Clostridium difficile Ribotyping Network (CDRN) for England and Northern Ireland
2015-2018
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Clostridium difficile Ribotyping Network (CDRN) for England and Northern Ireland 2015-2018

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Introduction

Since the introduction of The Clostridium difficile Ribotyping Network (CDRN) for England and Northern Ireland CDRN, coincident with the peak incidence of C. difficile infection (CDI) in England (and the UK), rates have fallen markedly.¹ CDRN continues to respond to a major public health need, by providing a molecular epidemiological service that enhances our understanding of C. difficile, which is recognised as a global threat.² CDI case fatality rates have also declined, notably in line with control of the epidemic ribotype C. difficile 027.³⁻⁵ It is not possible to determine which interventions have been particularly responsible for the decreased incidence of CDI and associated deaths. However, it is plausible that access to the ribotyping and enhanced fingerprinting results provided by CDRN have facilitated improved local investigation and control of CDI cases, clusters and outbreaks. CDRN has certainly contributed to a much improved understanding of the epidemiology of CDI, and its scope/coverage is unrivalled worldwide.

Samples are submitted to CDRN according to local clinical need. We aim to provide results within two weeks of sample receipt. We believe that the timely data provided by CDRN has enabled healthcare institutions to respond to changes in CDI presentation and/or incidence. We encourage all hospitals to consider submitting samples according to the CDRN criteria so that they can be best placed to continue to prevent and control CDI.

The CDRN currently comprises the following participating laboratories:

- Leeds (Leeds General Infirmary) [Yorkshire & Humber]; CDRN Reference Laboratory.
- Birmingham (Heartlands Hospital) [West & East Midlands Regions]
- Bristol [South West Region]
- Cambridge (Addenbrooke’s Hospital) [East of England Region]
- Manchester (Manchester Royal Infirmary) [North West Region]
- Southampton (Southampton General Hospital) [South East Region]
- Belfast (Royal Victoria Hospital) [Northern Ireland Region]
Accessing the service

The CDRN laboratories provide access to \textit{C. difficile} culture and ribotyping according to standardised criteria for submission of faecal samples. The number of samples to be submitted to the CDRN per scenario should be agreed prospectively with respective regional microbiologists, or a microbiologist from the CDRN laboratory, according to the extent and severity of CDI cases. The CDRN aims to provide timely information to help optimise the management of \textit{C. difficile} at a local level, with a turnaround time of less than two weeks (this includes the time to culture \textit{C. difficile}). It is recommended that the CDRN service is used by hospitals/infection control teams in England to investigate the following scenarios:

- Increased frequency of cases OR high baseline rates of CDI
- Increased severity/complications of cases of CDI
- Increased mortality associated with CDI
- Increased recurrence rate of CDI

We believe that the CDRN service can help local teams to meet targets that have been set for reducing the incidence of CDI. Additionally, we collect, via a mandatory request form, antibiotic risk and outcome data that can be used to provide more detailed information about CDI at a national level. Some requests provide few such data, which hinders this aim, and we therefore encourage all users of the CDRN service to submit the data requested.
Enhanced DNA fingerprinting

Since late 2008, CDRN has offered an enhanced DNA fingerprinting (multilocus variable repeat analysis, MLVA) service. This can be used to characterise and improve the understanding of the transmission of epidemic *C. difficile* strains within healthcare institutions. Importantly, the method can provide a high level of discrimination among epidemic *C. difficile* ribotypes, including 001, 027 and 106. For example, MLVA can distinguish more than 20 sub-types of *C. difficile* ribotype 027. MLVA is far superior to most other fingerprinting methods, including pulsed field gel electrophoresis, for analysing closely related *C. difficile* strains. MLVA has similar discriminatory power, as a typing/fingerprinting method, to whole genome sequencing, although the latter method provides considerable additional genetic information.

Institutions should consider the use of the CDRN MLVA Enhanced Fingerprinting service to optimise the control and prevention of CDI. As with the CDRN ribotyping service, there is currently no charge for the enhanced fingerprinting service for NHS hospitals in England. Access to the service is controlled, in the first instance by regional microbiologists, given its high cost and need to balance availability with the scale of CDI challenge. MLVA is available via the Leeds laboratory (based at Leeds General Infirmary), which acts as the reference laboratory for the CDRN service. Until recently, MLVA testing for the East and West Midlands was available via the Birmingham (Heartlands laboratory). From November 2018, MLVA services for the East and West Midlands (and so for the entire CDRN) will be delivered via the Leeds laboratory.

The criteria used to access the enhanced fingerprinting service are:

- a hospital/trust with a high rate of CDI as identified with local commissioners
- a hospital/trusts that is failing to meet its *C. difficile* target trajectory despite implementation and audit of control measures
- a declared outbreak of CDI as agreed with the local Health Protection Unit

In addition:

- ribotyping carried out by CDRN must have confirmed the presence of a dominant *C. difficile* ribotype
- a plan should be in place of how results of *C. difficile* enhanced fingerprinting will contribute to the control of CDI
- infection control teams/consultant microbiologists will first need to agree with the regional microbiologist that use of the *C. difficile* enhanced fingerprinting service is merited
- numbers of samples/isolates to be examined will be agreed with the MLVA laboratory on a case-by-case basis, taking account of the scale of CDI challenge
Antibiotic susceptibility testing

In order to determine the epidemiology of the susceptibility to metronidazole, vancomycin and fidaxomicin of C. difficile isolates from CDI cases, periodic prospective surveillance is performed on strains received by the CDRN Reference Laboratory in Leeds. Further such data are available via publications on a long-term European antibiotic susceptibility study.\(^9,10\)

Electronic Requesting & Reporting System

A dedicated electronic requesting and reporting system continues to be available for NHS trusts to complete electronic request forms and receive test results electronically, as well as access archived historical results. The service is accessible via the NHS N3 secure network and users must securely register on the site before making requests.

The service can be accessed at the following web address: https://cdrn.phe.nhs.uk

Historical data and user guides are available on the PHE webpages: https://www.gov.uk/government/collections/clostridium-difficile-ribotyping-network-cdrn-service

The electronic requesting and reporting system has been fully operational in all regions for several years and this service is completely electronic. The system employs heightened user notification via email, enabling faster reporting of results to assist outbreak investigation, and enhance data analysis capabilities.

We are collaborating with the PHE healthcare-associated infection surveillance team to streamline data collection. The aim is to enable different electronic data collection systems to communicate, and so minimise the duplication of data input by users of the different surveillance schemes.
Results for 2015/16, 2016/17 and 2017/18

In 2015/16, 2016/17, and 2017/18 CDRN processed 9,706 faecal samples from 132 healthcare facilities, 8,787 faecal samples from 129 healthcare facilities, and 8,326 faecal samples from 135 healthcare facilities, respectively. Data available since 2008 show slight regional differences in the number of samples submitted to the service (Figure 1). On average, 24, 33, 46, 40, 43, 51, 73, 68, and 62 samples were submitted to CDRN by each participating hospital in 08/09, 09/10, 10/11, 11/12, 12/13, 13/14, 14/15, 15/16, 16/17 and 17/18, respectively. Despite the decreasing number of reports of C. difficile recorded by the mandatory scheme in England (Figure 2), submissions to CDRN have continued to increase over time.

Figure 1: Distribution of CDRN Samples Submitted to the Service (2008/09 to 2017/18)

It should be noted that an epidemiological study took place in the North East region during 2009-11, which accounts for the larger numbers of samples processed here in these years.
Proportion of Mandatory CDI Reported Cases Ribotyped

Figure 3 below shows the ribotyping sample submission to CDRN by quarter, expressed as the proportion of mandatory *C. difficile* (all reported cases) on the Mandatory HCAI Data Capture System (DCS) in England from April 2008 to March 2018. The overall average annual proportion of *C. difficile* reported cases from whom samples were sent for ribotyping over the whole analysis period 2008-2018 was 43.4%. Usage of CDRN, expressed both in crude numbers and in terms of the proportion of all reported cases that are referred, has increased markedly since the service was launched.

Figure 2: Proportion of HCAI CDI Cases Submitted for Ribotyping to All Reported Cases of CDI to Public Health England (2008/09 to 2017/18)
Reasons for Sample Submission to CDRN Service

In 2015/16, 2016/17 and 2017/18, 9,706, 8,787 and 8,326 samples were submitted in response to clinical need. The reasons provided for sample submission are shown in Figure 3. The commonest reasons cited for sample submission was clustering of cases (47.4% of all samples cited this as a reason), followed by unexplained increase in CDI rate (20.0% of all samples), and severity of symptoms of CDI in the affected patient and in other patients (14.3% of all samples).

Figure 3: Reason for Sample Submission to CDRN (2008/09 to 2017/18)
C. difficile Recovery Rate

Figure 4 shows C. difficile recovery rates for samples submitted to the service since 2008/09. These data exclude samples not processed or rejected (not enough sample, duplicates, etc). There was a 29% increase between 2007/08 and 2008/09 in the proportion of (presumed toxin positive at the source laboratory) faecal samples submitted to CDRN that were C. difficile culture-negative (i.e. from 9.6% to 12.4%). This change may have reflected more false-positive samples, as CDRN examines samples that have tested locally as ‘toxin positive’.

Notably, the C. difficile recovery rate progressively increased in the early CDRN years, and has remained stably high at ~94% since 2012/13. These data are consistent with improved and then relatively consistent laboratory diagnosis of CDI. Guidelines for the diagnosis of CDI were issued in 2012.11

Figure 4: C. difficile recovery rate (2008/09 to 2017/18)

<table>
<thead>
<tr>
<th>Year</th>
<th>Total Samples</th>
<th>C. difficile Growth</th>
<th>Recovery Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>2008/09</td>
<td>4774</td>
<td>4175</td>
<td>87.45%</td>
</tr>
<tr>
<td>2009/10</td>
<td>5720</td>
<td>4995</td>
<td>87.33%</td>
</tr>
<tr>
<td>2010/11</td>
<td>7026</td>
<td>6202</td>
<td>88.27%</td>
</tr>
<tr>
<td>2011/12</td>
<td>5144</td>
<td>4761</td>
<td>92.55%</td>
</tr>
<tr>
<td>2012/13</td>
<td>5830</td>
<td>5523</td>
<td>94.73%</td>
</tr>
<tr>
<td>2013/14</td>
<td>7208</td>
<td>6781</td>
<td>94.08%</td>
</tr>
<tr>
<td>2014/15</td>
<td>8124</td>
<td>7609</td>
<td>93.66%</td>
</tr>
<tr>
<td>2015/16</td>
<td>8931</td>
<td>8335</td>
<td>93.33%</td>
</tr>
<tr>
<td>2016/17</td>
<td>7880</td>
<td>7405</td>
<td>93.97%</td>
</tr>
<tr>
<td>2017/18</td>
<td>7585</td>
<td>7079</td>
<td>93.33%</td>
</tr>
</tbody>
</table>
Ribotype distribution

Changes in ribotype prevalence

Figure 5 demonstrates the marked shifts in ribotype prevalences in the 40 quarters of CDRN operation between April 2008 and March 2018. The most prevalent ribotypes are shown i.e. those with an overall minimum of >2% prevalence in all regions for all years between 2008/09 and 2017/18 (n = 43 ribotypes). The isolates designated as “sporadic” represent the fact that the ribotypes in the commonly recognised ribotype group.

There has been a striking decrease in the prevalence of *C. difficile* ribotype 027, and also in ribotypes 001 and 106, with ‘compensatory’ increases in the other types. In general the pattern of ribotypes in England has become more heterogeneous. The relative prevalence rates for individual ribotypes has been relatively stable nationally and within regions for the past ~3 years; ribotypes 002, 014, 015, 005, 023 and 078 have become the most prevalent types in England.

These observations likely reflect the success of control measures to reduce cross-infection in hospitals caused by former predominant epidemic strains, especially ribotype 027. In some regions, ribotype 027 has almost completely disappeared, according to these CDRN data. With increased sample submission to CDRN, such an effect may be expected to accompany an increase in the relative contribution of other ‘emergent’ *C. difficile* ribotypes to overall disease burden.

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 determined 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. 125 distinct ribotypes were identified, with considerable intercountry variation in ribotype distribution. Ribotypes 027 (19%), 001/072 (11%) and 014/020 (10%) were the most prevalent, followed by ribotypes 002, 140, 010, 078, 176 and 018 (each <5%). The prevalence of ribotypes 027 and 176, but not other epidemic strains, was inversely proportional to overall ribotype diversity (R2 = 0.717). Importantly, there is increasing evidence that there are two distinct patterns of *C. difficile* ribotype spread – see ‘Enhanced fingerprinting’ section.
Figure 5 – Prevalence of *C. difficile* ribotypes in England by quarter (April 2008 – March 2018)

![Figure 5](image)

Figure 6 – Distributions of *C. difficile* ribotypes according to England region (April 2008 to March 2018)

![Figure 6](image)
Clostridium difficile Ribotyping Network (CDRN) for England and Northern Ireland 2015-2018

South East Region (1 April 2008 - 31 March 2018)

South West Region (1 April 2008 - 31 March 2018)
Enhanced fingerprinting

The Leeds CDRN Reference Laboratory has previously published an analysis of enhanced fingerprinting (MLVA) investigations for potential CDI case clusters/outbreaks in hospitals in England. Notably, despite sharing a common ribotype, 19% of these potential CDI case clusters/outbreaks comprised unrelated isolates, and 34% contained a mixture of highly related and distinct isolates, as shown by MLVA. These findings emphasise the value of enhanced fingerprinting to confirm or refute suspected CDI case clusters. In 2015-16, 2016-17 and 2017-18 Leeds carried out 93 (245 isolates), 77 (196 isolates) and 67 (155 isolates) MLVA based investigations, respectively.

We have continued to examine the utility of whole genome sequencing in comparison with MLVA for the examination of case clusters. These efforts were part of a UK-wide consortium, funded by the Wellcome Trust and MRC, between the University of Oxford, PHE and the Wellcome Trust Sanger Institute, to establish how revolutionary new technologies can be optimally integrated into medical microbiology.

We have examined C. difficile isolates from 61 suspected outbreaks affecting 2-41 patients in 31 UK hospitals (300 samples) using both 7-locus MLVA and WGS. Conclusions on whether potential outbreaks were confirmed were concordant in 58/61 (95%) of investigations.

We have also completed a front line service performance comparison of MLVA and WGS techniques. All isolates from MLVA-based cluster/outbreak investigations received by our testing laboratory over a period of 12 months were also subjected to WGS, in real time. 103 investigations (285 isolates (range 2-11 per investigation)) from 42 hospitals were examined. Outcome data generated by MLVA and WGS were concordant in 95/103 (92%) investigations. Using current strain relatedness criteria, all investigations of discordant outcome involved instances where WGS discriminated further than MLVA. Results for investigations using MLVA and WGS were available in 2 and 5 days, respectively.

Overall, when applied to outbreak investigation of CDI, findings using MLVA and WGS are very similar, despite these techniques analysing different parts of the bacterial genome. WGS offers marginally higher levels of discrimination than MLVA. Although WGS analyses take longer than MLVA, processing times associated with both techniques remain relevant for hospital outbreak investigations. With improvements in WGS technology, it is likely MLVA data will be available from WGS in the near future (achievable read lengths remain the limiting factor). Notably, WGS provides additional data, such as antimicrobial susceptibility genotype and the presence/absence of virulence genes. WGS has also been successfully utilised as a novel surveillance tool to establish rates of C. difficile transmission between healthcare institutions, to facilitate targeted efforts in the reduction of
CDI incidence. It is planned for CDRN to transition to using WGS instead of MLVA for the enhanced fingerprinting/investigation of *C. difficile*.

624 *C. difficile* isolates from 19 countries underwent WGS, which demonstrated that 5 ribotypes had within-country clustering: ribotype 356, only in Italy; ribotype 018, predominantly in Italy; ribotype 176, with distinct Czech and German clades; ribotype 001/072, including distinct German, Slovakian, and Spanish clades; and ribotype 027, with multiple predominantly country-specific clades including in Hungary, Italy, Germany, Romania, and Poland. By contrast, no within-country clustering was observed for ribotypes 078, 015, 002, 014, and 020, which is consistent with a Europe-wide distribution. These and other data support the existence of two distinct patterns of *C. difficile* ribotype spread, which are consistent with either predominantly healthcare-associated acquisition or Europe-wide dissemination via other routes/sources (for example, possibly via the food chain).
Outcome data

In 2015/16, 2016/17, and 2017/18 clinical follow-up data were available for ~46%, ~52% and ~49% of cases, respectively, although some follow-up data (e.g. mortality) was provided more commonly (35% of cases). Clinical follow-up data are shown in Figure 7 (these are for all referred cases, regardless of culture result); the data should be interpreted with caution given the partial response rate. Numbers of deaths and cases associated with either toxic megacolon or requiring surgery declined between 2008/09 and 2011/12 and have remained approximately stable thereafter; these observations are consistent with control and declining incidence of ribotype 027 CDIs.

Figure 7: Outcome data provided at the time of CDRN request submission (2008/09 to 2017/18)

A detailed analysis of risk factors associated with CDI, outcomes and specific ribotypes was presented in the 2009-10 CDRN report: https://www.gov.uk/government/publications/clostridium-difficile-ribotyping-network-cdrn-report

Further detailed information can also be found in two peer reviewed reports.20,21
Antibiotic Exposure

The interpretation of data on CDI risk associated with individual antibiotics is extremely difficult as commonly used agents may be reported as being associated with CDI more often than rarely prescribed antimicrobials, data often do not take into account duration of exposure or polypharmacy, and similarly may be confounded by other risks (patient age, co-morbidities, etc). Thus, the data in the following paragraphs need to be interpreted with caution; notably, the data should not be considered to be indicative of which agents actually caused CDI.

As in recent years, the most commonly reported antibiotics in 2015/16, 2016/1, and 2017/18 were piperacillin-tazobactam (n=1109, 815, 579) and co-amoxiclav (n=1080, 829, 895) (Figure 8). It is noticeable that the most commonly recorded antibiotics have changed markedly over the 10-year period that CDRN has been in existence. In 2007/08, cephalosporins were the most commonly cited agents, whereas these were uncommonly cited in subsequent reporting periods, and indeed have been numerically superseded by co-amoxiclav and piperacillin-tazobactam from 2008/09 onwards. These data likely reflect real changes in prescribing of systemic antibiotics as one of the control measures for CDI.

It is also noteworthy that there appears to have been a shift in the prescribing of CDI treatment antibiotics from metronidazole in favour of vancomycin (Figure 8). Such data are consistent with possible greater adherence to guidelines that advocate the choice of treatment agent according to severity of CDI. Indeed, more recent evidence shows that vancomycin is superior to metronidazole for CDI treatment.22
Figure 8 – Reported antibiotics associated with CDI episodes (2008/09 to 2017/18)
Metronidazole, Vancomycin and Fidaxomicin Susceptibility

Previously, targeted surveillance, based on investigation of cases suspected to represent cross-infection, has identified reduced metronidazole and vancomycin susceptibility amongst epidemic ribotypes. Epidemic ribotypes with reduced metronidazole or vancomycin susceptibility were associated with location clusters, as determined by MLVA. This may indicate expansion or selection of strains with reduced susceptibility within epidemic ribotypes.

A panel of 75 UK *C. difficile* isolates, collected between 2014-16, were uniformly susceptible to metronidazole, vancomycin and fidaxomicin (breakpoints <2 mg/L for metronidazole and vancomycin, and <1 mg/L for fidaxomicin). Geometric mean metronidazole, vancomycin and fidaxomicin MICs were 0.14, 0.65 and 0.02 mg/L, respectively, which were very similar to those found during 2014-16 in a recent Pan-European surveillance study of ~3500 *C. difficile* isolates (0.21, 0.59, 0.02 mg/L respectively). Four recent studies have described a small number of fidaxomicin resistant clinical *C difficile* isolates, but none of these was from the UK.
Summary

The *Clostridium difficile* Ribotyping Network (CDRN) for England & N. Ireland has for 10 years responded to a major public health need by providing a molecular epidemiological service that enhances our understanding of this pathogen. Since the introduction of CDRN the reports of *C. difficile* in England have fallen markedly. Reports of deaths associated with CDI also started to decrease the year after CDRN commenced, which is likely due to enhanced control of the epidemic ribotype *C. difficile* 027. It is plausible that timely data provision by CDRN has enhanced the capacity of healthcare institutions and infection control and prevention teams to control CDI incidence.

Continued referral to CDRN will afford the greatest chance of identifying emergent *C. difficile* ribotypes. However, despite the ~75% decline in CDIs in England since 2007, the number of cases in England for which samples are submitted to CDRN has continued to increase (Figure 2), and now accounts for half to two-thirds of all reported episodes. All NHS hospitals in England have been encouraged to submit samples, according to set CDRN criteria. The high proportion of all CDIs that are referred to CDRN for ribotyping means that is likely that, despite these criteria, they are not being followed consistently.

It is timely therefore that the function of CDRN should be reviewed to ensure that the service is cost effective. While this review is ongoing, we emphasise that all hospitals should submit samples according to the CDRN criteria (see page 3). Use of enhanced fingerprinted is recommended to optimise the control and prevention of CDI following discussion with CDRN/PHE personnel (see page 4).
References


Acknowledgements

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