Clostridium difficile Ribotyping Network for England and Northern Ireland

2008/09 report
Summary

The *Clostridium difficile* Ribotyping Network for England and Northern Ireland has continued to expand and to respond to a major public health need by providing a molecular epidemiological service that enhances our understanding of this pathogen. Since the introduction of CDRN(E) the reports of *C. difficile* in England have fallen markedly. Reports of deaths associated with CDI have also now started to decrease. It is not possible to determined which interventions have been particularly responsible for this decreased incidence of CDI. It is most notable that the epidemic ribotype *C. difficile* 027 has declined markedly. Samples are submitted to CDRN according to local clinical need; results are provided invariably 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.

Rationale

The Health Protection Agency funded the *Clostridium difficile* Ribotyping Network for England and Northern Ireland, delivered via the Regional Microbiology Network in England from April 2007. The CDRNE (renamed CDRN in 2009) initially comprised six regional microbiology laboratories in England: Leeds (Reference Laboratory, Leeds General Infirmary), Birmingham (Heartlands Hospital), London (HPA Collaborating Centre at University College Hospital), Manchester (Manchester Royal Infirmary), Newcastle (Newcastle General Hospital) and Southampton (Southampton General Hospital). From 2009, Cambridge (Addenbrooke’s Hospital) and Belfast (Royal Victoria Hospital) joined the network (Figure 1); it is planned that Bristol (Bristol Royal Infirmary) will also join within the next year. These additions will mean that all regions have a designated laboratory proving this service. Prior to this expansion all regions in England were still covered by the service through collaborative arrangements.

Given the inclusion of Northern Ireland, from spring 2009 the service was renamed as the *Clostridium difficile* Ribotyping Network (CDRN). The data within this report predates the inclusion of Cambridge and Belfast and is confined to those for England.
The CDRN laboratories provide access to *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 is agreed prospectively with respective regional microbiologists, or a microbiologist from the CDRN laboratory, according to the extent and severity of *C. difficile* infection (CDI) cases. The CDRN aims to provide timely information to help optimise the management of *C. difficile* at a local level, with a turnaround time of ≤2 weeks (this includes the time to culture *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, or
- 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. We stress that some requests provide few such data that hinders this aim, and we therefore encourage all users of the CDRN service to submit the data requested. The ongoing development of electronic requesting and reporting for CDRN will mean that in order for samples to be accepted completion of a minimum data set will be required.

**Additional CDRNE services**

**Enhanced DNA fingerprinting**

From winter 2008 CDRN 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; these accounted for approximately 70% of more than 2,000 *C. difficile* isolates ribotyped by CDRNE in 2007/08. 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.

As with the CDRN ribotyping service, there is 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. In the East and West Midlands MLVA is available via the Birmingham (Heartlands laboratory).

The criteria used to access the enhanced fingerprinting service are:

- A hospital/trust with a high rate of CDI as identified with the local SHA; or
- A hospital/trusts that is failing to meet its *C. difficile* target trajectory despite implementation and audit of control measures; or
- 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; and
- 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 and vancomycin of *C. difficile* isolates from CDI cases, prospective surveillance is performed on strains received by the CDRN Reference Laboratory in Leeds.

**Results for 2008-09**

In 2008/09 CDRN processed 4,682 faecal samples from 190 healthcare facilities (Figure 2); this is an ~100% increase over 2007/08 when 2318 samples were received from 152 healthcare facilities. Thus, on average 24.6 samples were submitted to CDRN by each participating hospital in 2008/09. Hence, despite the decreasing number of reports of *C. difficile* recorded by the mandatory scheme in England (Figure 3), submissions to CDRN have increased markedly. In 2008/09 about one out of every eight or nine *C. difficile* cases in England were examined by CDRN. During the near same 12 month period CDRN received samples from 4,682 patients in 192 hospitals, giving an average sample size of 24.6 samples per hospital per year. Males accounted for 43% of cases and the age range was 1-105 years; with a mean of 76 years and a median of 81 (80) years.
Figure 2
Distribution of samples processed by CDRN region in 2008/09

Figure 3
Sample submission to CDRN by quarter year, expressed as the proportion of mandatory *C. difficile* reports in England in 2007-2009
Reason for sample submission to CDRN

The CDRN samples were submitted in response to clinical need. The reasons provided for sample submission are shown in Figure 4; as some respondents gave more than one reason, the total number of responses (5,131) is greater than the sample total of samples processed (4,682). The commonest reasons cited for sample submission was clustering of cases (55% of all samples cited this as a reason), followed by unexplained increase in CDI rate (15% of all samples), and severity of symptoms of CDI (10% of all samples); 17% of requests did not answer this question.

Figure 4
Reason for sample submission to CDRN 2008/09

<table>
<thead>
<tr>
<th>Reason</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Did not answer</td>
<td>819</td>
<td>16.0%</td>
</tr>
<tr>
<td>Because of severity of symptoms in this patient</td>
<td>451</td>
<td>8.8%</td>
</tr>
<tr>
<td>Because of severity of symptoms in other patients</td>
<td>61</td>
<td>1.2%</td>
</tr>
<tr>
<td>Other reason</td>
<td>512</td>
<td>10.0%</td>
</tr>
<tr>
<td>Because of unexplained increase in CDI rate</td>
<td>703</td>
<td>13.7%</td>
</tr>
<tr>
<td>Because of cluster/s of cases</td>
<td>2585</td>
<td>50.4%</td>
</tr>
</tbody>
</table>

*C. difficile* recovery rate by CDRN

These 4,682 faecal samples yielded 4,101 *C. difficile* ribotype results (*C. difficile* recovery rate 87.6%). Notably, 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 reflect more false positive samples, as CDRN examines samples that have tested locally as ‘toxin positive’.4-6
Changes in ribotype prevalence

CDRN obtained 4,101 *C. difficile* isolates which yielded 66 formally identified ribotypes plus a further 329 isolates that were designated as sporadic (and thus there was no evidence of clustering of these); 37 ribotypes were represented by at least 4 isolates.

There were marked changes in ribotype prevalence in 2008/09 compared with the previous 12 months (Table 1). Figure 5 demonstrates the shifting ribotype prevalences in the eight quarters (2 years) since CDRN(E) was introduced. There was a striking decrease in the prevalence of *C. difficile* ribotype 027, with ‘compensatory’ increases in the other main types. This phenomenon may reflect the success of control measures to reduce cross-infection in hospitals caused by the predominant epidemic strain. With increased sample submission to CDRN, such an effect may be expected to be accompanied by increases in the relative contribution of other ‘emergent’ *C. difficile* ribotypes to overall disease burden.

Table 1
Changing prevalence of most common *C. difficile* ribotypes detected by CDRN in 2007/08 and 2008/09

<table>
<thead>
<tr>
<th>Ribotype</th>
<th>2007/08 (n,%)</th>
<th>2008/09 (n,%)</th>
<th>Prevalence change (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>027</td>
<td>1152 (55.3%)</td>
<td>1468 (36.1%)</td>
<td>- 19.2%</td>
</tr>
<tr>
<td>106</td>
<td>270 (13.0%)</td>
<td>517 (12.7%)</td>
<td>- 0.3%</td>
</tr>
<tr>
<td>001</td>
<td>181 (8.7%)</td>
<td>297 (7.3%)</td>
<td>- 1.4%</td>
</tr>
<tr>
<td>002</td>
<td>57 (2.7%)</td>
<td>231 (5.7%)</td>
<td>+ 3.0%</td>
</tr>
<tr>
<td>014/020*</td>
<td>57 (2.8%)</td>
<td>218 (5.4%)</td>
<td>+ 2.6%</td>
</tr>
<tr>
<td>015</td>
<td>50 (2.4%)</td>
<td>215 (5.3%)</td>
<td>+ 2.9%</td>
</tr>
<tr>
<td>078</td>
<td>37 (1.8%)</td>
<td>144 (3.5%)</td>
<td>+ 1.7%</td>
</tr>
<tr>
<td>005</td>
<td>29 (1.4%)</td>
<td>118 (2.9%)</td>
<td>+ 1.5%</td>
</tr>
<tr>
<td>023</td>
<td>21 (1.0%)</td>
<td>109 (2.7%)</td>
<td>+ 1.7%</td>
</tr>
<tr>
<td>026</td>
<td>5 (0.2%)</td>
<td>87 (2.1%)</td>
<td>+ 1.9%</td>
</tr>
</tbody>
</table>

The top 10 most prevalent ribotypes are shown i.e. those with >2% prevalence in 2008/09. In 2007/08 and 2008/09, 7.2% and 8.1%, respectively, of all isolates were designated as sporadic i.e. these were not one of the commonly recognised ribotypes.

* Data for ribotypes 014 and 020 are combined.
It is notable that one these ‘emergent’ *C. difficile* ribotypes is 078. This ribotype has become much more prevalent in the Netherlands, and has been recovered from several animal sources. Currently there is no definitive evidence to link food sources and human *C. difficile* infection. The prevalence of *C. difficile* ribotype 078 over time within individual regions in England does not reveal a clear pattern of increased incidence during 2008/09 (Figure 6).
There were clear regional differences in ribotype prevalence e.g. CD 027 was the commonest in each region except the North East (CD 001, 20.0% vs CD 027, 12.6%; p<0.001). However, interpretation of regional differences in ribotype prevalence needs to be made with caution because sample submission patterns and thus the influence of individual hospitals (for example, because of outbreaks) on data may vary between regions. Nevertheless, such analyses provide useful indicators of possible regional differences and time trends to indicate potential emergent or declining *C. difficile* ribotypes (Figures 7a-i).
Figures 7a-i
Distributions of the 10 most common *C. difficile* ribotypes within each region in England (April 2007 to March 2009)
A European Centre for Disease Prevention and Control-sponsored *C. difficile* period prevalence study was carried out in 69 hospitals in 28 countries in Europe in the month of November 2008. The eight most common ribotypes detected were ribotypes 001, 002, 012, 014, 015, 018, 027 and 078. Of these eight types commonly seen across Europe, six featured in the top eight most prevalent ribotypes in England in 2008/09 (the exceptions being ribotypes 012 and 018). The most prominent ribotype that is seen in England but rarely elsewhere is 106.

**Antibiotic exposure**

Although 21% of request forms failed to provide any data on recent antibiotic exposure, 1,874 cited previous exposure to at least one antibiotic. Of those who did report recent antibiotic exposure, 61% and 25% listed use of >1 and >3 antibiotics, respectively. Such observations make interpretation of CDI risk associated with individual antibiotics extremely difficult. Thus, the data in the following paragraph need to be interpreted with caution.

Request forms that indicated the use of a specific antibiotic reported that co-amoxiclav (n=618), cephalosporin (n=580), piperacillin-tazobactam (n=444), ampicillin/amoxicillin (n=379), macrolide (n=255), trimethoprim (n=252) and fluoroquinolones (n=239) were most frequently implicated.

Of those cases where data were provided (n=1,206) on whether metronidazole or vancomycin were used to treat CDI, 70% and 30% respectively cited these antibiotics. Data analyses to determine whether specific antimicrobials are associated with particular *C. difficile* ribotypes have not been presented here because of concerns about potential reporting bias. As data capture improves we envisage greater power to determine antimicrobial-ribotype and other relationships.
Outcome data

Complete follow-up data were available for ~22% of cases, although some follow up data (e.g. mortality) was provided more commonly (see below). Clinical follow-up data are shown below (Figure 8). These data should be interpreted with caution given the limited response rate.

Figure 8
Outcome data provided at the time of CDRN request submission (2008/09)

In those cases where the answer to the question on whether the patient died within 30 days of onset of CDI was provided (43%, n=2,034), the mortality was 20.3%. Multivariate analysis revealed that only *C. difficile* ribotype 027 was associated with all-cause mortality (OR = 1.9; p<0.001), independent of whether toxic megacolon or pseudomembranous colitis was present. The increased mortality associated with ribotype 027 cases helps to explain the increased recorded deaths in the UK as the predominant ribotype switched from 001 to 027. Furthermore, Office for National Statistics data have recently shown that the number of death certificates mentioning *C. difficile* in England and Wales has decreased for the first time since 2004. Given the increased risk of death associated with *C. difficile* 027, the large decrease in the prevalence of this ribotype in 2008/09 in comparison with 2007/08 as measured by CDRN, is thus consistent with the recent decline in *C. difficile* associated deaths. Such data emphasise the importance of surveillance that can provide timely data on the identity of *C. difficile* strain types.

Metronidazole and vancomycin susceptibility

A total of 1,010 *C. difficile* isolates from CDRN samples received during 2008/09 from patients in four regions in England were screened for susceptibility to metronidazole and vancomycin. Overall geometric mean (GM) MICs of M and V were 0.9 and 0.7 mg/L. However, epidemic *C. difficile* PCR ribotypes 027, 106 & 017 had significantly higher metronidazole MICs (GM = 1.73, 1.56, 1.15 mg/L; p << 0.0001) than the remaining top ten most prevalent ribotypes (GM 0.39-0.54 mg/L). Metronidazole MICs = 8 mg/L were seen in five CD ribotypes (027, 016, 015, 118) with evidence for clustering in some cases, as has been demonstrated previously using MLVA. Within one institution (Leeds) comparison of MICs for *C. difficile* isolated in 2008/09 vs 2005/06 showed significant increases in MICs for CD 027 and 106 (p <<0.001). Vancomycin MICs varied little among the top 10 most common
ribotypes (GM 0.62-0.81 mg/L), but 12 isolates had MICs = 4 mg/L, with evidence for clustering of cases in at least one institution.

These data show decreasing susceptibility to metronidazole among epidemic *C. difficile* strains, including *C. difficile* PCR ribotype 027. Metronidazole achieves relatively poor gut concentrations and thus even modest shifts in MICs may be clinically relevant, although at present there is no evidence to link therapeutic failure of metronidazole to decreased susceptibility. The evidence of clustering of *C. difficile* isolates with raised MICs of metronidazole or vancomycin emphasises the need for control of transmission to minimise the dissemination of epidemic strains. The data emphasise the need to carry out continued surveillance susceptibility testing of *C. difficile*, preferably prospectively so that clustering of isolates with reduced susceptibility can be identified.

**Future developments of CDRN**

A web-based electronic requesting system (ERS) has been successfully piloted in London, and is currently being rolled out across all remaining regions. The CDRN ERS allows requesting NHS laboratory and infection control staff to complete an electronic ribotyping request form, receive notifications from the CDRN laboratory as the test status and ribotyping results. The system is fully searchable, has archived official laboratory results by NHS trust, and data can be securely downloaded at a trust, regional or national level.

The CDRN ERS will fully replace the paper-based system once implemented in each region, and it will enable faster reporting of results to assist outbreak investigation, as well as enhance data analysis capabilities.

We are collaborating with the HPA 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.
References


Acknowledgements

We thank everyone who has contributed to the successful development of the CDRN. A full list of contributors is shown below.

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