emergence of new unreferenced RT in a timely manner.

2.2 Other Methods Used for *C. difficile* Typing

Toxins A and B, which are considered as the main virulence factors of *C. difficile* (Pruitt and Lacy 2012), are encoded by *tcdA* and *tcdB* genes located within a locus of pathogenicity (PaLoc). The PaLoc also contains *tcdR* (positive regulator of toxin expression), *tcdE* (holin required for toxin secretion), and *tcdC* (potential negative regulator). The genetic polymorphism of the

PaLoc can be explored by toxinotyping, which is a PCR-restriction based method (Rupnik et al. 1998). Toxinotypes are defined according to differences in the PaLoc compared to the reference strain VPI 10463 (nonvariant toxinotype 0). To date, 34 toxinotypes have been described (Rupnik and Janezic 2016; http://www.mf.um.si/mf/tox/profile.html). Toxinotyping and PCR ribotyping are well correlated since most of the strains in a given RT have similar changes in the PaLoc and thus belong to a single toxinotype. The analysis of 123 strains showed that in a few cases, PCR ribotyping can be more discriminatory than toxinotyping, whereas RT include several toxinotypes less frequently (Rupnik et al. 2001). To avoid ambiguities, a revised toxinotyping nomenclature was recently published (Rupnik and Janezic 2016).

PFGE (Pulsed-field gel electrophoresis) is a genotype-based typing method developed in the 1980s and mostly used in North America. There is good concordance between results of PFGE and PCR ribotyping (Bidet et al. 2000). PFGE has a higher discriminatory power than PCR ribotyping (Killgore et al. 2008) but the interpretation of genetic relatedness is comparable between both typing methods. However, some strains are non-typeable with this method, and degradation of genomic DNA can hinder the analysis (Kristjánsson et al. 1994). PFGE is also very labour-intensive and the lack of standardisation makes inter-laboratory data comparison difficult.

The discriminatory power of PCR ribotyping is not sufficient to prove the nosocomial transmission of a strain, particularly when a RT is endemic at a regional or national level. In that case, another more discriminant typing method has to be used, such as multilocus variable-number tandem repeat (VNTR) analysis (MLVA). MLVA relies on the variability of the VNTR at different loci. The genetic relatedness of isolates is appreciated through the sum of tandem repeat number differences (STRD) (Marsh et al. 2006).

Whole genome sequencing (WGS) can distinguish between strains at the single nucleotide level, highly increasing the discriminatory power over other typing schemes. Given the transferability of data and the diversity of potential applications, such as comparative genome analysis and lineages analysis, this method is increasingly being used for *C. difficile* typing and could spread widely in the coming years (Knetsch 2013). WGS has successfully and rapidly identified transmission of healthcare-associated infection and could become a valuable tool in routine clinical practice (Eyre et al. 2012).

3 Global Distribution of *C. difficile* PCR Ribotypes in Europe

The European *C. difficile* infection study (Bauer et al. 2011) and the EUCLID study (Davies et al. 2014, 2016b) are two major European surveys aiming at describing the epidemiology of CDI including prevalence, diagnosis and RT distribution.

The first pan-European study on *C. difficile* was performed in 2008 in 106 laboratories from 34 countries (Bauer et al. 2011). The incidence of CDI and the RT distribution varied greatly between hospitals, as well as the density testing for CDI. The authors could differentiate 65 RT among 389 *C. difficile* isolates. One of the main findings of this study was that RT 027 was not predominant in 2008, representing only 5% of the isolates. The most common RT were 014/020 (16%), 001, (9%), and 078 (8%). Some RT seemed to spread regionally, such as RT 106 mostly described in UK and Ireland.

The EUCLID study (European, multicentre, prospective, biannual, point-prevalence study of CDI in hospitalized patients with diarrhoea) was conducted in 2012–2013 and included 482 hospitals from 19 European countries (Davies et al. 2016b). The objectives were to measure the underdiagnosis of CDI and to assess the diversity of RT repartition in Europe. During two sampling days (one in winter and one in summer), participating hospitals sent every diarrhoeal stool sample, irrespective of the request to test for *C. difficile* by the physician, to a national coordinating laboratory. The RT diversity was much higher than in the previous study, with 125 RT identified among 1196 isolates.

Interestingly, the most common RT was 027 (19%), highlighting the rapid spread of this strain at a global scale. An inverse correlation was noted between the rate of testing and prevalence of ribotype 027 across north, south, east, and west quadrants of Europe, which suggests that increased awareness of CDI and use of optimum testing methods and policies can reduce the dissemination of epidemic strains (Davies et al. 2014). The comparison with the 2008 data indicated a shift in the frequency of RT 027 from UK and Ireland (decreasing prevalence) to Eastern Europe countries (increasing prevalence). RT 001/072 (11%) and 014/020 (10%) were the second and third most prevalent RT, consistent with the 2008 results, however the prevalence of RT 078 dropped from 8% in 2008 to 3% in 2012–2013. The distribution of causative RT was country-specific as shown in the Fig. 1 (Davies et al. 2016b).

Besides these two large epidemiological studies, several other recent European studies analysed RT distribution at a national level. The results of these national studies are summarized in the Table 1.

A multicentre study characterized 3333 toxigenic strains isolated between 2010 and 2015 in 110 Belgian hospitals (Neely et al. 2017). RT 027 (4.2%) and 078 (7.0%) were associated with a higher rate of complications (unadjusted data) and higher levels of *in-vitro* toxin production from cultured isolates.

A study compared epidemiological data for community-associated (CA)-CDI and healthcare-associated (HA)-CDI in 113 laboratories across England between 2011 and 2013 (Fawley et al. 2016). A total of 703 *C. difficile* toxin-positive faecal samples from CA-CDI cases were analysed and the results were compared to HA-CDI data (n = 10,754) obtained from the *C. difficile* Ribotyping Network. RT distribution was similar in cases of CA- and HA-CDI, but RT 002 was more likely to cause CA-CDI, while RT 027 was more often associated with HA-CDI.

In Spain, Alcalá et al. performed *C. difficile* cultures on 807 unformed stool specimens sent to 118 Spanish microbiology laboratories on a single day, regardless of the prescription by the clinician (Alcalá et al. 2012). Among 42 toxigenic strains, RT 014/020, 001 and 078/126 were the most prevalent (20.5%, 18.2% and 18.2% respectively). RT 027 was not found.

The characterization of 498 clinical isolates from 20 hospitals in Portugal showed that RT 027 was predominant with 18.5% of all the strains, and 19.6% of HA-associated CDI. RT 014 was the second most frequent overall (9.4%) and the most frequent among CA-CDI (12%). The prevalence of RT 126 and 078 was low (3.8% and 2.8% respectively) (Santos et al. 2016). The authors described a great heterogeneity of the RT distribution through the country with a higher diversity in the north, where RT 027 was not predominant.

The geographic distribution of *C. difficile* genotypes in Germany was assessed using 393 isolates sent to the national advisory laboratory for diagnostic reason between 2011 and 2013 (von Müller et al. 2015). The typing method used was surface-layer protein A sequence typing, with strain assignment to RT for better comparison with international data. RT 001 (35%) and 078 (8%) were prevalent nationwide; RT 027 (26%) and 014/066 (9%) were detected in almost all regions.

In France, a multicentre study conducted in 2009 in 78 healthcare facilities showed that the most prevalent RT were 014/020/077 (18.7%), followed by 078/126 (12.1%) (Eckert et al. 2013). The prevalence of RT 027 strains remained low (3.1%), and they were only isolated in Northern France, where RT 027 emergence was first described in 2006 (Coignard et al. 2006; Birgand et al. 2010). These results are consistent with the more recent LuCID (Longitudinal European *Clostridium difficile* Infection Diagnosis) surveillance study (Davies et al. 2016a), during which RT 014/020/077 and 078/126 were the most prevalent in France (21.9% and 9.5% respectively) (Eckert et al. 2015).

In conclusion, RT 014/020 and 001/072 are endemic in almost all European countries while there is a national or regional specificity for other RT. Moreover, the RT diversity is significantly increasing across Europe.

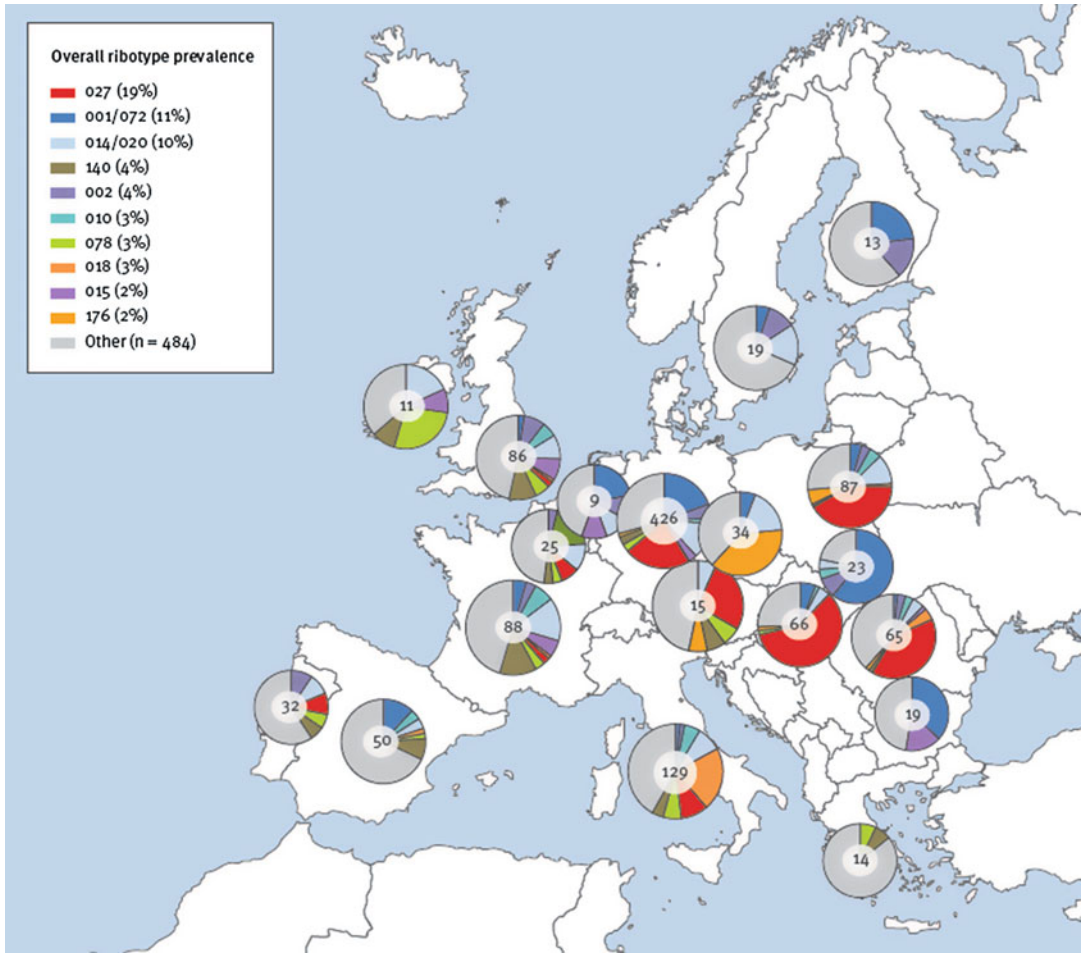

Fig. 1 Geographical distribution of *C. difficile* PCR ribotypes, by participating European country, EUCLID 2012–2013 and 2013 (n = 1196) (Reproduced with permission from Davies et al. 2016b) Pie charts show the proportion of the most common ribotypes per country and the number in the centre of the charts is the number of typed isolates in the country

4 Emerging PCR Ribotypes

4.1 PCR Ribotype 176

RT 176 strains are closely related to RT 027 (Stabler et al. 2006). They belong to toxinotype III, produce the binary toxin and bear a deletion at position 117 of the *tcdC* gene, leading to a potential RT 027 misidentification with commonly used molecular assays such as Xpert® *C. difficile* (Cepheid). Moreover, their similar banding pattern (only one band difference) after gel electrophoresis can be confusing for RT attribution (Valiente et al. 2012).

The first cases of RT 176-associated CDI were described in 2008 in Poland (Obuch-Woszczatyński et al. 2014), in 2009 in the Czech Republic (Nyč et al. 2011) and in 2015 in Croatia (Rupnik et al. 2016). The first RT 176-related outbreak was recently described in France (Couturier et al. 2017). Four strains isolated in two geographically close hospitals, previously identified as RT 027 with the agarose gel method, were reassigned as RT 176 by capillary gel-based electrophoresis. MLVA analysis showed that those four strains formed a clonal complex (STRD $\leq$2), and were genetically related to RT 027 strains (STRD $\leq$10).

Table 1 National epidemiological studies on *Clostridium difficile* PCR ribotypes repartition

Country	N strains	PCR ribotyping method	Most prevalent RT (%)	References
Belgium	3333	Agarose gel electrophoresis	014 (11.6), 020 (8.5), 002 (7.6), 078 (7.0), 027 (4.2), 005 (3.5), 106 (3.4)	Neely et al. (2017)
United Kingdom	11,457	Agarose gel electrophoresis	015 (10.2), 002 (9.1), 014 (9.1), 078 (8.0), 005 (7.4) and 027 (6.4)	Fawley et al. (2016)
Spain	42	Agarose gel electrophoresis	014/020 (20.5), 001 (18.2), 078/126 (18.2)	Alcalá et al. (2012)
Portugal	498	Capillary electrophoresis	027 (18.5), 014 (9.4), 020 (5.6), 017 (5.2)	Santos et al. (2016)
Germany	393	slpAST with assignment to RT	001 (35), 027 (26), 014/066 (9), 078 (8)	von Müller et al. (2015)
France	224	Agarose gel electrophoresis	014/020/077 (18.7), 078/126 (12.1), 015 (8.5), 002 (8), 005 (4.9)	Eckert et al. (2013)
Czech Republic	774	Capillary electrophoresis	176 (29), 001 (24)	Krutova et al. (2016)

slpAST surface-layer protein A sequence typing

The results of the EUCLID study showed a regional specificity of RT 176, isolated mostly in the Czech Republic where it accounted for 38% of the strains (Davies et al. 2016b). In 2014, a study among 18 Czech hospitals showed that 29% of *C. difficile* isolates belonged to RT 176, and 24% to RT 001 (Krutova et al. 2016, Table 1). Further typing analysis by MLVA, indicated that both RT formed clonal complexes in several hospitals, suggesting a rapid spread of these clones at a national level.

These results suggest a rapid nosocomial spread of RT 176 strains through Europe, stressing the need for a common data base for PCR ribotyping.

4.2 PCR Ribotype 078

RT 078 strains can produce toxins A and B, as well as the binary toxin and belong to toxinotype V. They are characterized by a 39 bp deletion in *tcdC*. RT 078 was reported as predominant in Greece in 2005 (Barbut et al. 2007), and was the third most common RT in the 2008 European study (Bauer et al. 2011). A recent study showed that RT 078 strains co-circulate with the hypervirulent 027 strains in Southern France (Cassir et al. 2017). While 027 strains are mostly responsible for outbreaks of HA-infections in the elderly, 078 strains are more frequently associated with CA-infections in a younger population. CA-CDI due to 078 strains were also described in England (Fawley et al. 2016) (see "*Clostridium difficile* infection in the community" below). Finally, RT 078 strains are frequently resistant to fluoroquinolones and erythromycin, partly explaining this epidemiological success (Baldan et al. 2015).

4.3 PCR Ribotype 126

RT 078 and 126 are highly related: they share similar banding patterns in agarose gel electrophoresis method, and can only be differentiated with the capillary gel-based electrophoresis. Consequently, they are often reported together as RT 078/126. Like RT 078 strains, RT 126 strains belong to toxinotype V and are considered as "hypervirulent" (Knetsch et al. 2011). They also produce the binary toxin and are characterized by a 39 bp deletion in *tcdC*.

The prevalence of RT 126 strains in animals in Germany is high, suggesting the potential zoonotic spread of this RT (Schneeberg et al. 2013). MLVA analysis showed that most of those strains are genetically related to RT 078 strains (STRD $\leq$10), and some of them belong to the

same clonal complex (STRD $\leq$2). RT 126 strains are also frequently resistant to antibiotics, including erythromycin, moxifloxacin and tetracyclin (Álvarez-Pérez et al. 2017).

4.4 PCR Ribotype 033/Toxinotype XI

PCR ribotype 033 strains belong to toxinotype XI. They are characterized by the absence of TcdA and TcdB expression and therefore cannot be detected by EIA (enzyme immunoassay) methods for toxins. These strains were first described in 2001 (Rupnik et al. 2001). In 2014, six symptomatic CDI cases due to toxinotype XI strains were reported by the French National Reference Laboratory for *C. difficile* (Eckert et al. 2014). In four cases, the patient was successfully treated by oral metronidazole. These strains were characterized by PCR ribotyping, amplification of *tcdA, tcdB, cdtA* and *cdtB* genes and toxinotyping. The six strains were defined as RT 033 (or 033-like) and were negative for TcdA and TcdB. The binary toxin genes were present and a 39 bp deletion was identified in the *tcdC* gene. The six strains were characterized by major deletions of the 5′ region of the PaLoc including *tcdB, tcdE* and *tcdR*; only a remnant part of *tcdA* (A2 and A3 fragments) and *tcdC* could be amplified.

The pathogenicity of toxinotype XI strains remains controversial. Studies on the role of the binary toxin as a virulence factor in animal models gave contradictory results. In the rabbit ileal loop model, an enterotoxic response was observed after inoculation of supernatants from culture of $A^-B^-CDT^+$ strains. However, despite colonization, no symptoms occurred in clindamycin-treated hamsters challenged with these strains (Geric et al. 2006). Although the prevalence of $A^-B^-CDT^+$ strains in Europe seems rather low (Barbut et al. 2007; Bauer et al. 2011), surveillance of this unusual strains is required. Indeed, the atypical genomic organization of the PaLoc can lead to a false negative diagnosis, more particularly when methods relying on the presence of toxin A and/or toxin B only are used. However, the increasing use of the Xpert® *C. difficile* assay, which detects binary toxin genes, will possibly enable a better identification of toxinotype XI strains.

4.5 PCR Ribotype 018

RT 018 has recently been reported as an emerging RT responsible for outbreaks in Italy, where RT 126 was previously predominant (Spigaglia et al. 2010). The EUCLID study (Davies et al. 2016b) showed that prevalence of RT 018 was high in Italy (22%), as opposed to other European countries. In addition, Baldan et al. characterized 312 *C. difficile* isolates from a large Italian teaching hospital between 2009 and 2013, and observed that RT 018 was predominant. After epidemiological investigation of the outbreaks, RT 018 represented 42% of index CDI cases and virtually all secondary cases (due to nosocomial transmission). The transmission index (number of secondary cases divided by number of index cases) of RT 018 was significantly higher than that of RT 078 (0.640 and 0.0606, respectively) (Baldan et al. 2015). Another study comparing RT 018, RT 126 and RT 078 demonstrated that RT 018 strains produced higher levels of toxins, showed increased adhesion to cells and became endemic in a short time (Barbanti and Spigaglia 2016). Moreover, RT 018 strains were all multidrug resistant (resistance to erythromycin, clindamycin and moxifloxacin). Together, these results suggest that RT 018 strains have phenotypic traits conferring an adaptive advantage and are able to spread widely. RT 018 strains were indeed reported in Southern Europe (Spain, Austria and Slovenia) and are associated with a higher rate of complicated infections (Bauer et al. 2011).

4.6 PCR Ribotype 017

RT 017 strains belong to toxinotype VIII and are part of *C. difficile* clade 4; they lack toxin A production and binary toxin genes (Cairns et al. 2012). The clinical relevance and the prevalence of this clone has been unclear for many years,

since it was mainly found in asymptomatic infants (Depitre et al. 1993; Kato et al. 1998). However, it has now been established that RT 017 strains are predominant in Asian countries such as Korea, China and Japan (Collins et al. 2013), and that they have spread worldwide. RT 017-related outbreaks have been reported in England (Cairns et al. 2015), The Netherlands (Kuijper et al. 2001), Poland (Pituch et al. 2001), and Ireland (Drudy et al. 2007). RT 017-related CA-CDI appear to be more likely to affect younger patients (Fawley et al. 2016). Severe RT 017-related CDI have been described in Germany, although RT 027 was the most prevalent strain in this study (Arvand et al. 2009).

4.7 Other Emerging PCR Ribotypes

RT 244 strains belong to the same hypervirulent clade as RT 027 (clade 2) (Lim et al. 2014). They produce the binary toxin and bear a single nucleotide deletion at position 117 in *tcdC*. Severe CA-CDI and outbreaks due to RT 244 strains were recently reported in Australia and New Zealand, where it was previously uncommon (De Almeida et al. 2013; Eyre et al. 2015). Eyre et al. showed that a strain isolated in a patient recently returned from Australia to the UK was phylogenetically related to their outbreak, highlighting the potential rapid spread of RT 244 via international travel.

The previously quoted French multicentre survey showed that among 224 toxigenic strains, 19 (8.5%) belonged to RT 015 which was the third most frequent RT (Eckert et al. 2013). Fawley et al. showed that RT 015 was also predominant in England (Fawley et al. 2016). Although RT 015 accounted for only 2% of the strains analysed in the EUCLID study, it seems that RT 015 strains can spread and become predominant at a national scale.

RT 106 strains represented 5% of all toxigenic isolates in the 2008 hospital-based European study, but their distribution showed a regional spread: among 20 strains, 13 were isolated in the United Kingdom and 5 in Ireland (Bauer et al. 2011). In a Southern England healthcare facility, 38% of *C. difficile* isolates (n = 97) were identified as RT 106, the second most prevalent RT after 027 (45%) (Sundram et al. 2009). Almost all of these RT 106 strains were resistant to ciprofloxacin and erythromycin. Moreover, in the Belgian multicentre study (Neely et al. 2017), recurrences were more frequent with RT 106-related CDI.

Other data reported the emergence of RT 001 strains with reduced susceptibility to metronidazole, raising concerns about the potential spread of these strains due to this selective advantage (Baines et al. 2008). In Southern Germany, the prevalence of RT 001 strains exhibiting resistance to erythromycin, ciprofloxacin and moxifloxacin is high in both in- and out-patients (Borgmann et al. 2008; Arvand et al. 2009).

Given their pathogenic and epidemic potential, the emergence of these RT should be closely followed in European countries.

The genetic and epidemiological features of the emerging RT described above are summarized in the Table 2.

4.8 Emerging Strains with a A+B-CDT- Unusual Profile

Recently, three clinical strains with an atypical PaLoc structure were described in France (Monot et al. 2015), including the first variant strain producing only toxin A ($A^+B^-CDT^-$). WGS analysis of this strain showed that its PaLoc only contained *tcdA* and *tcdR*. None of the three strains belonged to any of the most frequent RT. Moreover, the authors described variability in the sequence of the toxin genes, which may lead to potential false negative results with the most commonly used diagnostic methods (immunoenzymatic or molecular assays).

Table 2 Characteristics of currently circulating and emerging PCR ribotypes in Europe

RT	Toxinotype	Toxins A and B	Binary toxin	Deletion in *tcdC*	Main circulation area
027	III	+/+	+	−18 bp/ Δ117	Europe, mostly Eastern Europe Davies et al. (2016b)
176	III	+/+	+	−18 bp/ Δ117	Poland, Czech Republic Nyč et al. (2011), Obuch-Woszczatyński et al. (2014)
078	V	+/+	+	−39 bp/ A117T	Community-onset infections Eckert et al. (2011), Fawley et al. (2016)
126	V	+/+	+	−39 bp/ A117T	Eckert et al. (2011)
033	XIa/XIb	−/−	+	−39 bp	Low prevalence in Europe Eckert et al. (2014)
018	XIX	+/+	−	ND	Italy Spigaglia et al. (2010), Rupnik and Janezic (2016)
017	VIII	−/+	−	ND	Asia Collins et al. (2013), Ireland Drudy et al. (2007), England (Cairns et al. (2015), The Netherlands Kuijper et al. (2001), Poland Pituch et al. (2001), Germany
244	IXb	+/+	+	ND/ Δ117	Australia Lim et al. (2014), Rupnik and Janezic (2016)
015	NA	+/+	−	−18 bp or ND	France Eckert et al. (2013)
106	NA	+/+	−	−18 bp or ND	United Kingdom, Ireland Bauer et al. (2011)
001	XXIX	+/+	−	ND	Germany, multidrug resistant strains Borgmann et al. (2008), Rupnik and Janezic (2016)

ND not deleted, *NA* not available

5 *C. difficile* Infection in the Community

The epidemiology of CA-CDI is poorly known, since *C. difficile* testing is rarely requested in stool samples from community patients. However, recent data suggest that the incidence of CA-CDI is rising (Chitnis et al. 2013). In addition, CDI were recently described among young patients from community settings without the traditional risk factors (antibiotic exposure, recent hospitalization, co-morbidities) (Wilcox et al. 2008; Gupta and Khanna 2014).

Fawley *et al.* showed that RT 002, 020 and 056 were largely responsible for CA-CDI, whereas RT 027 was most associated with HA-CDI (Fawley et al. 2016). RT 078 strains have been reported in animals in the Netherlands (Goorhuis et al. 2008), and by using MLVA analysis, Debast *et al.* showed that RT 078 strains found in animals and in humans were genetically highly related, suggesting a foodborne interspecies transmission of *C. difficile* (Debast et al. 2009). In Canada, RT 078 epidemic strains (identified as pulsotype NAP7 by PFGE) were found in vegetables from grocery stores (Metcalf et al. 2010). RT 078 has also been described in the environment; it was the most frequently isolated RT in wastewater treatment plants in Switzerland (Romano et al. 2012). RT 078 was the commonest (19.0%) in 42 CA-CDI cases in a prospective study conducted in Scotland, followed by RT 014/020 (16.7%), 015 (14.3%) and 001 (11.9%) (Taori et al. 2014). However, in a US study of 984 CA-CDI cases, NAP1/RT 027 was the most frequent strain isolated (21.7%), while less than 7% of the isolates belonged to NAP7/RT 078 (Chitnis et al. 2013). In 2011, population- and laboratory-based surveillance for CDI was conducted in 10 US areas (Lessa et al. 2015). A total of 1364 strains were characterized. The most common strains were NAP1/RT 027 (18.8% of CA-CDI and 30.7% of HA-CDI), NAP4/RT 020 (11.4% and 10.3%) and NAP11/RT 106 (10.7% and 10.0%). Less than 4% of the strains in both settings belonged to NAP7/RT 078. These

results show a large overlapping of the RT distribution in HA- and CA-CDI, suggesting the existence of common reservoirs and multiple transmission routes between community and hospital settings.

6 Conclusion

In conclusion, there is a large diversity of RT across Europe, although some specific RT are able to disseminate at a regional or national level. A national and European clinical surveillance system, associated with microbiological characterization of strains, is essential in order to monitor the constantly changing epidemiology of CDI. A common European data base of the circulating RT would be very helpful to detect emergence of new virulent clones in a timely manner.

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