**Operational direction Public Health and surveillance**

**STUDY PROTOCOL**

Transmission of *Clostridium difficile* infection (CDI) in Belgian hospitals

2015_CDIF_transm research

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<tr>
<td>Written by</td>
<td>Dr Fiona Neely</td>
<td>Project responsible</td>
<td>12 Mar 2015</td>
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<td></td>
<td>Dr Marie-Laurence Lambert</td>
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| Approved by   | Boudewijn Catry           | Head of Unit   | 12 Mar 2015 |

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1. **GENERAL INFORMATION**

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2. **OBJECTIVES OF THE STUDY**

2.1 **Primary objectives**

- To measure what proportion of clinical *Clostridium difficile* infections (CDI) with hospital onset, result from transmission from symptomatic cases within the same hospital
- To study how transmission varies between hospitals in Belgium and measure the association between CDI incidence in different hospitals and the proportion of cases acquired from another symptomatic case

2.2 **Secondary objectives**

- To calculate the mean reproductive number (R) for transmission of CDI within Belgian hospitals
- To ascertain what proportion of second episodes in the same hospitalised patient are recurrences with the same ribotype i.e. relapse, or are re-infections with a different ribotype
3. **SCIENTIFIC BACKGROUND AND PUBLIC HEALTH RELEVANCE**

Studies in the 1990s\(^1,2\) indicated that many cases of hospital diagnosed CDI were acquired through hospital transmission between symptomatic cases. In addition, the large number of hospital outbreaks documented in the early 2000s throughout North America and Europe was seen to support this view.

Transmission from asymptomatic carriers has also been implicated in in-hospital transmission and although asymptomatic carriage is associated with demonstrable skin and environmental contamination\(^3\), estimates of the contribution of asymptomatic carriers to onward transmission have varied considerably\(^1,4\) and Eyre et al\(^5\) found no evidence of onward transmission in their recent study using whole genome sequencing.

In addition, the same group of researchers used whole genome sequencing to analyze transmission patterns between symptomatic hospitalized patients.\(^6\) They concluded that in an environment where standard infection control precautions were effectively implemented, only 35% of cases of CDI were genetically related to at least one previous case.

The implication is that many patients have acquired their *Clostridium difficile* outside the hospital and go on to develop symptomatic CDI once their endogenous *Clostridium difficile* is exposed to exacerbating factors such as antibiotics or proton pump inhibitors.\(^7\)

The importance of identifying the contribution of each of these pathways is vital to effective targeting of prevention and control interventions. Standard infection, prevention and control interventions largely target the pathway between symptomatic patients and non-colonized patients. Isolation of asymptomatic colonized patients is currently not feasible, as they remain mostly unidentified. In settings where standard infection, prevention and control interventions are already effectively in situ, it may be that interventions that target the pathway between colonization and emergence of *Clostridium difficile* associated diarrhea, such as antibiotic stewardship, should assume a higher priority.

Using the highly discriminatory method of Multi-Locus Variable number tandem repeat Analysis (MLVA) testing\(^8,9\) this research, therefore, aims to ascertain the proportion of cases attributable to transmission within Belgian hospitals and whether this varies substantially between hospitals, in order that interventions to prevent CDI may be more appropriately targeted.
4. METHODS

4.1 Overall study design

The study will be based on data arising from the existing national surveillance programme for Clostridium difficile infection (CDI), capitalizing on the clinical, laboratory and information technology networks that are currently in routine use.

4.2 Data collection

Case-based reporting of CDI in hospitalized patients was mandatory by law in Belgian acute care hospitals between 2007-2014. For a minimum period of at least 6 months per year, every acute hospital had to report online (via the NSIHweb1 user interface) all symptomatic, toxin-positive cases and send five consecutive isolates to the national reference laboratory (NRC) for ribotyping. Since 2015, this surveillance has become one of an obligatory choice of four hospital associated infection surveillance programs. A detailed protocol for the CDI surveillance program is available on the website of the Scientific Institute for Public Health (WIV-ISP) http://www.nsih.be/surv_cdif/download_fr.asp or http://www.nsih.be/surv_cdif/download_nl.asp.

For this research, Belgian hospitals will be invited to participate if the number of cases previously registered on NSIHweb1 in 2013 exceeded 30 for the whole year or 15 per semester for those hospitals participating for just one semester. The only additional requirements on top of the already existing ones are for participating hospitals to provide surveillance data for the complete year of 2015 and to send cultures from all their cases to the reference laboratory for Clostridium difficile typing. Each participating centre will be asked to keep isolates and to send them by batches following phone or email agreement with the NRC.

Key variables that are collected as part of the routine surveillance are: sex, age, date of hospital admission; date of admission to department where CDI diagnosed; date of discharge; date sample taken for lab; toxin positive (stools & culture); diarrhoea on admission; date of onset of diarrhoea; likely origin of infection – home, declaring hospital, other hospital, long term care facility, other institution; recurrent case; clinical evolution – death within 30 days as direct, indirect cause of or not related to CDI, pseudomembranous colitis, admission to ICU, surgical intervention, non-complicated.
4.3 Laboratory methods

For occurrences of \( \geq 2 \) of the same ribotype within the same hospital within 3 months of each other in 2015, the ribotyped samples will be further analysed by the more discriminatory test Multiple-Locus Variable number tandem repeat Analysis (MLVA) to identify whether the samples have arisen from the same clone.

For occurrences of a second episode of infection in the same patient, ribotyping, and if necessary MLVA testing, will be undertaken to identify whether the subsequent episode is a re-infection with a different genetic strain or a relapse (i.e. recurrence with the same genetic strain as the previous episode).

Ribotyping will be carried out by extracting DNA with chelex and 16S - 23S rRNA intergenic spacer regions will be amplified using primers as described by O’Neill et al.\(^{10}\) Amplicon sizes will be analysed by capillary electrophoresis using an automatic sequencer (ABI 3100 Automated Capillary DNA Sequencer) and GeneMapper Analysis (Applied Biosystems, Inc.). A 35–500 bp ROX ladder (ABI) will be used as internal marker. Profiles will be analyzed by comparison with those of reference strains from the European collection (Brazier classification) and with our own database.

MLVA is a method used to perform molecular typing of microorganisms and has been applied to \textit{C. difficile}. It utilizes the naturally occurring variation in the number of tandem repeated DNA sequences found in the microbial genome of most bacterial species. A series of PCR amplifications are performed in several loci selected because they contain characteristic repetitive sequences. Depending on the allele, the number of these repetitive sequences varies giving amplicons of different sizes. Applied to \textit{C. difficile} it allows to discriminate isolates of the same ribotype.\(^{8,9}\)

4.4 Linkage between laboratory and epidemiological data

Linkage will be established between laboratory and epidemiological data using the automatically generated unique patient identity code on NISHweb1, which has been used since 2011 for this purpose. The combined data will be the basis for subsequent analysis.

4.5 Data management

Data collection, management, quality assessment and data confidentiality and security will be according to the national surveillance protocol as described in section 4.2 and in the quality assurance documents for the CDI surveillance programme (https://intranet.wiv-isp.be/quality/epidemiotoxicology/Health%20care%20associated%20infections/index.asp). In addition, the quality and completeness of data entry into NSIHweb1 and of sending specimen cultures appropriately to the reference laboratory will be checked manually by WIV-ISP every one to three months.
4.6 Definitions

**CDI case:** an episode of culture-confirmed, symptomatic CDI (symptomatic: at least 3 loose stools in less than 24 hours or pseudomembranous colitis or characteristic colonic histopathology)

**Genetically linked case:** case of symptomatic CDI sharing the same ribotype and MLVA patterns

**Serial interval:** number of days between dates of onset of diarrhoea in two genetically linked cases

**Secondary case:** a case with hospital onset of symptoms which is genetically linked to an earlier symptomatic case in the same hospital, and with a serial interval of no more than three months

**Index case:** the first occurrence of a case amongst genetically linked cases in the same hospital in the calendar year 2015.

**Recurrent case** (as per surveillance protocol): a second CDI episode in a patient with onset of symptoms within 8 weeks of the start of a previous episode, as long as there has been a complete resolution of symptoms between episodes (with or without treatment) and will be designated as recurrent by the clinician encoding the case on NSIHweb1.

Recurrence can be divided into re-infection and relapse; where **re-infection** is a subsequent infection in the same patient with a different ribotype and/or MLVA and **relapse** is a recurrence with the same ribotype and same MLVA as the previous episode.

4.7 Data analysis

On the basis of the number of cases reported by the invited hospitals in 2013, we expect the reference laboratory to receive approximately 1000 specimens from 36 hospital sites for typing and suitable for analysis during the whole of year 2015.

We will provide descriptive epidemiology of hospitals, cases and ribotypes

*Primary outcome measures:*

- Proportion of the total number of episodes of symptomatic CDI attributable to local transmission (from symptomatic cases) will be computed as the number of secondary cases / total cases with hospital onset.

- Correlation coefficient for association between incidence of CDI and the proportion of secondary cases (with evidence of local transmission) within participating Belgian hospitals in 2015

*Secondary outcome measures:*

- Mean reproductive number for transmission of CDI within participating Belgian hospitals in 2015 = mean number of secondary cases per index case of CDI

- For pairs of samples provided from two different episodes in the same patient for which a diagnosis of recurrence has been made, we will calculate the proportion that are true relapse, and the proportion that are reinfection with another strain. i.e.

  Proportion of relapse among recurrent cases = number of recurrent episodes genetically linked / total number of recurrent episodes
5. **ETHICAL REVIEW**

This study has been approved by the Université Catholique de Louvain ethical committee (CEHF Comité d’Ethique Hospitalo-Facultaire Saint Luc).

6. **TIMETABLE**

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<th>Task</th>
<th>Start date</th>
<th>Completion date</th>
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<tr>
<td>Agree principals with reference lab (NRC)</td>
<td>Nov 14</td>
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<td>Y</td>
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<tr>
<td>Send out invitation to hospitals</td>
<td>Dec 14</td>
<td>Dec 14</td>
<td>Y</td>
</tr>
<tr>
<td>Data collection begins</td>
<td>1/1/2015</td>
<td>31/12/2015</td>
<td>Begun</td>
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<tr>
<td>Send repeat invitation to non-responders</td>
<td>Jan 15</td>
<td>Jan 15</td>
<td>Y</td>
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<tr>
<td>Deadline for agreement of participation</td>
<td>End Jan 15</td>
<td>End Jan 15</td>
<td>Y</td>
</tr>
<tr>
<td>Lab to send sample labels to participating hospitals</td>
<td>Jan 15</td>
<td>mid-Feb 15</td>
<td>Y</td>
</tr>
<tr>
<td>Write protocol &amp; agree with lab</td>
<td>Dec 15</td>
<td>Start mar 15</td>
<td>y</td>
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<tr>
<td>Apply ethical approval from UCL</td>
<td>Mar 15</td>
<td>End mar 15</td>
<td>y</td>
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<tr>
<td>Check data collection from hospitals (every 1-3 months)</td>
<td>Start Feb 15</td>
<td>Jan 16</td>
<td>Begun</td>
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<tr>
<td>Lab analysis of <em>C. difficile</em> cultures – ribotyping &amp; MLVA tests</td>
<td>Feb 15</td>
<td>May 16</td>
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<tr>
<td>Data analysis</td>
<td>Mar 16</td>
<td>Jul 16</td>
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<td>Write up</td>
<td>Jul 16</td>
<td>Sep 16</td>
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<td>Submit for publication</td>
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7. **STRENGTHS AND LIMITATIONS**

7.1 **Strengths of study**

- The study will make use of existing networks and data collection. No additional patient information will be required over and above the normal epidemiological data collection.
- The study will type complete hospital cohorts of CDI patients arising over a wide geographic distribution throughout Belgium.
- A large number of cases will be available for typing.
- Epidemiological and laboratory typing information can be linked.

7.2 **Benefits and risks to participating hospitals**

- Each hospital will be able to review the distribution of strains of CDI and transmission patterns within their hospital. This should assist them in targeting prevention and control interventions against CDI.
- Hospitals will be required to send cultures to the reference laboratory for all their patients with CDI, compared to the normal surveillance requirement of 5 consecutive samples per semester. This will entail an extra workload for those who participate and will exclude a small number of hospitals which no longer carry out cultures for the diagnosis of CDI.

7.3 **Benefits and risks to participating patients**

- There will be no direct benefit or risk to patients in the short term from this study but in the long term this study should contribute to more effective targeting of interventions to the prevention CDI.

7.4 **Limitations of study**

- This study will miss transmissions which have occurred in the first or last months of the study (secondary cases with an index case before the study started, or index case during the study but secondary cases after the study). Analyses can take these limitations into account.
- This study will miss transmissions with a serial interval of > 3 months. These are expected to be very rare, however. Depending on laboratory capacity to carry out a large number of MLVA tests, by extending the serial interval cut-off to 6 months or the whole year to define possible transmissions, we plan to do a sensitivity analysis in order to identify how many transmissions are missed.
- No variables regarding the antimicrobial consumption, an important trigger for CDI, will be available/colllected.
- This study necessarily encompasses the limitations of the national surveillance system itself, such as differences in case detection between hospitals.

8. **COMMUNICATION OF THE RESULTS AND REPORTS**

- Individualised reports to participating hospitals
- A full study report will be written (anonymising hospitals)
- The study will be submitted for peer review publication
9. **REFERENCES**


