Established in 1871
Swiss Medical Weekly
Formerly: Schweizerische Medizinische Wochenschrift
The European Journal of Medical Sciences

Review article: Medical intelligence | Published 10 September 2014, doi:10.4414/smw.2014.13995
Cite this as: Swiss Med Wkly. 2014;144:w13995

Molecular epidemiology of *Clostridium difficile* for clinical practice

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Summary

During the last decade, the world has witnessed numerous outbreaks of *Clostridium difficile* infections (CDI) in healthcare settings. Increasing incidence rates of CDI have been mainly attributed to the successful global spread of a more virulent strain of *C. difficile* – namely PCR-ribotype 027. More recent studies, however, point to the emergence of other ribotypes as the main cause of the continuing epidemic. This review summarises the current literature on the molecular epidemiology of *C. difficile* as well as its implications for clinical practice.

Key words: *Clostridium difficile; PCR-ribotype 027; PCR-ribotype-078; molecular epidemiology*

Methodology

The search engine PubMed (www.pubmed.com) was used to identify publications. Reference lists of selected reports were searched to identify additional publications.
Publications were collected from 1980–2014. Studies published in English were identified and results of publications written in other languages were included if their abstracts were in English. Search terms included "*Clostridium difficile*", "*C. difficile*", "epidemiology", "ribotype", "strain", and "North American pulsed field type".

Global spread of different *C. difficile*-ribotypes

Clostridium difficile is the most frequent cause of healthcare associated diarrhoea resulting in significant morbidity and mortality among hospitalised patients [1]. In the United States, diagnoses of CDI doubled from 31/100,000 discharges in 1996 to 61/100,000 in 2003 – the overall rate during this period being several-fold higher in persons greater than 65 years of age [2]. Concurrently, an epidemic of CDI associated with a high case-fatality rate emerged in Canada in 2002 [3]. These developments were largely attributed to the emergence of a more virulent strain of *C. difficile* – namely PCR-ribotype 027 causing more severe and recurrent CDI [4, 5]. First reports of the further spread of this ribotype to the European continent derived from Great Britain in 2005 [6] and consecutively from the Netherlands [7]. Over time, numerous European countries – including Switzerland [8] – reported epidemics [9]. Evidence for the worldwide spread of this organism derives from reports of PCR-ribotype 027 from different Asian countries [10–12], Australia [13] and South America [14].
The successful establishment of PCR-ribotype 027 in healthcare settings has been attributed to its fluorochinolone-resistance, toxin-hyperproduction and the potential of this strain to produce binary toxin, possibly explaining more severe and recurrent cases of CDI. Fluorochinolone-resistance may have played a crucial part in the distribution of this strain as the increasing use of these antibiotic agents in highly susceptible patients precipitated the rapidly emerging international epidemic [15]. Overcrowding of hospitals and understaffing may have additionally contributed. To understand the evolution of PCR-ribotype 027, historic non-endemic 027 *C. difficile* strains were compared to recent epidemic strains by performance of comparative genome and phenotypic analysis. The genome of the epidemic PCR-ribotype 027 strain has five additional genetic regions compared with its historic counterpart (i.e., non-epidemic 027 strain) including a novel phage island, a two component regulatory system and transcriptional regulators possibly contributing to its successful emergence [16].
After its recognition as one of the principal drivers of the ongoing CDI-epidemic, ribotype 027 still accounts for the majority of all hospital-acquired CDI cases (62.7%) in Canada – this strain being predominant among patients with CDI, whereas asymptomatic patients were more likely to be colonised with other strain-types [17]. A comprehensive survey on the distribution of different *C. difficile* ribotypes was performed in 34 different European countries in 2008 [18]. Incidence of CDI was 4.1 per 10'000 patient-days, ranging from 0.0–36.3 and varying across hospitals. Interestingly, PCR-ribotype 027 only accounted for 5% of all strains, other ribotypes – most commonly 014/020, 001 and 078 being predominant in European hospitals [18]. These results differed from a previous report demonstrating a predominance of PCR-ribotypes 001 and 014 followed by 027 and 020 in 14 European countries [19]. PCR-ribotype 078 was the third most commonly identified strain correlating with reports on the emergence of this ribotype

in the Netherlands [20]. Patients infected with PCR-ribotype 078 present with similar disease severity as patients infected with PCR-ribotype 027, but are younger and more commonly diagnosed with community-associated disease [20]. Table 1 summarises the global distribution of the two hyper-virulent PCR-ribotypes 027 and 078.

It is likely that other epidemic strains of *C. difficile* will emerge in the future [21] owing to the highly fluid genome of this pathogen, resulting in its adaptability to environmental changes. In a study on the molecular epidemiology of *C. difficile* over a 10–year course at a tertiary care hospital, years with high CDI-incidence were associated with large clusters of specific strains that changed yearly. The molecular epidemiology of CDI in this hospital was characterised by a wide diversity of *C. difficile* types and an ever-changing dominance of specific *C. difficile* types over time – leading to the conclusion that the molecular epidemiology of CDI is expected to continuously evolve [22].

The role of different ribotypes in community-associated disease

CDI is classified as community-associated in up to 41% of all cases [23]. In a population-based study, patients with community-acquired CDI were significantly younger, had lower co-morbidity scores, and lower rates of antibiotic exposure [23]. These findings have been supported by further studies reporting that otherwise healthy people without prior exposure to antibiotics [24, 25], peripartum women [26–28] and children [29] are increasingly at risk for CDI. Few studies have analysed the correlation of such trends with the circulation of more virulent *C. difficile* ribotypes in the community. A recently published article aimed to identify epidemiological and clinical characteristics of community-associated CDI and to explore potential sources of *C. difficile* acquisition in the community [30]. A total of 984 patients from eight US states diagnosed with community-associated CDI were included and North American pulsed-field gel electrophoresis (NAP) 1, corresponding to PCR-ribotype 027 was the most commonly (21.7%) isolated strain. Exposure to antibiotics during the

Table 1: Global distribution of the hyper-virulent *Clostridium difficile* ribotypes 027 and 078.

Strain	Distribution	
PCR-ribotype 027	**Asia**	China [10] Japan [11] Korea [12] Singapore [63]
	Australia [13]	Australia
	Europe [9]	Austria Belgium Denmark Finland France Germany Ireland Luxembourg Netherlands Norway Poland Spain Sweden Switzerland United Kingdom
	Middle East	Israel [64]
	North America	Canada [4] United States [5]
	South America	Costa Rica [14]
PCR-ribotype 078	**Europe** [18]	Austria Belgium Denmark Hungary Ireland Italy France Germany Greece Netherlands Norway Portugal Slovenia Spain Switzerland United Kingdom
	Middle East	Iran [65] Kuwait [66]
	Australia	New Zealand [67]

last 12 weeks prior to diagnosis of CDI was not reported for 35.9% of all patients and only 18% had no outpatient exposure. Patients having CDI with no or low-level outpatient health care exposure were more likely to be exposed to infants younger than 1 year and to household members with active CDI compared with those having high-level outpatient health care exposure [30]. These findings were also supported by one European study suggesting that PCR-ribotype 027 is most commonly acquired in the community [31]. Potential reservoirs and vectors for community-associated CDI may include colonised asymptomatic infants [32]. A recent study reported an acquisition rate of 100% in infants followed during their first year of life from the age of 6 months [32]. Food borne transmission may be an additional explanation for increasing rates of community-associated CDI and affection of populations previously considered being at low risk. *C. difficile* has been recovered from retail meat and food-animals in Europe [33] – including Switzerland [34], the United States [35] and Canada [36]. *C. difficile* PCR-ribotype 078 has been isolated from piglets with diarrhoea possibly suggesting ongoing transmission by introduction to the food chain, as isolates from humans and pigs were found to be highly genetically related [20]. A community component to ribotype 078 infections was also reported in a recent study deriving from Northern Ireland, where almost a 1:1 ratio of cattle to humans and a large pig population are present [37].

Associations between different ribotypes with disease severity and recurrence

Disease severity

The emergence of PCR-ribotype 027 not only resulted in an overall increase of CDI-incidence in many industrialised countries, but was also associated with an alarming increase in disease severity. High case-fatality rates were reported from Canada [3, 38, 39] and increasing colectomy- and death rates were reported from the United States [40]. These reports were mirrored by studies emerging from Europe also relating to higher case-fatality rates than previously experienced [15, 41]. In the UK, a six-fold increase in CDI-related mortality was observed from 1999–2006 [15]. In the Canada-wide CDI study performed in 2005 by the Canadian Nosocomial Infection Surveillance Program, 12.5% of the infections due to the NAP1 strain (corresponding to PCR-ribotype 027) resulted in a severe outcome, compared with only 5.9% of infections due to the other types. Patient's age was strongly associated with severe outcome, and patients 60–90 years of age were approximately twice as likely to experience a severe outcome if the infection was due to NAP1, compared with infections due to other types [42]. In a recent study from the Netherlands, high mortality rates were reported for patients infected with ribotype 027 – however, underlying conditions were not accounted for [43]. In contrast, in a study performed at a teaching hospital in the United Kingdom assessing the relationship between strain type, clinical factors and outcome, strain type was not associated with any outcome measure [44]. This result is supported by the fact that the mortality rate among patients included in this study was typical of studies in which outbreaks of PCR-ribotype 027 have been reported [44]. More recent data deriving from the United States have also challenged the concept of disease severity being linked to strain-type. In a cross-sectional study including 310 independent cases of CDI, the association between ribotypes 027 and 078 and severe CDI was not significant after correcting for any of the other clinical covariates. After full adjustment, severe cases were predicted only by patients' white blood cell count and albumin level. This result was supported by analysis of a validation data set containing 433 independent CDI cases [45] – an approach clearly underscoring the robustness of these findings. The authors concluded that presence of binary toxin or tcdC mutations may not be useful to guide patient care. No associations of ribotypes 027 and 078 with more complicated CDI were observed in the large European survey including 389 patients with CDI and characterisation of their respective strain-type [18]. However, PCR ribotypes 018 and 056 were significantly associated with complicated disease outcome after adjustment for possible confounders. This association has not been shown previously and the results should be interpreted with caution given the small number of cases with complicated CDI in the cohort [18]. A recent study from Austria confirms the lack of association between different ribotypes (including ribotype 027) and disease severity [46]. An outbreak with two different *C. difficile* PCR-ribotypes (017 und 027) occurred in the Netherlands providing a unique opportunity to asses ribotype-specific outcomes [47]. Thirty-day mortality rates were strikingly higher in patients infected with one of the two outbreak strains (23% for PCR-ribotype 017 and 26% for PCR-ribotype 027) compared to patients infected with other ribotypes (3%). This result is surprising as PCR-ribotype 017 does not harbour the gene encoding for toxin A and contains none of the proposed virulence markers typical for ribotype 027 [47]. One possible explanation provided by the authors may be that both ribotypes 017 and 027 are markers for underlying disease severity. Possibly, more severely ill patients are at higher risk for transmission of *C. difficile* in outbreak settings.

Increased disease severity associated with the emergence of ribotype 027 is thought to be related to binary toxin production and genetic mutations in a toxin regulator gene (tcdC) resulting in hyperproduction of toxins A and B [48, 49] – the primary virulence factor of *C. difficile* [50, 51]. A more recent study, however, suggests that PCR-ribotype 027 isolates do not produce more toxins *in vitro* than other strains but produce significantly more spores, which may in itself explain the successful spread of this ribotype in healthcare settings [52]. Similarly, functional status of tcdC based on nucleotide sequencing does not necessarily correlate with disease severity [53]. Another study found deletions in tcdC genes being common among *C. difficile* isolates but not associated with more severe disease [54].

The role of binary toxin in conferring virulence and contributing to disease severity remains conflicting. Higher mortality rates have been reported for patients infected with binary toxin-producing strains – however, it remains unclear if it is just a marker for more virulent strains or if it contributes directly to disease severity [55].

In conclusion, most of the evidence regarding increased virulence of specific ribotypes derives from settings in which such strains emerged rapidly, often resulting in outbreaks. In endemic settings, the association of disease severity has not been confirmed.

Recurrence

High rates of recurrence – defined as an episode of CDI occurring within 8 weeks after the onset of a previous episode, provided that CDI symptoms from the earlier episode resolved, with or without therapy are one of the hallmarks of CDI and approximately 20% of patients are affected [56]. Two phase 3 treatment trials with the same study design compared the safety and efficacy of fidaxomicin compared to vancomycin, and the combined study population from both trials was analysed with respect to treatment outcomes in relation to different *C. difficile* strain types [57]. Recurrence rates for patients infected with the B1 strain (corresponding to PCR ribotype 027) were higher than for patients infected with other strain types (27.4% versus 16.6%).

Suggested associations between different ribotypes with disease severity and recurrence are summarised in table 2.

Ribotypes and treatment of *C. difficile* infection-implications for clinical practice

Guidelines issued by both American (The Infectious Diseases Society of America (IDSA), the Society for Healthcare Epidemiology of America (SHEA)) [58] and European societies (European Society of Clinical Microbiology and Infectious Diseases [ESCMID]) [59, 60] do not make specific recommendations regarding treatment-adaption in accordance to different *C. difficile* ribotypes. All societies, however, recommend treatment with vancomycin instead of metronidazole for patients diagnosed with severe CDI. Given the conflicting data on the associations of different ribotypes with disease severity, it seems prudent to tailor treatment to clinical presentation rather than strain characteristics.

Regarding recurrent CDI, treatment with Fidaxomicin was associated with a significantly lower rate of recurrence of *CDI* associated with non–North American Pulsed Field type 1 strains. These findings, however, need to be confirmed [60, 61]. Treatment with monoclonal antibodies against *C. difficile* toxins A and B tended to show lower recurrence rates for patients infected with PCR ribotype 027 [62]. No specific recommendations – taking specific ribotypes into account – can be made at this point regarding further treatment options for CDI as fecal microbiota transplantation. The European Society (ESCMID) states that the value of the PCR ribotype as a prediction marker for disease severity may be limited, as the ribotype involved in an infection is commonly not known upon diagnosis. However, in an epidemic situation the PCR ribotype may be taken into account in deciding on the choice of empirical treatment regimen [60].

Conclusions

The molecular epidemiology of *C. difficile* is expected to continuously evolve. Ongoing surveillance of the distribution of different ribotypes and their independent associations with disease severity and outcome are crucial to drive future recommendations regarding prevention of CDI.

Community-associated CDI accounts for a high proportion of all CDI cases also affecting patients previously considered as being at low risk. Food borne transmission may be an important source for infection in this setting and requires further investigation.

Most of the evidence regarding increased virulence of specific ribotypes derives from settings in which such strains emerged rapidly, often resulting in outbreaks. In endemic settings, associations of disease severity with specific ribotypes are conflicting and require further confirmation.

Given the conflicting data on the associations of different ribotypes with disease severity, it seems prudent to tailor treatment to clinical presentation rather than strain characteristics. In epidemic settings, PCR ribotype may be taken into account for decisions on empirical treatment regimens.

Funding / potential competing interests: No financial support relevant to this article was reported. S. Tschudin-Sutter is a member of the Fidaxomycin Advisory Board (Astellas) and received an unrestricted educational grant from Pfizer International Operations. Further, she is a recipient of a Swiss National Foundation grant.

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Table 2: Suggested associations between different ribotypes with disease severity and recurrence.

Ribotype	Disease severity	Recurrence
PCR-ribotype 017	X	
PCR-ribotype 018	X	
PCR-ribotype 027	X	X
PCR-ribotype 056	X	
PCR-ribotype 078	X	

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