Diversity in Prevalent PCR Ribotypes of Clinical Strains of C. difficile

Abstract:
E Brabazon, M Carton, R Sheehan, P Finnegan, D Bedford
Department of Public Health, HSE North East, Railway St, Navan, Co Meath

In 2009, a programme of Clostridium difficile ribotyping was established in the north east. The aim of this project was to profile circulating ribotypes in the region. In all, 50 notified north east Clostridium difficile cases were ribotyped. The majority of cases occurred in patients over 70 years and in hospital in-patients. The most common ribotype identified was O27 (n=12, 24%) and O05 (n=8, 16%). Ribotype O78 was also detected (n=5, 10%). Comparison with a 2009 national ribotyping study demonstrated that there were a number of ribotypes identified in the north east that were not identified during the national study and visa versa. The results of this study point to the existence of regional variation in circulating Clostridium difficile strains in Ireland. A reference Facility for Ireland is urgently required to provide a central point for enhanced testing and epidemiological analysis of national and regional Clostridium difficile trends.

Introduction
Clostridium difficile is a gram positive, anaerobic spore forming bacillus. The organism can cause a wide spectrum of disease including antibiotic associated diarrhoea or colitis and pseudomembranous colitis with toxic megacolon. Acquisition by vulnerable hospitalised patients has serious implications for both the patient in terms of outcome and the hospital in terms of subsequent transmission to other patients and possibility of outbreaks. In all, 150 different PCR ribotypes and 25 different toxinotypes of C. difficile have been described and recently a hypervirulent strain (PCR ribotype O27) which is associated with high morbidity and mortality has also emerged[1]. Clostridium difficile associated disease, CDAD, became notifiable in Ireland on 4th May 2008. Between this date and 31st December 2009, 5,538 laboratory-confirmed CDAD cases were notified nationally providing a national crude incidence rate of 56.9 per 100,000 population. The publication of national guidelines on the surveillance, diagnosis and management of CDAD has also allowed a greater understanding and response to the problem of CDAD in Ireland.

The Health Protection Surveillance Centre (HPSC), St. Vincent’s University Hospital and University College Dublin undertook a one-month national enhanced surveillance, typing and antimicrobial susceptibility study of all cases of CDAD identified in March 2009. Of the 80 new C. difficile cases ribotyped during this national study, from 33 Irish healthcare facilities, ribotype O27 (16%) and O106 (14%) were most common. Less than ten C. difficile cases occurred in the north east region during the national project. The north east region comprises a population of 440,698 and covers the counties of Cavan, Louth, Meath and Monaghan. The region is served by four laboratories which manage the diagnostic needs of the hospitals and local communities. Therefore, in order to extend the mapping of circulating strains of C. difficile in the north east region, a six month ribotyping project of C. difficile from notified hospital in-patients and long term care facility residents was established. The project received financial support of the north east regional SARI (Strategy for the Control of Antimicrobial Resistance in Ireland) committee. The aim of this study was to identify the common strains of C. difficile circulating in the north east during the latter half of 2009 and to compare the results to data from the national project.

Methods
In 2009, all four laboratories in the north east performed testing for C. difficile toxin on faecal samples using Meridian ImmunoCard Toxin A&B (Meridian Bioscience Inc.) or Techlab (TECHLABfi, Inc) enzyme immunoassay methodology. Laboratories in the region tested on request for C. difficile in patients over two years but culturing of isolates was not routinely performed. A six month surveillance project was established in July 2009. Diarrhoeal samples from the laboratories in the north east region which were found to be toxin positive for C. difficile by immunoassay were centralised and dispatched in a monthly shipment to the C. difficile Ribotyping Network for England (CDRNE), Microbiology Reference Laboratory, Leeds General Infirmary, UK. Some samples prior to July 2009 which had been stored appropriately were also sent for ribotyping. Only samples that were either toxin positive by immunoassay in the local laboratory or that were toxin positive following cytotoxic culture testing by the reference laboratory were included in this study in order to analyse only clinically relevant strains.

Data on all notified cases of acute infectious gastroenteritis (AIG) due to CDAD from the north east region were obtained from the Computerised Infectious Diseases Reporting (CIDR) System. Data on all new C. difficile cases from the 2009 national ribotyping study were used for comparison with the north east study.

Results
Overview
There were 84 C. difficile cases notified in the north east region during 2009, 50 of which were ribotyped (Table 1). This represents 60% of all C. difficile cases notified in the region for 2009. In all, 18 different ribotypes were identified. The most common ribotype was O27 (n = 12, 24%), followed by O05 (n = 8, 16%), O02 (n = 6, 12%) and O78 (n = 5, 10%). Together, these four ribotypes account for over half (62%) of all the ribotypes identified.

Age Profile
The majority of notified cases were aged 70 years or over (n= 62, 73.8%) (Figure 1). A variety of ribotypes were identified from this cohort but the most common ribotypes were O27 and O05 (Figure 1 & Table 1). In patients under 55 years of age (n = 4), only ribotypes O27 or O05 were identified.

Distribution over Time
The distribution of cases by quarter is shown in Figure 2. The months with the largest number of cases was October and November and coincides with an outbreak of C. difficile over two of the hospitals in the region. This outbreak was associated with ribotype O27.

Patient Type
The majority of notified C. difficile cases were classified as hospital in-patients (n=53, 63%). There were 15 notified cases for which their main residence was recorded in CIDR as a long term care facility (17.8%). Of these 15 C. difficile cases, nine were ribotyped. The most common ribotype was O27 (n=3). The other ribotypes identified for these long term care facility residents included O05, O27, O05, O14, O20, O78. The remaining cases were classified with patient type as Not Specified or Other.

Comparison with National 1 month typing project (2009)
Table 1 compares the ribotyping results from all new cases of C. difficile during the 2009 one month national project with the ribotyping results from notified patients during the six month north east project. The most common ribotype

Diversity in Prevalent PCR Ribotypes of Clinical Strains of C. difficile
Discussion

This study has documented for the first time the diversity of circulating C. difficile ribotypes in the north east in 2009. The most common ribotype found was the hypervirulent strain, ribotype O27, followed by ribotype O05. In all, 19 ribotypes were identified over the course of this study, including ribotypes O27 and O05, both of which are associated with increased morbidity and mortality and have been responsible for C. difficile outbreaks in the Irish context. Indeed, the ribotype O27 was also found to be associated with an outbreak in the north east region during this period.

In this study, the most common age for CDAD cases was 70 years and over with the majority of patients classified as hospital in patients (63%). However, almost 18% of patients had their main residence recorded as a long term care facility and from this group, ribotypes O27 and O05 were also identified. Although patient type or residence does not necessarily ascribe origin of infection, the age profile of these patients would suggest that LTCFs may be important reservoirs for C. difficile. Clearly, there is potential for interventions in both the hospital and long term care setting (e.g. review of antibiotic stewardship) which may impact on the epidemiology of C. difficile and improve overall patient morbidity and mortality.

It is not surprising that ribotype O27 was found to be the most common ribotype in both the national project and north east project as it appears that this strain is well established in Ireland. The second most common ribotype identified in the national project was only present to a minor extent in the north east. Furthermore, one of the most common ribotypes identified in the north east during the six month study was not identified at all during the national project. These results, combined with other variations in distribution of ribotypes (i.e. 11 north east ribotypes that were not identified in the national project and eight national ribotypes that were not identified in the north east) points to the existence of significant regional variation and possibly an over representation of particular co-horts in the national data. Regional variations in the distribution of ribotypes within a country have been demonstrated in both the UK and Hungry.

There have only been a small number of publications on the prevalence of C. difficile PCR ribotypes in Ireland1,5-9,11-13. The majority of these publications relate to ribotyping which resulted from outbreak situations. Until now, systematic ribotyping under non-outbreak periods in Ireland has been limited to the national one month project conducted in March 2009. However, this type of short snapshot favours collection of samples from large facilities with high throughput of patients and may therefore reflect regional variations. Furthermore, if large scale national studies are to be undertaken in the future, longer time periods for sample collection may need to be considered in order to allow the opportunity to utilise the data to provide a basis for implementing interventional strategies. The results of this study suggests that ribotyping of C. difficile samples is an important exercise and review of guidance at regional level will take cognisance of these findings. It would appear that there is quite a wide diversity of ribotypes circulating in Ireland and both the national and north east project has only provided an initial insight into the true extent of this variation.

During the timeframe of this study, the interpretation of the national guidelines by laboratories in the region meant that specimens were tested on request for C. difficile. This testing strategy based on clinical suspicion may have underestimated the overall number of cases and was the main limitation of this study. The national one month study was conducted in 2008 and at the time was the only study in Ireland (to our knowledge). Hence, the results from both studies are still comparable. The current national guidelines on the management of C. difficile recommend that all diarrheal specimens in patients over two years should be tested for C. difficile regardless of request.

Once off studies such as this one and the one month national surveillance project are important in the characterisation of Irish C. difficile isolates so that regional, national and international comparisons can be made. However, these type of studies cannot substitute for a dedicated C. difficile reference facility for the Republic of Ireland. Such a facility would allow rapid identification of circulating strains, would track changes in ribotype distribution and type of studies cannot substitute for a dedicated reference facility for the Republic of Ireland. Such a facility would allow rapid identification of circulating strains, would track changes in ribotype distribution and improve our understanding of the Irish C. difficile reservoirs of infection.

It is not surprising that ribotype O27 was found to be the most common ribotype in both the national project and north east project as it appears that this strain is well established in Ireland. Clearly, there is potential for interventions in both the hospital and long term care setting (e.g. review of antibiotic stewardship) which may impact on the epidemiology of C. difficile and improve overall patient morbidity and mortality.

Correspondence: E Brabazon
Department of Public Health, HSE North East, Railway St, Navan, Co Meath
Email: Elaine.Brabazon@hse.ie

Acknowledgements

M Wilcox and his team in the C. difficile reference facility in Leeds for helpful discussions and advice throughout this project and during outbreak periods in 2009. This work was supported through funding provided by the north east regional SARI committee.

References


