FULL STUDY PROTOCOL for COMBACTE-CDI

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COMBACTE-CDI

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1. Introduction

1.1 Study rationale

COMBACTE-CDI addresses the specific challenge related to CDI as formulated in the Innovative Medicines Initiative (IMI) 2 grant call text,(http://www.imi.europa.eu/apply-funding/closed-calls/imi2-call-9) and which the consortium was successfully awarded in 2017. The public-private partnership in COMBACTE-CDI will quantify the burden of CDI via a large, complex, multi-centre, multi-country study, describe current management practices and set-up a consolidated, European, CDI, clinical research platform. As indicated within the call, the need and opportunity for public-private collaborative research is important to meet the clinical challenges provided by CDI in European healthcare systems. Therefore, different perspectives and a broad range of diverse evidence sources will be gathered and synthesised. A number of key stakeholders have been incorporated into this public-private partnership to ensure the success of the collaboration. In addition, mapping of and coordination with all stakeholders is integral in our proposal.

COMBACTE-CDI fully addresses the specific scope as formulated. The better quantification of CDI burden, understanding of transmission and more complete description of control practices across Europe will provide a basis for the further development of public health interventions and practices.

1.2 Objectives and scope

This project, termed Combatting Bacterial Resistance in Europe – *Clostridium difficile* Infections (COMBACTE-CDI), aims to develop a detailed understanding of the epidemiology and clinical impact of CDI. More specifically, this project's objectives are to:

- Align and understand the unmet public health needs relating to CDI;
- Identify and quantify the direct and long-term burden of CDI on healthcare systems;

COMBACTE-CDI will synthesise all efforts made at European Union (EU) level on CDI, and crucially will align clinical/research consortia that have developed independently, one focussing on CDI and the other on AMR, and the CDI-related expertise of 7 EFPIA (industry) partners. This alignment between existing research consortia and EFPIA partners will optimize research and managerial efficiency. In addition, COMBACTE-CDI will perform a large, complex study to enable the delivery of data to answer multiple research questions on the epidemiology of CDI.

1.2.1 Work-package 1 objectives

The aim of this work-package is to deliver a more complete understanding of CDI epidemiology, risk factors and burden of disease across European countries, via a consistent, comprehensive data collection approach that builds on and extends existing information, and notably addresses knowledge gaps according to the whole healthcare economy within each country

Objective 1.1	Collection and testing of residual diagnostic tissue to determine 'missed' cases
Objective 1.2	Strain distribution (characterisation of strains)
Objective 1.3	Collection of participant data regarding outcomes of CDI (case/control study)
Objective 1.4	Collection of participant data regarding risk factors for CDI (Case/control study)
Objective 1.5	Transmission model constructed from datasets collected in objectives 1.1-1.4.

1.2.2 Work-package 2 objectives

The aim of this work-package is to highlight current guidelines for CDI diagnosis and treatment, at both European and national levels, and measure compliance with these. We will also measure the impact of CDI on healthcare systems, and determine if better compliance with guidelines leads to a

lower burden in terms of reducing cases and costs. Objectives and deliverables for WP2 will be closely aligned with those for WP1, using a synergistic approach to study design (see Figure 1). The timing of the objectives has been planned so that data and analyses generated from WP1 will be available for analyses in the required objectives in WP2. In addition, much of the analysis in the cost-effectiveness objective will be based on data generated from earlier objectives within WP2; the timing of these two objectives (O2.5 and 2.6) has therefore been planned accordingly.

Objective 2.1	To highlight current guidelines for disease detection, notification and management/control
Objective 2.2	To determine the heterogeneity in testing density and in following the existing guidance (including identification of missed diagnoses and empiric treatment approach practices)
Objective 2.3	To determine the heterogeneity in current surveillance practices and ways to improve them.
Objective 2.4	To describe current treatment pathways for patients with CDI infection and recurrences.
Objective 2.5	To elucidate the overall costs of CDI, including management of multiples episodes and re-hospitalisation (healthcare perspectives incl. indirect financial impact on the healthcare system).
Objective 2.6	To measure the impact of CDI management on the healthcare system and the impact of current control measures (infection control/antibiotic stewardship).

1.3 Study design of COMBACTE-CDI (overview)

To achieve our objectives, a large, complex study will be carried out (Figure 1), using proven methodology from a previous European wide (20 countries) CDI surveillance project (EUCLID network)[1]. In short, hospitals/laboratories of interest which carry out diagnostic testing of samples from both in-patients and community patients (including Long-Term Care Facilities patients) will be approached for inclusion. Hospitals/laboratories of interest will be selected to give respective coverage, based on population size, in the countries of interest and to represent North, South, East and West Europe.

Two study days will be selected, and all diarrhoeal stool samples received on those days by the hospital/laboratory in the study will be sent (regardless of age of patient or test requested) to the European Coordinating Laboratory (Leeds), where samples will be tested for CDI using the recommended 'optimal' testing methodology (ESCMID guidelines) (O1.1). Relevant ethical approvals will be sought (please see ethics section 1.4). Results will be compared to those from the submitting hospital/laboratory, including if the sample was even tested. Missed cases will be identified, enabling an estimation of the true burden of disease in both hospitals and the community. Isolates of *C. difficile* identified from the community and hospital samples will be sent for further analysis; including PCR-ribotyping, toxinotyping and whole genome sequencing to identify factors that may be driving clustering of strains (O1.2). Additional strains of animal and food origin collected from an existing network (NLZOH) will be added to the analysis for comparison between human and animal strains.

A clinical report form (CRF) will be designed to capture data for risk factor analysis and outcome data on all positive (ECL defined) and a proportion of CDI negative samples; completed 6 months after sample collection data (O 1.3 and 1.4). Please see section 1.4 for relevant ethical considerations.

All data collected from O1.1, 1.2, 1.3 and 1.4 will be brought into the analysis for transmission modelling in O1.5.

Hospitals/laboratories and their associated community practices will complete a surveillance questionnaire regarding:

- Testing policy and methodology;
- Surveillance practices;
- Guidelines available and compliance;
- The criteria for stratification of patients into 'severe' or 'non-severe' cases

All these data will be uploaded (by the national coordinators) to a central website, thereby providing data for O2.1, 2.2, 2.3, 2.4

All of the data from the previous objectives in WP1 and WP2 will be fed in to the cost effectiveness analysis in O2.5 and 2.6

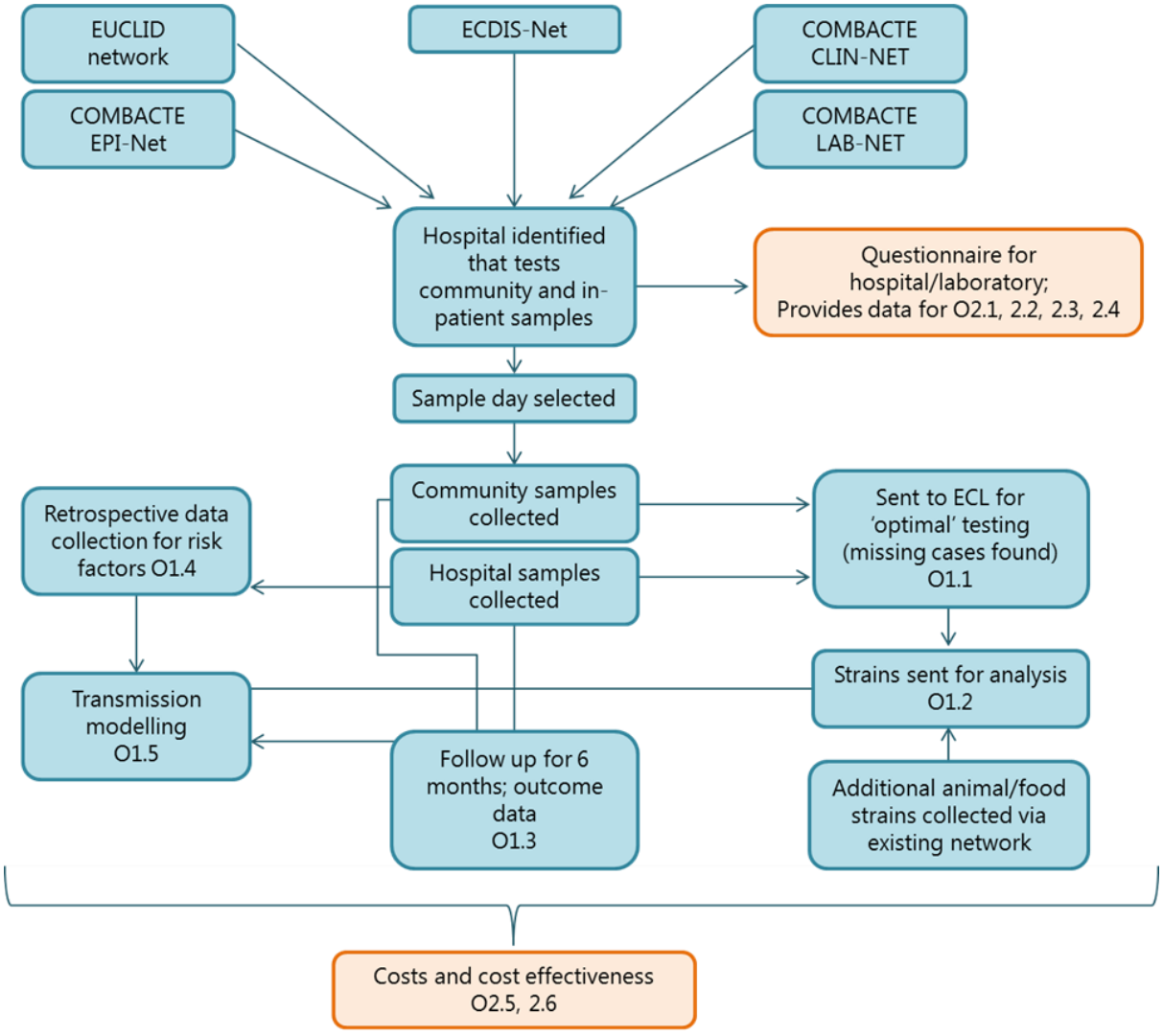

Figure 1. Schematic representation of study design (WP1 &WP2). A synergistic approach will enable completion of all the objectives from WP1 and WP2, with data created from a single, large complex study flowing into other objectives for further results analysis. Boxes in blue denote objectives in WP1, and those in orange denote for objectives in WP2. Due to the inter-related nature of the objectives within both WP1 and WP2, the flow of work has been planned to ensure maximum outputs within the timeframe of the grant. The directions of the arrows indicates data flow and, therefore, also the timing of activities;. i.e. Strains cannot be analysed (1.2) until after they have been collected (1.1). This ambitious comprehensive project design takes influence from several

existing, separate studies; the scope of producing all of these is data from one, well-designed and far-reaching study is novel and innovative.

1.4 Ethical and regulatory approvals

1.4.1 General overview

The research involves residual diagnostic tissue (non-interventional study) and collection of clinical data from participants notes on outcomes and risk factors; therefore, the following ethical aspects have been considered:

- Research on humans (clinical studies);
- Observational, non-interventional, research using data from the treatment of children;
- Use of human tissues (biological samples);
- Privacy/data collection (data collected from participant databases).

Ethical standards and guidelines compatible with, and equivalent to, those of Horizon2020 will be rigorously applied, regardless of the country where the research is taking place. Every partner will agree to follow all relevant national and EU legislation, and associated guidelines, relating to the conduct of project work. We will ensure that as per H2020 guidelines we adhere to the prevailing EU law, being mindful of the fact that the exemptions under Article 89 of the GDPR 2018 may vary between countries. We have however tried to mitigate for this during the initial proposal, as we sought advice from several countries on their local laws, and this has helped to determine in which countries this research will be feasible. Ethical approval will be sought from every country taking part in the study. All of the ethical implications of this project were reviewed by the ethical review board of IMI, as part of the grant application, to ensure that it met with all the relevant regulations (current and upcoming).

1.4.1.1 Key EU legislation, standards, and guidelines for human research

Key references that govern the conduct of human research are listed below. This list is not intended to be all-inclusive.

- ICH GCP International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use: Good Clinical Practice Guidelines; (2016)
- CIOMS: (1) International Guidelines for Ethical Review of Epidemiological Studies (1991); and (2) International Ethical Guidelines for Biomedical Research Involving Human Subjects (2002);
- WMA: Declaration of Helsinki (2008);
- UNESCO: Universal Declaration on Bioethics and Human Rights (2005);
- CoE: (1) Convention on Human Rights and Biomedicine (Convention of Oviedo), Articles 15-18 (1997); and (2) Additional Protocol on Biomedical Research (2005);
- EMA/219517/2012 publication ”Towards a robust global framework for conduct and oversight of clinical trials”.
- EU General Data Protection Regulation (GDPR) 2018

1.4.2 Description of studies within COMBACTE-CDI and their associated ethical considerations

Work-package 1

Objective 1.1 Collection and testing of residual diagnostic tissue to determine 'missed' cases

A large study will be carried out, using proven project methodology from a previous European wide (20 countries) CDI surveillance project[1]. Hospitals/laboratories of interest which carry out diagnostic testing of samples from both in-patients and community patients (including Long-term Care facilities patients) will be approached for inclusion. The number of hospitals/laboratories will be selected to give proportional representation for each country included (with one study site/3 million population). In addition, the countries selected will proportionally represent each of the four zones of Europe (North, South, East and West, as defined by the UN Geoscheme for Europe), as stark differences between hospital CDI burden in these zones was shown in the previous study[1].

Two study days will be selected, and all diarrhoeal stool samples received on that day by the hospital/laboratory in the study will be sent (regardless of test requested) to the European Coordinating Laboratory (Leeds), where samples will be tested for CDI using the recommended 'optimal' testing methodology (ESCMID guidelines). Results will be compared to those from the submitting hospital/laboratory, including if the sample was even tested. Missed cases will be identified, enabling an estimation of the true burden of disease in both hospitals and the community.

All data held by the study team will be pseudonymised to a study number. Pseudonymisation of data in this way is in-line with the current EU Data Protection Directive and the upcoming General Data Protection Regulation (Spring 2018) specifically Article 89 (1), and all sites will abide by the principals of these legislative requirements regarding confidentiality and security of data. Only the original submitting laboratory will hold the code to link back to the patient. All samples will be sent for processing under the study number only. The link to the participant will be stored at the participating hospital/laboratory until after data verification (see objectives 1.3 and 1.4), and then destroyed. The anonymised research data (with no personal identifiers) will be securely stored for 10 years. It is an widely accepted ethical principal to ensure that there is the maximum scientific output from the data collected, and future research may re-use the data that has been collected; this will only be permitted after this has been agreed by the data guardian for the project and full ethical approval for the new analysis has been granted after ethical/regulatory review.

Informed consent is one of the cornerstones of the declaration of Helsinki. However, as detailed in the Recommendation Rec (2006)(4) of the Council of Europe Committee of Ministers to member states on research on biological material of human origin, Article 22 para 1 (i) 'If contacting the person concerned is not possible with reasonable efforts, these biological materials should only be used in the research project subject to independent evaluation of the fulfilment of the following conditions;

- The research addresses important scientific interest
- The aims of the research could not reasonably be achieved using biological materials for which consent can be obtained and
- There is no evidence that the person concerned has expressly opposed such research'.

Informed consent is not required for the use of pseudoanonimised residual diagnostic material. In order to truly assess the burden of CDI we need to alleviate selection bias, which may be introduced by 'selecting' samples to include in the study. Understanding the true burden of CDI is one of the main objectives of the IMI2 grant award. It is also imperative for detection of *C. difficile* toxin that samples are tested within 3 days of receipt (from the participant) or the degradation of toxin may

lead to false negative results. It would be impossible therefore to contact each patient (after their sample has been identified) and ask for informed consent to use their sample within this time window, especially given that many of these samples will have come from patients in the community, who would need to be contacted by post. We feel therefore that it is justified not to obtain informed consent to use the residual diagnostic tissue (stool samples) for further CDI testing, subject to full ethical approval to do so (after ethical review). Articles 12(1) and 13(2) of the GDPR require 'appropriate measures' to inform participants of their inclusion in research and their related rights. In addition, under Article 9(1) 'Processing of personal data revealing racial or ethnic origin, political opinions, religious or philosophical beliefs, or trade union membership, and the processing of genetic data, biometric data for the purpose of uniquely identifying a natural person, data concerning health or data concerning a natural person's sex life or sexual orientation shall be prohibited'. However, Article 9(2) states 'Paragraph 1 shall not apply if' one of a number of clauses are met. We believe that Article 9 para 2(j)) applies here; 'processing is necessary for archiving purposes in the public interest, scientific or historical research purposes or statistical purposes in accordance with Article 89(1) based on Union or Member State law which shall be proportionate to the aim pursued, respect the essence of the right to data protection and provide for suitable and specific measures to safeguard the fundamental rights and the interests of the data subject.' Under article 14, para 5(b) there is an exemption from providing information to potential participants regarding the research if this would require a disproportionate effort. Given that potential participants could be anyone within the catchment area of a particular hospital laboratory (both in-patients and community patients) it would be exceedingly difficult to ensure that we could inform every one of the activity. We therefore believe, based on Article 9 para 2(j) and Article 14 para 5(b) the decision to waive consent and waive the requirement to take reasonable measures to inform participants of their inclusion, is compliant with the upcoming regulations. There is provision within the sub-contracts to ensure that the study sites comply with GDPR, and they should ensure they have appropriate measures in place to ensure data confidentiality and non-disclosure.

Collection of human tissue

We will not be prospectively collecting additional clinical samples. We will collect residual diagnostic stool samples that have been submitted to laboratories for microbiological testing. The following general principles will apply to all research conducted in COMBACTE-CDI;

- A human sample is defined as any human tissue or biological material, including any portion of an organ, any tissue, skin, bone, muscle, connective tissue, blood, cerebrospinal fluid, cells, or any derivative of such human biological material such as stem cells or cell lines; and any human biological product, including, but not limited to, hair, nail clippings, teeth, urine, faeces, breast milk, and sweat. Our study is limited to collecting residual diagnostic stool samples.
- No additional samples will be collected.
- In all cases, following initial planned analysis, remaining aliquots will be stored centrally at University of Leeds, UK, for the duration of COMBACTE-CDI to ensure samples can be re-analysed as new data emerges to maximise benefit to society. At the end of the project, additional provisions will be made for storage or disposal as appropriate. This will be made clear in the ethical approval application.
- All processing of clinical samples and experiments will be performed in laboratories equipped with class II safety cabinets.
- When working with biological material, each partner must ensure that strict safety procedures are in place and in compliance with national and EU regulations on biosafety.

Residual diagnostic faecal samples will be sent to the University of Leeds (European Coordinator) from each of the participating sites. Samples will be processed and stored within the Healthcare

Associated Infections Research Group Facilities at University of Leeds for the period of the COMBACTE-CDI project. A small portion of a selected number of samples will be sent to another COMBACTE-CDI partner (bioMerieux, France) for analysis by the SiMoA test, as this novel technology is not yet available elsewhere. Once SiMoA testing is complete any residual material will be destroyed.

Key EU legislation for human tissue use

Key references that govern the conduct of research on human tissue samples are listed below. This list is not intended to be all-inclusive.

- Directive 2004/23/EC on Setting Standards of Quality and Safety for the Donation, Procurement, Testing, Processing, Preservation, Storage, and Distribution of Human Tissues and Cells
- CoE: (1) Convention on Human Rights and Biomedicine (Convention of Oviedo), Articles 21-22 (1999) and (2) Recommendation Rec (2006) 4 of the Committee of Ministers to Member States on Research on Biological Materials of Human Origin (2006)
- WHO: Guideline for Obtaining Informed Consent for the Procurement and Use of Human Tissues, Cells, and Fluids in Research (2003)
- ITA: Infectious Substances and Diagnostic Specimens Shipping Guidelines (2005)
- ISBER: Best Practices for Repositories I: Collection, Storage and Retrieval of Human Biological Materials for Research (2005).
- EU General Data Protection Regulation (GDPR) 2018

Objective 1.2 Strain distribution (characterisation of strains)

C. difficile isolates collected from the study samples will be typed by various methods (PCR-ribotyping, toxinotyping, whole genome sequencing), to determine strain relatedness and distribution. All of the residual diagnostic stool samples will be processed in the laboratory facilities at the Healthcare Associated Infection Research Group (HCAI) at the University of Leeds. The HCAI laboratory is also the *Clostridium difficile* reference laboratory for the UK and has >20 years of experience in handling stool specimens and bacterial isolates derived from the samples. The laboratory follows all relevant regulatory processes for the UK, and all staff are fully trained in the relevant health and safety procedures. All work is risk-assessed for hazards (microbiological, chemical, and procedural) before commencement. Samples are handled in a class II Microbiological safety cabinet to protect the researcher. Isolates of *C. difficile* will be sent to the National laboratory for health, environment and food (NLZOH), in Slovenia, a COMBACTE-CDI partner. Again the NLZOH has >20 years of experience in handling the organism *C. difficile* and the laboratory follows all relevant regulatory processes, and all staff are fully trained in the relevant health and safety procedures. All work is risk-assessed for hazards (microbiological, chemical, and procedural) before commencement. All laboratory work will be performed in the facilities mentioned above, which are containment level 2 facilities, who comply with all local and national laboratory health and safety regulations. Both laboratories follow strict waste disposal policies to ensure the correct disposal of microbiological material, to prevent accidental environmental contamination.

No participant identifiable data is associated with the strains; each is identified by a sample code only. A dataset from Leeds will be provided to NLZOH which will give the origin (sample source site) of the sample from which the isolate came, so that comparisons can be made between isolates derived from different sample source sites. This dataset will not contain any personal or health data. Bacterial isolates derived from human biological materials do not require consent and/or IEC approval for use in research.

Objective 1.3 Collection of participant data regarding outcomes of CDI (case/control study) and Objective 1.4 Collection of participant data regarding risk factors for CDI (Case/control study)

All CDI positive cases (as determined by the ECL), and a proportion of negative cases (at a ratio of 1 case to 3 controls) will have clinical data collected (after 6 months) on patient outcomes (O1.3) and risk factors for disease (O1.4). Data will be compared to identify differences between countries and healthcare providers.

Informed consent

Informed consent will not be sought, as this is not required for collection of already recorded health data, that has been robustly pseudonymised at source. The researchers will never have access to personal identifiable information. CDI occurs in all ages, but the prevalence increases in the elderly, especially in those with co-morbidities and who are often frail. Recurrent visits to hospital are common in elderly patients who lack capacity, due to the complicated nature of their on-going care. This places them at increased risk of CDI and to exclude them from this project would be unethical. It is imperative that we understand the true burden of disease in these individuals, not just in hospital but within the community and the impact it has on their lives in term of morbidity and mortality. Whilst their inclusion in the testing of residual diagnostic tissue will allow us to estimate the burden of disease, only the case/control study will allow us to measure the impact it has on these individuals. The same arguments hold true for children, in which CDI may be an under-recognized condition. Understanding the true impact of CDI in these vulnerable groups will inform better diagnostic and treatment options for these groups in the future.

Data protection

The Data Protection Directive is the cornerstone of EU personal data protection. However, in January 2012 the European Commission proposed a comprehensive reform of the data protection rules, to update and modernise the principles enshrined in the 1995 Data Protection Directive to guarantee privacy rights in the future. Central to these proposals is a new regulation (EU General Data Protection Regulation (GDPR) 2018), specifically Articles 12 (1), 13 (1) and 89 (1), which to set out a general EU framework for data protection and which includes special rules on the collection and use of personal data for medical research and public health purposes. Emerging legislation will be monitored to ensure the long-term and on-going legitimacy of COMBACTE-CDI approaches to data privacy.

The following general principles will apply to all research conducted in COMBACTE-CDI.

Data handling will comply with current national and EU legislation, including:

- Personally Identifiable Information (PII) is adequately protected;
- Anonymisation/de-identification is conducted appropriately;
- Ethical review is completed as required.

Each COMBACTE-CDI member will be responsible for the confidentiality and security of personal identifiable information and for complying with their own SOPs and any shared COMBACTE-CDI SOPs.

In general terms, appropriate data protection principles will be observed, including:

- Data are fairly and lawfully processed;
- The amount of data collected is relevant and not excessive;
- All reasonable efforts are taken to ensure data accuracy;
- The data are used in accordance with the rights of the study participant;

- The data are stored securely;
- Relevant international and national guidance will be consulted.

All data held by the study team will be pseudonymised to a study number. Only the original submitting laboratory will hold the code to link back to the participant. All samples will be sent for processing under the study number only. After 6 months the original hospital will be asked to collect health data on a pseudonymised (study number only) clinical report form on all cases and a proportion of controls (CDI negative samples) identified by the central testing laboratory. Under GDPR pseudonimised data is classified as personal data. After collection of the data and verification/data querying has been completed (within one month of collection) the link will be destroyed at the local study sites, to ensure there cannot be any link to participants, rendering the data anonymous at this point. To add a further layer of protection all participating sites are also pseudonymised, to prevent link back to the site by consortium partners. The University of Leeds will hold the link back to the site code on a password protected spreadsheet on secure University of Leeds servers during the collection of the data; this will be destroyed after data collection and will not be archived.

Key EU legislation for human data use

Key references that govern the use of human data are listed below. This list is not intended to be all inclusive.

- Data Protection Directive 95/46/EC of the European Parliament and of the Council (1995)
- Convention for the Protection of Individuals with Regard to Automatic Processing of Personal Data (1985)
- Recommendation No. R (97) 5 on the Protection of Medical Data (1997)
- Declaration on Ethical Considerations Regarding Health Databases (2002)
- Data protection and privacy ethical guidelines of the Experts Working Group on data protection and privacy (2009)
- Article 29 Data Protection Working Party, Vademecum on Notification Requirements (2006)
- Article 29 Data Protection Working Party, Working Document on the processing of personal data relating to health in electronic health records (EHR) (2007)
- EU General Data Protection Regulation (GDPR) 2018) , specifically Articles 12 (1), 13 (1) and 89 (1),

Objective 1.5 Transmission model constructed from datasets collected in objectives 1.1-1.4.

An anonymised dataset, derived from the previous objectives, will inform the transmission modelling. The dataset will be anonymous at this point, as the link-back to the participants will have been destroyed. No participant identifiable data will be included in this dataset. All data will be held against a study number based on the sample number from objective 1.1. These data may include but may not be limited to demographic information, co-morbidities, patient outcomes (mortality/morbidity), recurrence of CDI, linked clinical and laboratory tests and results. The principals of data protection discussed above in O1.3 and 1.4, are also applicable to this objective.

Work-package 2

Objectives 2.1-2.4 Collection of surveillance data from participating sites (hospital and community practices)

Each participating centre and their associated community practices (sample source sites) will also be asked to complete a web-based questionnaire on guideline knowledge, compliance, testing policy and methodology, surveillance practices, and stratification of 'severe' patients. In addition, 10

nursing homes in each of the four regions of Europe will be asked to complete the surveillance form to address the knowledge gap with these facilities. Data will be compared to identify differences between countries and healthcare providers. Collection of surveillance data from participating sites and their associated community practices does not require approval from an ethical review board.

Objectives 2.5 Evaluation of costs based on data collected from O2.1-2.4.

The dataset used for this data analysis will be derived from objectives 2.1-2.4. This data was collected as part of surveillance and therefore does not require approval from an ethical review board.

Objective 2.6 Construction of cost effectiveness model based on data in O2.5 and dataset generated from work-package 1.

An anonymous dataset, derived from the previous objectives in both work-package 1 and work-package 2, will inform the cost-effectiveness model. No participant identifiable data will be included in this dataset. All data will be held against a study number based on the sample number from objective 1.1. These data may include but may not be limited to demographic information, co-morbidities, patient outcomes (mortality/morbidity), recurrence of CDI, linked clinical and laboratory tests and results. The principals of data protection discussed above in O1.3 and 1.4, are also applicable to this objective.

2. COMBACTE-CDI organisational structure

2.1 Funding and Sponsorship

COMBACTE-CDI receives support from the Innovative Medicines Initiative Joint Undertaking under grant agreement no 115523 115620 115737 777362 resources of which are composed of financial contribution from the European Union Seventh Framework Programme FP7/2007-2013) and EFPIA companies in kind contributions.

2.2 European coordinator

The lead partner for COMBACTE-CDI is University of Utrecht; however work packages 1 and 2 are led by the University of Leeds and will be coordinated from the Healthcare Associated Infections Research Group in The University of Leeds. The European Coordinator for the main study that forms the basis for objectives 1.1-1.4 is the University of Leeds.

2.2.1 European Coordinator role

- Identify National coordinators and oversee study
- Write standard operating procedures
- Write materials required for submission for ethical approval
- Design data capture sheets (manual and electronic) and databases
- Collate data from all national coordinators
- Data analysis and report writing
- Control of budget

2.3 National coordinating centres

One national coordinating centre will be recruited per country. Each national coordinating centre will correspond with the European coordinator directly. Each national coordinator will receive a budget to cover participating site recruitment, obtaining in-country ethical approval and coordinating all study activities within their country including distribution of study transport

packaging to participating sites. The national coordinator will also be responsible for the payments to participating hospitals for sample collection and follow up Clinical report forms and questionnaires. A full list of national coordinators can be found at the beginning of this document.

2.3.1 National Coordinator role

- Obtain ethical approval for the study in their country
- Recruit participating sites in their own country to the study
- Distribution of study transport packaging to participating sites
- Coordination of sampling from all participating sites within their country.
- Distribute CRF packs to sites
- Coordinate the distribution of surveillance forms to participating sites (and nursing homes in selected countries)
- Report to the European coordinator

2.4 Participating Sites

A small fee will be paid to each participating site to cover labour costs incurred in sending the samples. An additional small fee will be payable for each CRF completed as part of the case/control study, and the completion of the surveillance form for their site and coordination of surveillance form completion (by all associated community practices) as part of work package 2.

2.4.1 Participating Site recruitment

The number of participating sites to recruit for each country is shown in table 1 in the appendices. Participating sites should be selected to cover as broad a geographical area of each country as possible, with all geographical regions within a country represented (dependent upon the number of participating sites to be included). In addition, the countries selected will proportionally represent each of the four zones of Europe (North, South, East and West, as defined by the UN Geoscheme for Europe), as stark differences between hospital CDI burden in these zones was shown in the previous study. The letter to invite the site to participate should be sent to the laboratory manager/lead Clinician of the Microbiology/Pathology department of the chosen site. A copy of the template Participating Site invitation letter can be found in Appendix 2.

2.4.2 Participating Site definition

A **participating site** is a hospital/laboratory facility that provides enteric testing and fulfils the criteria below.

A **sample source site** is any location that sends a sample to the participating site to be tested on the sampling day (e.g. hospital, community doctor, nursing home, mental health facility).

All **participating sites** must meet the following criteria:

- Sites must carry out diagnostic testing of faecal samples from both in-patients and community patients
- Sites must be able to collect residual diagnostic tissue of all faecal samples received by the laboratory on a specified date in May/June and October 2018, and export this to the UK the day after collection (transport provided)
- Sites must be able to collect prospective patient data (outcome data) linked to the faecal samples provided during the sampling period (based on a provided Case Report Form) either directly or via the **sample source site**
- Sites must be able to collect retrospective patient data (risk factor data) linked to the faecal samples providing during the sampling period (based on a provided Case Report Form) either directly or via the **sample source site**

- Sites must be able to collect data on attributable costs of CDI at their site (based on a provided Case Report Form)
- Sites must be able to provide data in regards to their CDI testing policy and methodology, CDI surveillance practices, knowledge of national CDI guidelines and their local compliance, and CDI treatment (including according to patient level risk e.g. CDI severity) (based on a provided questionnaire).

2.4.3 Participating Site packs

Each participating site should be allocated a date, following the site sampling plan provided to each National Coordinator. Each participating site should be sent a pack containing; the instruction leaflet to explain how to process the samples for transport to the European Coordinator, transport materials, the sampling date and copy of the data collection sheet (sample information only) at least a week before the date of sampling. Samples should be stored at 4-8°C until transport.

2.4.4 Materials required

2.4.4.1 Provided by European Coordinator

- National coordinator study packs (sample collection)
    - Site list and sampling plan
    - Template site invitation letter
    - National Coordinator instructions for sample collection
- Participating site study packs (sample collection)
    - Participating site cover letter following confirmation of participation
    - Participating site sampling and shipping instructions
    - Sample collection spreadsheet
    - CDI sample result spreadsheet
    - Template material transfer agreement
- Packaging materials for distribution to participating sites (to send samples to the European coordinator via DHL).
- National Coordinator study pack (case control study)
- Participating site study pack (case control study)
- National Coordinator study pack (surveillance study)
- Participating site study pack (surveillance study)
- Copy of UK ethical approval

Study packs will be distributed to National Coordinators prior to the relevant sub-study (sample collection/case control/surveillance).

3.2.2 Provided by National Coordinator

- Completed site list and sampling plan
- Copy of in-country ethical approval

3.2.3 Provided by Participating site

- Completed and signed material transfer agreement
- Faecal samples and completed CDI sample result spreadsheet
- Clinical data on case control studies (CRF completion)
- Data for surveillance questionnaire

3. Study methods

3.1 Sample collection study (objective 1.1 and 1.2)

3.1.1 Study rationale

Only two studies (in the UK & the Netherlands) have determined incidence rates, clinical characteristics and outcome of community-acquired CDI (CA-CDI), both reporting CDI positivity in 1.5-2.1% of patients attending a general practitioner because of diarrhoea[1,3]. Given the number of patients that may never even be tested, this may well represent an underestimate of the true incidence of CA-CDI. The burden of CDI in LTCF and its relation with that in neighbouring hospitals is largely unknown. A systematic review identified only 8 studies for critical appraisal with none containing economic evaluations of interventions[4]. The estimated incidence of CDI in LTCF was 0.41/10,000 residents per year. ECDC initiated surveillance of HAIs and antimicrobial use in European LTCF (HALT) project, including two point prevalence surveys[5,6] in >750 LTCF in ~20 European countries. This study reported HAI prevalence of 2.4-3.4%, of which 5.1% represented gastrointestinal infections; of which 18% were due to *C. difficile*. However, CDI was not surveyed systematically, and no information was collected on the diagnostic methodology. We aim to address this gap.

PCR ribotyping is the method of choice for typing *C. difficile* strains. Currently, >800 PCR ribotypes, (designated 001, 002…) are known; the reference ribotype collection is held by the co-applicant's (MHW) laboratory in Leeds, UK. Knowledge of strain distribution improves understanding of epidemiology (outbreaks), transmissions (within hospital, between reservoirs, e.g. animal to human) and potential differences in virulence. Indeed, some PCR ribotypes (e.g. ribotype 027, 078) appear more virulent, with emerging evidence that some are also more transmissible[7].

PCR ribotype distribution varies between European countries and over time, with some types remaining prevalent for decades (e.g. ribotypes 014, 002, 027, non-toxigenic 010)[8-11] However, these studies were based on isolates from hospitalised patients collected within limited time intervals. Notably, comparable data on a European scale are lacking for CA-CDI and CDI in non-hospital settings. From studies at local or national level, it is known that different strains would be more likely associated with hospital (e.g. ribotypes 027, 176) and community (e.g. ribotypes 078, 244) infection, although there is also some overlap[12].

Strains collected from the main study (O1.1) will be compared, by PCR-ribotyping, along with basic clinical data. Comparisons will be made between strains identified from the community and those from hospital settings, including specific analysis of those within long-term care facilities. Comparison will be made between the strains isolated from cases and those who are colonised (no *C. difficile* toxin detected). Previous work within the consortium has shown that there is variable performance for current toxin assays for different PCR-ribotypes of *C. difficile*, but lack of a highly sensitive toxin detection method has so far hampered efforts to investigate this further[13]. Further analyses of those strains that were isolated from patients with toxin levels that were only detectable by the innovative highly sensitive SiMoA assay will help to elucidate a correlation between certain strains and low levels of toxin produced in-vivo.

Recent data show that certain *C. difficile* strains may cause disease and yet only produce binary toxin, but not toxins A and B (A-B-CDT+ strains). These strains are likely to be missed by conventional microbiological testing which detects the main toxins A and/or B[14]. Other binary toxin producing strains (A+B+CDT+, A-B+CDT+) are associated with increased relapse rates. More data are needed to determine the prevalence of such strains and what their true potential is to cause CDI. In addition, therefore, strains will be toxinotyped; identification of the variation in toxin genes. Comparisons will be made between those strains seen within hospital and community settings, with emphasis on

long-term healthcare facilities. In addition, as with the PCR-ribotyping results, analyses will determine if there is any correlation between different toxinotypes and the healthcare setting from which they are isolated. Molecular variations in toxin genes and toxins could influence molecular diagnostic assays and vaccine approaches. The current European toxinotyping scheme is run by MR (NLZOH), who will be leading this task.

Further analysis of strains will be completed by using highly discriminatory fingerprinting (whole genome sequencing), comparing within and between healthcare settings. Whole genome sequencing (WGS) studies have recently demonstrated the value of this approach for supplementing conventional surveillance based on ribotyping, and so determine the extent of true linkage between CDI cases and other patients. We will employ WGS to examine the true relatedness of strain types within and between CDI settings and identify factors that may be driving clustering of strains. Unpublished pilot data show that for some, but not all, common ribotypes there is evidence for widespread dissemination of very closely related strains. We will seek to validate these findings to deliver innovative information about potential reservoirs for infection and strain transmission.

There is emerging evidence that transmission of *C. difficile* is more complex than originally assumed; hence, CDI case-case transmission, outside of the outbreak setting, is not the dominant route of spread of *C. difficile*. This has led to several on-going research projects by the participants (some with manuscripts submitted) to examine other possible reservoirs/routes of transmission of *C. difficile.* There is increasing recognition that the food chain may be an important vehicle for *C. difficile* dissemination, which we recognise. Additional strains (matched in size with strains from main study) from animal and food sources will be collected (contemporaneously) from an existing network, collated by NLZOH. These strains will also be analysed, as above, and then comparisons made between strains of human and animal origin.

3.1.2 Outcome measures

Primary outcome measure: Number of missed cases of CDI - Comparison of test result at participating site and test result at European Coordinating Laboratory (using optimised testing). Objective 1.1

Secondary outcome measures:

- Number of missed cases of CDI - Comparison of test result at participating site and test result at European coordinating laboratory (using novel highly sensitive C. difficile assay for toxins A and B).
- Types of *C. difficile,* as measured by PCR-riboyping, toxinotyping and whole genome sequencing. Objective 1.2

3.1.3 Sampling Criteria for sample collection study

3.1.3.1 Inclusion criteria

ALL unformed faecal samples (takes the shape of the container and/or stick will not remain vertical when inserted into the sample) from **ALL** patients (ALL ages) sent to the microbiology department of participating sites on their allotted testing day (**irrespective of test requested**).

3.1.3.2 Exclusion criteria

Only one sample per patient can be included

3.1.4 Study methods

Two study days will be selected, and all diarrhoeal stool samples received on that day by the participating site in the study will be sent (regardless of test requested) to the European Coordinating Laboratory (Leeds), where samples will be tested for CDI using the recommended 'optimal' testing methodology (GDH/Toxin (Techlab, US) testing on the DS2, as per ESCMID guidelines), reference methods (cell-cytotoxicity neutralisation assay and cytotoxigenic culture) and a novel, Single-molecule Array (SiMoA) immunoassay platform for C*. difficile* toxins A and B. Samples will also be tested for other potential gastrointestinal pathogens (using the bioFire Film array molecular method), to investigate either co-infections or alternative cause of diarrhoea (where *C. difficile* negative). Results will be compared to those from the submitting hospital/laboratory, including if the sample was even tested. The SiMoA test will be carried out at bioMerieux (France), a COMBACTE-CDI partner, as the novel technology is currently not available elsewhere. A small portion of selected samples will be sent to BioMerieux for this purpose. They will only be labelled with the study number.

All data held by the study team will be pseudoanonymised to a study number. Only the original submitting laboratory will hold the code to link back to the patient. All samples will be sent for processing under the study number only.

All of the residual diagnostic stool samples will be processed in the laboratory facilities at the Healthcare Associated Infection Research Group (HCAI) at the University of Leeds. The HCAI laboratory is also the *Clostridium difficile* reference laboratory for the UK and has >20 years of experience in handling stool specimens and bacterial isolates derived from the samples. The laboratory follows all relevant regulatory processes for the UK, and all staff are fully trained in the relevant health and safety procedures. All work is risk-assessed for hazards (microbiological, chemical, and procedural) before commencement. Samples are handled in a class II Microbiological safety cabinet to protect the researcher.

C. difficile isolates collected from the study samples will be typed by various methods (PCR-ribotyping, toxinotyping, whole genome sequencing), to determine strain relatedness and distribution. Isolates will be processed at both the Healthcare Associated Infection Research Group (HCAI) in Leeds and the National laboratory for health, environment and food (NLZOH), in Slovenia, a COMBACTE-CDI partner. Again the NLZOH has >20 years of experience in handling the organism *C. difficile* and the laboratory follows all relevant regulatory processes, and all staff are fully trained in the relevant health and safety procedures. All work is risk-assessed for hazards (microbiological, chemical, and procedural) before commencement. All laboratory work will be performed in the facilities mentioned above, which are containment level II facilities, who comply with all local and national laboratory health and safety regulations. Both laboratories follow strict waste disposal policies to ensure the correct disposal of microbiological material, to prevent accidental environmental contamination.

In addition, NLZOH will collect contemporaneous isolates of *C. difficile* from environmental and food sources, within the same countries as the human samples. These will be typed using the methodologies described above.

3.1.5 Sample size

It is impossible to accurately predict the number of samples that will be collected, as this is based on what is collected on those selected days. However, given our previous experience of collecting samples from 482 hospitals across 20 European countries during the EUCLID study[1], we are able to estimate that we would receive 1620 samples from the 120 hospitals included (see Table 1) on each sampling day, with an average positivity rate (for toxin positive samples) of 10%, which would give 240 toxin positive samples. The culture positive rate will likely be ~15%; 252 strains would therefore

be collected for further typing per sampling day. The number of samples and the positivity rate for CDI will be examined after the first sample day collection, to decide if the next sampling day should be carried out on one (as planned) or two days. Contingency has been added to the budget to cover the additional costs of adding a testing day to increase sample numbers.

We will aim to collect a similar number of contemporaneous isolates from the environmental and food samples.

3.1.6 Data collection

In addition to the laboratory data, data will be collected on the following variables;

- Sample source sites (hospital/GP, nursing home)
- CDI test result for that sample at the submitting laboratory

3.1.7 Data analysis

Results of the CDI testing at the ECL will be compared with the original result at the participating site, with 'missed' diagnoses recorded (either due to false negative result at participating site, or sample not tested at participating site). Results of 'missed' diagnoses will be compared between countries and sample source sites (hospital/GP/nursing home). In addition, the number of co-infections and alternative GI pathogens (in CDI negative samples) will be compared between countries and sample source sites, to determine difference between in-patient and community populations.

Types of *C. difficile* (by PCR-ribotyping, and toxinotyping) will be compared between countries, settings (community/hospital), cases/colonised participants and those detected by different commercial testing methodologies (optimised method vs SiMoA). Whole genome sequencing will be used to examine the true relatedness of strain types within and between settings (community/hospital). Comparison will be made between the types of *C. difficile* isolated from human, environmental and food sources. In addition, whole genome sequencing will be used to examine the true relatedness of strain types between sources.

3.2 Case control study- objective 1.3 (outcomes)

3.2.1 Study rationale

Morbidity and all-cause mortality in relation to CDI within hospitals have been extensively studied[8, 15-17]. However, CDI outcomes in community settings, including nursing/convalescent homes, are poorly understood. In addition, research studies have concentrated on one area of healthcare, i.e. either a hospital or a community setting, without examining the interplay between these two settings. It is plausible that a patient with hospital acquired CDI, who recovers and is discharged into the community, may suffer a recurrence that is diagnosed empirically (i.e. without testing) or via a different laboratory from the index diagnosis. Currently, such outcomes are not captured by either surveillance or research. Given that CDI cases occur within all parts of the healthcare system, it is imperative that data relating to recurrence, morbidity and mortality from the healthcare system as a whole are studied to understand the true disease burdens and the interplay between settings.

3.2.2 Outcome measures

Primary outcome measure: Crude mortality at 30 days and 6 months after the sample was taken will be compared between case and controls, those that received a correct diagnosis at the original hospital and those that were 'missed' (either due to lack of testing or inadequate testing) and between community and hospitalised patients.

Secondary outcome measures: Patient length of stay, severity of infection (e.g. WCC, serum albumin, temperature), need for escalation of medical/surgical care due to gastrointestinal disease, post-infectious sequelae.

3.2.3 Sampling criteria for case/control studies (objectives 1.3)

3.2.3.1 Inclusion criteria

The study will collect data on cases/controls identified during the sample collection study (section 3.1 above)

1. Any patient who had a sample sent to the European coordinating laboratory and was positive for CDI (GDH positive/toxin positive) at the European coordinating laboratory
2. A matched (age/gender) patient from the same participating site as a patient in criteria 1, but that had a negative result for CDI at the European Coordinating Laboratory.

3.2.3.2 Exclusion criteria

None

3.2.4 Study methods

All CDI positive cases (as determined by the ECL), and a proportion of negative cases (at a ratio of 1 case to 3 controls) will have clinical data collected (after 6 months) on participant outcomes. Data will be compared to identify differences between countries and healthcare providers.

3.2.5 Sample size

We estimate, based on previous experience (please see section on sample size in 3.1.4), that we would receive 480 *C. difficile* toxin positive samples over the two sampling days. These would form the cases for the case/control study. Controls (participants with *C. difficile* negative samples submitted during O1.1) would be recruited at a rate of 1:3, from the same participating centre. Where possible these will be age and gender matched. Given that we will only have a predefined group of participants to select from; it is possible that we are unable to completely match all controls, apart from at the geographical level.

3.2.6 Data collection

Data will be collected via a standardised CRF, with a full supporting SOP. The CRF will be completed by the clinical care team at the participating site that submitted the sample (during O1.1). The CRF will not contain any participant identifiable data; all data will be held against a pseudoanonymised study code, with the link back held by the clinical care team only. COMBACTE-CDI investigators will not access participant's notes or access the link back. Once the CRF data has been collected and validated and verified the link-back will be destroyed by the local study site.

Data will be collected on the following variables on outcomes of CDI;

- Evidence of other enteric pathogens (+/- 7 days of the original sample)?
- Symptoms at time of the sample
- Alternative cause for the patient's diarrhoea documented (e.g. laxatives, pathogen)?
- Duration of diarrhoea/non-normal bowel habit?
- Primary/recurrent CDI
- Antibiotics at the time of the sample or the preceding 3 months?
- Evidence of colitis?
- Evidence of post-infectious diarrhoea

- CDI Severity markers (Temperature (nearest reading +/- 3 days of the sample), serum creatinine (nearest reading +/- 3 days of the sample), White cell count (nearest test +/- 3 days of the sample)
- Treatment for CDI episode
- Was treatment for CDI/diarrhoea escalated – medical/surgical?
- Outcome 30 days and 6 months after the sample (discharged, in-patient, died)
- Length of stay

3.2.7 Data analysis

Comparisons will be made between recurrence/Morbidity/Mortality (crude and attributable) rates in different healthcare settings and countries. Outcomes will also be compared for different strain types (as identified in O1.2).

3.3 Case control study- objective 1.4 (risk factors)

3.3.1 Study rationale

Many studies have examined risk factors associated with CDI; there are, however, three major deficiencies of existing data; (1) most studies have been performed in the epidemic setting and so may be biased by a preponderance of cases caused by one or a few strain types; (2) most studies are in hospitalised patients; (3) most were biased to patients receiving antibiotics, identified antibiotic use as the most important risk factor, and so reduced the potential to identify other modifiable risk factors. Conversely, several studies have shown that exposure to prescribed antibiotics was not present in up to 50% of CA-CDIs[2,3]. CA-CDI, therefore, may be linked to other microbiota disturbing factors (other drugs, diet, etc.) currently not recognised as risk factors for CDI. Recent data have questioned whether PPIs are truly associated with CDI; earlier results may be confounded by choice of control patients[19].

A recent systematic review of risk factors associated with CDI recurrence and mortality noted that important variables were inconsistently reported, such as previous episodes and antibiotic use[17]. Also, there was substantial heterogeneity and methodological limitations, including sample sizes, definitions of outcomes and follow-up periods, thus precluding a meta-analysis. We have considerable experience of determining risk factors associated with CDI[2,3,20]. Notably, a recent University of Leeds study has determined CDI risk factors for cases determined according to a standardised diagnostic algorithm (data unpublished).

3.3.2 Outcome measures

Primary outcome measure: Episode of CDI

3.3.3 Sampling Criteria for sample collection study (objective 1.4)

3.3.3.1 Inclusion criteria

The study will collect data on cases/controls identified during the sample collection study (section 3.1 above)

1. Any patient who had a sample sent to the European Coordinating Laboratory and was positive for CDI (GDH positive/toxin positive) at the European Coordinating Laboratory
2. A matched (age/gender) patient from the same participating site as a patient in criteria 1, but that had a negative result for CDI at the European Coordinating Laboratory.

3.3.3.2 Exclusion criteria
None

3.3.4 Study methods
All CDI positive cases (as determined by the ECL), and a proportion of negative cases (at a ratio of 1 case to 3 controls) will have clinical data collected (after 6 months) on possible risk factors for CDI. Data will be compared to identify differences between countries and healthcare providers.

3.3.5 Sample size
We estimate, based on previous experience (please see section on sample size in 3.1.4), that we would receive 480 *C. difficile* toxin positive samples over the two sampling days. These would form the cases for the case/control study. Controls (participants with *C. difficile* negative samples submitted during O1.1) would be recruited at a rate of 1:3, from the same participating centre. Where possible these will be age and gender matched. Given that we will only have a predefined group of participants to select from; it is possible that we are unable to completely match all controls, apart from at the geographical level. The same participants will be recruited for both case/controls studies (O1.3 and 1.4), using harmonised documentation (PIS, ICF and CRF).

3.3.6 Data collection
Data will be collected via a standardised CRF, with a full supporting SOP. The CRF will be completed by the clinical care team at the participating site that submitted the sample (during O1.1). The CRF will not contain any participant identifiable data; all data will be held against a pseudoanonymised study code, with the link back held by the clinical care team only. COMBACTE-CDI investigators will not access participant's notes or access the link back. Once the CRF data has been collected and validated and verified the link-back will be destroyed by the local study site.

Data will be collected on the following variables to examine risk factors for CDI;

- Prior treatment with cytotoxic drugs
- Prior treatment with PPIs
- Underlying diagnoses
- Co-morbidities
- Previous antibiotics (preceding 12 weeks)
- Previous hospitalisation (preceding 12 weeks)
- Admission history (if an in-patient)
- Residential status (if in the community), e.g. home/nursing home
- Previous CDI
- Kissing contact with an infant
- Known contact with CDI case
- Previous wards/movement within hospital

3.3.7 Data analysis
Odds ratios for different risk factors for CDI will be compared between settings (community/hospital) and countries. Risk factor comparison will also be made between different strain types (as identified in O1.2).

3.4 Transmission model (Objective 1.5)

3.4.1 Study rationale

Consideration of patient movement in hospitals, LTCFs and the community is crucial in understanding the transmission dynamics and control of *C. difficile*[21]. Data–driven estimates of CDI burden outside hospital settings are lacking (see 1.1). However, frequent interactions between hospital and LTCF populations mean their dynamics are inherently linked. Asymptomatic colonisation in LTCF is a potential reservoir for transmission[22]; reported rates vary widely (4-50%)[23], likely due to differences in healthcare system structures, and variations in definitions and long-term care case-mix[24]. Overall, *C. difficile* transmission dynamics are poorly understood. Our molecular studies and statistical modelling[17,29] have questioned the importance of symptomatic hospital patients, the traditionally assumed primary source of CDI acquisition

3.4.2 Outcome measures

Primary outcome measure: A *C. difficile* transmission model describing the relatedness of each part of each target country's healthcare economy
Secondary outcome: A modelling tool that enables country and setting-specific CDI burden estimation and evaluation of intervention (cost-) effectiveness.

3.4.3 Sampling Criteria for sample collection study (objective 1.5)

3.4.3.1 Inclusion criteria

Data generated from the sample collection study and the case/controls studies described above.

3.4.3.2 Exclusion criteria

None.

3.4.4 Study methods

All CDI positive cases (as determined by the ECL), and a proportion of negative cases (at a ratio of 1 case to 3 controls) will have clinical data collected (after 6 months) on possible risk factors for CDI. Data will be compared to identify differences between countries and healthcare providers.

3.4.5 Sample size

This is based on the collection of data from the previous objectives.

3.4.6 Data collection

An anonimised dataset of the combined data from objectives 1.1-1.4 (as above) will be included in the transmission modelling. No additional data will be collected.

3.4.7 Data analysis

We will use novel Markov chain Monte Carlo algorithms, to quantify transmission from different settings across different countries, using the repeated point-prevalence data collected in O1.1, along with the analysis on strain distribution (O1.2), impact on patient outcomes (O1.3) and risk factors (O1.4).

In addition, we aim to develop an innovative tool to enable country and setting-specific CDI burden estimation and evaluation of intervention (cost-)effectiveness. The model framework will be easily adaptable to different countries and settings. For example, so that settings of high antimicrobial use or CDI incidence can be simulated. The model could also provide a unique framework to perform

transmission dynamic model-based (cost-)effectiveness evaluations of interventions such as antimicrobial stewardship (e.g. targeting in- vs. outpatient prescribing) or admission screening

3.5 Surveillance study of current CDI detection, surveillance, treatment and attributable costs

3.5.1 Study rationale

This part of COMBACTE-CDI (work-package 2) will highlight current guidelines for CDI diagnosis and treatment, at both European and national levels, and measure compliance with these. We will also measure the impact of CDI on healthcare systems, and determine if better compliance with guidelines leads to a lower burden in terms of reducing cases and costs. Objectives and deliverables for WP2 will be closely aligned with those for WP1, again using a synergistic approach to study design (see Figure 1). The timing of the objectives has been planned so that data and analyses generated from WP1 will be available for analyses in the required objectives in WP2. In addition, much of the analysis in the cost-effectiveness objective will be based on data generated from earlier objectives within WP2; the timing of these two objectives O2.5 and 2.6 has therefore been planned accordingly.

Highlight current guidelines for disease detection, notification and management/control Objective 2.1

Five international societies have recently issued/updated guidelines on diagnosis, prevention, treatment and control of CDI.20,34-37 This reflects the increasing burden of CDI worldwide, new knowledge, and because some issues remain contentious. Underdiagnosis, as reported by our EUCLID study[1] is a main contentious area. Underdiagnosis can depend on suboptimal laboratory testing for CDI and/or lack of suspicion by some clinicians, which could be overcome by automatic testing for CDI on every liquid stool sample. Overdiagnosis of CDI is also a challenge; it is important to distinguish CDI infection from colonization in hospitalised patients with diarrhoea[25].

One of the pillars of control of CDI is communication through reporting, but notification represents another conundrum not addressed by guidelines. In many countries, both in community and healthcare settings, there is mandatory notification for infectious diarrhoea, but not for CDI. In a recent systematic survey of clinical practice guidelines, showing that recommendations are often not consistent with available evidence, there is no mention of notification strategies for CDI[26].

Determine the heterogeneity in testing density and in following the existing guidance on testing practices (objective 2.2)

We will aim to fill the current knowledge gaps about hospital identification of missed diagnoses (either due to lack of clinical suspicion or inadequate laboratory diagnostics). Existing programmes collecting European data on CDI testing heterogeneity are limited by either the number of hospitals surveyed per country or the number of countries[13,27]. Also, current programmes focus on hospital acquired CDI; thus, data for the entire healthcare economy are lacking. More robust data comparisons could be made by expanding existing networks.

Determine the heterogeneity in current surveillance practices and ways to improve them (objective 2.3)

An evaluation of changes in local laboratory CDI diagnostics in the period 2011-14 concluded that an increased use of 'optimal' diagnostics for CDI surveillance should be further promoted[27]. Results of a

web-based electronic form sent to national coordinators showed that surveillance methods also varied considerably between countries[27]. This illustrates the need for a harmonised European protocol to allow consistent monitoring of the epidemiology of CDI at European level. To support this further, a new CDI diagnostic guidance document has been published in 2016[28]. We aim to determine the heterogeneity in current surveillance practices within the study source sites recruited for the prospective study in O1.1, and ways to improve them (by compiling and comparing information on the current surveillance systems at both European and country levels, and to determine the best strategy for future surveillance.

Describe current treatment pathways for patients with CDI infection and recurrences (objective 2.4)

Current European treatment guidelines were published in 2014[29], but there are a paucity of data on national guidance and compliance with these guidelines. Guidelines often focus on in-patients and we will explore whether altered guidance is needed for different healthcare settings (see WP1). By building on networks used to establish variations in CDI diagnosis (EUCLID, LUCID, CLOSER), we will measure variability in treatment policies across Europe, focussing on the treatment/prevention of recurrent CDIs. By using the network of hospitals/laboratories established for the main study (O1.1.2), and their associated community practices, we will close the knowledge gaps within the community setting, with focus on long-term healthcare facilities. Comparisons will be made between healthcare locales/types and countries. Because information of outpatient costs of CDI are scarce, these new networks will also be used for further health-economic evaluations to analyse direct and indirect costs of patients with CDI outside hospitals (this data will feed in to O2.5 and 2.6).

Current guidelines on CDI treatment take into account both initial and recurrent episodes. For initial CDI treatment, pathways are mainly based on stratification on the severity of the disease; for recurrence, on the frequency of CDI episodes. The main concerns/knowledge gaps of these guidelines are represented by 1) heterogeneous definitions of severity [1,30] increasing rates of resistance to the often 1st-line treatment option metronidazole [18,31,32] lack of data on the risk of poorer outcomes in selected populations with CDI e.g. inflammatory bowel disease[33]. Recently, Kanhafer *et al*[30] demonstrated that the proportion of severe CDI cases varied widely (11-59%) depended on the definitions/risk factors used. This is important not only for stratifying patients for management strategies, including new treatments, but also for comparing data across studies. Some risk factors are also controversial; albumin levels could be the cause or effect of severe CDI[30]. Studies should be addressed to better identify the role of these factors.

Elucidate the overall costs of CDI, including management of multiples episodes and re-hospitalisation (healthcare perspectives incl. indirect financial impact on the healthcare system). (Objective 2.5)

There are current knowledge gaps regarding the costs of managing CDI in various healthcare settings. While some data exist on European tertiary care hospitals[34,35], the majority of CDI patients receive treatment in other hospital categories. Outside hospitals, little is known about costs in outpatients and nursing homes/LTCF. There are further knowledge gaps regarding costs of recurrent CDI.

3.5.2 Outcome measures

This section of COMBACTE-CDI is surveillance of existing guidelines, including local knowledge and compliance. There is no fixed outcome measure for this.

3.5.3 Sampling Criteria for surveillance studies (objectives 2.1-2.5)

3.5.3.1 Inclusion criteria

All participating sites (hospital based), and their associated community healthcare providers who submitted samples on the sampling days (sample source sites). In four selected countries (representing the four zones of Europe) 10 nursing homes that did not submit samples on the sampling days will also be asked to complete the surveillance questionnaire, as there are a paucity of data on CDI regarding these facilities.

3.5.3.2 Exclusion criteria

None

3.5.4 Study methods

All included sites (see definition in inclusion criteria 3.5.3.1) will be asked to fill in a surveillance questionnaire asking about knowledge and compliance with current guidelines on CDI diagnosis, notification and control, surveillance, treatment and will collect attributable costs. Data will be compared to identify differences between countries and healthcare providers.

For cost data we will gather data on direct medical and non-medical costs of CDI in different healthcare settings to address the gaps and so elucidate the costs in all healthcare settings. Direct medical costs will include personnel (i.e. medical, nursing and technical service) as well as material costs (e.g. material and drug products). Direct non-medical costs will include for example, costs for carriage and expenditures of relatives

3.5.5 Sample size

This is based on the number of participating sites (n = 120) and the number of associated community healthcare providers that submit samples on the sampling days, plus up to 40 nursing homes.

3.5.6 Data collection

An online data capture system will be used to collect the data, built within the COMBACTE online network. This data will not contain any personal data, but details about individual sites knowledge and compliance with existing guidelines. Each site will have a unique anonymised study identifier for the online data capture system; investigators will therefore not be able to identify sites.

The main dataset will then be accessed via the online portal by the investigators for each of the objectives. Rule bases will be added to ensure that each objective investigator can only access data relevant to their objective.

3.5.7 Data analysis

Comparisons of knowledge and compliance with guidelines for disease detection, notification and management/control will be made between countries and healthcare settings (community/hospital). Particular attention will be focussed on the dates of policies and the length of time it takes for European/national guidance to filter down to a local level. This may highlight a possible area for improvement in guideline communications.

Comparisons between testing policies and methodologies will be made between countries and healthcare settings (community/hospital). In addition, data from 1.1 on ‘missed’ case rates in these settings will allow identification of ‘poor practice’.

Data will be compiled on current surveillance programmes for CDI and compliance with these, in different countries and settings. This will be used to determine a 'best-practice model' for surveillance and investigate mechanisms for increasing engagement with surveillance.

Comparisons of knowledge and compliance with guidelines for treatment pathways will be made between countries and healthcare settings (community/hospital). In particular comparisons will be made of risk stratification of patients (via severity criteria) between different healthcare settings and countries. Again this will be used to determine a 'best-practice model' for risk stratification.

We will aim to elucidate the overall costs in all healthcare settings. Data will be compared on the costs of CDI in different healthcare settings, and to address knowledge gaps regarding managing CDI outside hospitals, including in nursing homes. For the most comprehensive analyses on overall costs associated with CDI, a micro-costing approach will be combined with published data of the respective health systems for all health-economic evaluations. Additionally, by using data from Objective 1.3, indirect costs, i.e. productivity losses due to illness related disability and death before retirement age, in inpatient and outpatient settings will be addressed from societal perspectives to measure the full impact of CDI. The human capital and/or friction cost approaches will be used. All calculated costs will be expressed in an appropriate currency (e.g. EURO, €). Furthermore, discounting of all calculated costs will be performed by taking account of country and annual discount rates (normally 3% - 5% per year). Multivariate analyses will be performed to estimate relevant components of cost. Sensitivity analyses will be performed by using different annual discount rates.

3.6 Measure the impact of CDI management on the healthcare system and the impact of current control measures (infection control/antibiotic stewardship). (Objective 2.6)

3.6.1 Study rationale

Although it appears self-evident that CDI can be prevented by infection control and antimicrobial stewardship, this has only been demonstrated in the setting of outbreaks by selected strains and only for complex intervention bundles[36]. It is currently unknown which measures are best at preventing CDI and which are most cost-effective. For CDI treatment, calculations have been modelled [37,38], but for preventive measures such data need to be generated. Using data from Objectives 1.5, 2.1, 2.2 & 2.4, we will determine the diagnosis and treatment costs for those cases managed vs. not managed according to current guidelines. Results of cost-effective measures will be presented/visualized by using threshold analyses. Potential cost-savings are likely by e.g. shortened overall length of stay, prevention of severe and multiple CDI, transfer to (expensive) intensive care unit (ICU), and morbidity/mortality due CDI. Calculation of overall costs (direct and indirect costs) will be based on methodology as stated in O2.5.

3.6.2 Outcome measures

This section of COMBACTE-CDI is surveillance of existing guidelines, including local knowledge and compliance. There is no fixed outcome measure for this.

3.6.3 Sampling Criteria for surveillance studies (objective 2.6)

3.6.3.1 Inclusion criteria

All data generated from samples and isolates collected on the sample days (O1.1 and 1.2), all participants from the case/controls studies (O1.3 and 1.4), all participating sites (hospital based), and their associated community healthcare providers who submitted samples on the sampling days (sample source sites). In four selected countries an additional 10 nursing homes that did not submit samples on the sampling days will also be asked to complete the surveillance questionnaire, as there are a paucity of CDI data regarding these facilities.

3.6.3.2 Exclusion criteria

None

3.6.4 Study methods

The investigators will a execute cost-benefit analyses of the different CDI management policies/pathways highlighted from Objectives 2.1, 2.2, 2.3 and 2.4, and identify the most cost-effective. The most cost-effective policy is expected to differ between healthcare settings as identified in 2.5. Cross-country comparisons of prices for health services will be based on OECD data. Data from Objective 1.2 will enable us to calculate differential costs per *C. difficile* strain/ribotype, and to what extent cost-effectiveness of infection control/antimicrobial stewardship (AMS) measures is driven by local CDI epidemiology. So-called hypervirulent strains 027/078/244 may be pooled for such analysis. It is well known that CDI due to hypervirulent strains leads to high rates of morbidity and mortality. Excessive direct costs may be expected, especially if patients are treated on an Intensive Care Unit e.g. due to toxic megacolon or pseudomembranous colitis). Further indirect costs may arise in case of productivity losses as stated in O2.5.

The investigators will calculate the value of infection-control measures including the identification of high-risk patients and, using data from Objective.1.5, we will analyse the economic value of preventing transmission. Using data from Objective 2.5, we will determine the potential reduction in CDI economic burden in individual healthcare settings by introducing or changing infection control/AMS measures. Therefore, expenditures for direct cost factors (e.g. personnel costs for Infectious Diseases specialists) will be compared with in- and outpatient cost-savings (e.g. due to prevention of CDI). Based on these results we will calculate the optimal approach, taking into account an institution's economic burden. This could be helpful to implement standardised and effective infection control/AMS measures into clinical practice to improve patient outcomes and reduce overall costs associated with CDI across Europe in all healthcare settings. Furthermore, these results could be of interest for consideration of updated guidelines. Calculation of costs will be based on methodology as stated in O2.5.

3.6.5 Sample size

As this analysis requires data from the objectives O1.1-15 and O2.1-2.5, the sample size is dependent on the sample size of these previous objectives. It will include;

- The number of samples collected on the sampling days (O1.1) and
- The number of isolates of *C. difficile* (O1.2),
- The number of participants in the case/control studies (O1.3 and 1.4),
- The data generated in the transmission modelling (O1.5),
- The number of completed surveillance questionnaires (O2.1-2.5) as described above.

3.6.6 Data collection

The anonymised datasets generated from work-package 1 (O1.1-1.5) will be combined by the University of Leeds (overall guardian of the data) and sent to University of Cologne (a COMBACTE-CDI partner) for analysis as part of the cost-benefit modelling. This dataset will contain anonymised health information; it will not contain any personal identifiable data. This will be combined with the surveillance data (O2.1-2.5).

As described above, for the surveillance data, an online data capture system will be used to collect the data, built within the COMBACTE online network. This data will not contain any personal data, but details about individual sites knowledge and compliance with existing guidelines. Each sample source site will have a unique anonymised study identifier for the online data capture system; investigators will therefore not be able to identify the sample source sites.

The main dataset will then be accessed via the online portal by the investigator for this objective. Rule bases will be added to ensure that each objective investigator can only access data relevant to their objective.

3.6.7 Data Analysis

A simulation of the economic impact of novel CDI treatment options will be made using data from Objectives 1.4 & 1.5.

For the simulation of costs, a decision-analytic model (e.g. decision tree analysis, markov model) will be appropriate. Therefore, a separate analysis of direct/indirect costs factors as well as further methodology as stated above will be performed/used to determine the impact of treatment interventions as well as changes in diagnostic pathways.

Building on data collected in WP1 (esp. the modelling in 1.5) and in O2.5, resource-utilization analyses will be performed for different CDI management approaches according to healthcare setting.

Data from O1.2 will be used to calculate differential costs per *C. difficile* strain/ribotype, and to what extent cost-effectiveness of infection control/antimicrobial stewardship (AMS) measures are driven by local CDI epidemiology.

The value of infection-control measures including the identification of high-risk patients will be calculated, and, using data from O1.5, the economic value of preventing transmission will be assessed.

4.0 Data management

All data generated during work package 1 will be under the guardianship of the University of Leeds. It will be held on secure, password protected encrypted University servers. No personal identifiable data will be held, only health data against a pseudoanonymised study number. The link back to the participant will only be held by the clinical care team, this link back will never be disclosed to the investigators or leave the clinical participating site. The link back (retained at the clinical site) is essential however to enable the clinical care team to fill in the follow up CRFs for the case/control study. Once the CRF data has been collected and validated and verified the link-back will be destroyed by the local study site.

This anonymised dataset will also be transferred to a sub-contractor (still to be defined) for the transmission modelling. The sub-contractor will be within the EEA, and will be subject to a full

document review by the University of Leeds, to ensure they comply with all of the relevant regulatory requirements. Their responsibilities will be clearly defined within the sub-contract and data transfer agreement between the University of Leeds and the sub-contractor. Once finalised, the data required for, and generated by the transmission modelling will transfer back to the University of Leeds for storage on secure, password protected encrypted University servers. No data will be held locally by the sub-contractor after completion of the modelling.

In work package 2, an online data capture system will be used to collect the surveillance data, built within the COMBACTE online network. The data will be held on secure, password protected servers at the University of Utrecht (a COMBACTE-CDI partner). This data will not contain any personal data, but details about individual sites knowledge and compliance with existing guidelines. Each sample source site will have a unique anonymised study identifier for the online data capture system; investigators will therefore not be able to identify sample source sites. The main dataset will then be accessed via the online portal by the investigators for each of the objectives. Rule bases will be added to ensure that each objective investigator can only access data relevant to their objective.

The anonymised datasets generated from work package 1 (O1.1-1.5) will be combined by the University of Leeds (overall guardian of the data) and sent to University of Cologne for analysis as part of the cost-benefit modelling. This dataset will contain anonymised health information; it will not contain any personal identifiable data. This will be combined with the surveillance data (O2.1-2.5). These data will be held on the secure, password protected servers at the University of Cologne (a partner within COMBACTE-CDI). Data transfer agreements are in place between the University of Leeds and the University of Cologne, as part of the consortium agreement. As the University of Cologne is a partner within COMBACTE-CDI, this data will be archived locally, not transferred back to University of Leeds.

5.0 Reporting

This is strictly an observational (surveillance) study. Results of additional testing will not be reported back to participating laboratories. The study is therefore 'non-interventional' as the participant samples will be processed as per local policy at each hospital and those results acted upon; i.e. additional COMBACTE-CDI study results will NOT be available for patient management.

Study reports and conclusions based on analysis of data at a national and European level will be prepared by the lead investigator for each objective.

A full study report will be presented at the end of each objective. Results will be submitted for presentations at scientific meetings followed by preparation of manuscript and publication whenever applicable. Authorship will be led by the lead investigator for each objective (see consortium agreement).

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Appendix 1

Country	Recruitment of study sites which test both in-patient and community (GP, long term healthcare facilities) stool samples	Collection of residual diagnostic tissue and export to the UK	Collection of prospective patient data (outcome data)	Collection of retrospective patient data (risk factor data)	Collection of attributable costs of CDI data	Collection of data regarding testing policy and methodology, surveillance practices, national guidelines and compliance and patient testing and treatment stratification (severity markers)	Region of Europe[a]	Population (average from two sources[b])	Study sites 1 per 3m population
Slovakia	Yes	Yes	Yes	Yes	Yes	Yes	Eastern	5463801	2
Poland	Yes	Yes	Yes	Yes	Yes	Yes	Eastern	38421407	13
Romania	Yes	Yes	Yes	Yes	Yes	Yes	Eastern	20486235	7
UK	Yes	Yes	Yes	Yes	Yes	Yes	Northern	64770786	22
Ireland	Yes	Yes	Yes	Yes	Yes	Yes	Northern	4833233	2
Sweden	Yes	Yes	Yes	Yes	Yes	Yes	Northern	9511327	3
Spain	Yes	Yes	Yes	Yes	Yes	Yes	Southern	47314040	16
Italy	Yes	Yes	Yes	Yes	Yes	Yes	Southern	60904272	20
Greece	Yes	Yes	Yes	Maybe	Yes	Yes	Southern	10846356	4
France	Yes	Yes	Yes	Yes	Yes	Yes	Western	65617845	22
Netherlands	Yes	Yes	Yes	Yes	Yes	Yes	Western	16998349	6
Austria	Yes	Yes	Yes	Yes	Yes	Yes	Western	8397080	3

Table 1. Results of an initial feasibility assessment for conducting the study in O1.1 in Europe.
a = UN geocheme for Europe, zones of Europe; b= bwww.geoba.se, and www.worldmeters.info.

Appendix 2 Participating site invitation letter

Dear Sir/Madam,

We are writing to invite you to be a participating site in the Combatting Bacterial Resistance in Europe – *Clostridium difficile* Infections (COMBACTE-CDI) project which aims to develop a detailed understanding of the epidemiology and clinical impact of CDI across Europe.

This research project received support from the Innovative Medicines Initiative Joint Undertaking under grant agreement, resources of which are composed of financial contribution from the European Union Seventh Framework Programme (FP7/2007-2013) and EFPIA companies in kind contributions.

We are the national coordinating laboratory for XXXXXXX and are recruiting sites to take part in this study.

All sites **must** meet the following criteria:

- Sites must carry out diagnostic testing of faecal samples from both in-patients **and** community patients
- Sites must be able to collect residual diagnostic tissue of all faecal samples received by the laboratory on a specified date in May/June and October 2018, and export this to the UK the day after collection (transport provided)
- Sites must be able to collect prospective patient data (outcome data) linked to the faecal samples provided during the sampling period (based on a provided Case Report Form) either directly or via the **sample source site**
- Sites must be able to collect retrospective patient data (risk factor data) linked to the faecal samples providing during the sampling period (based on a provided Case Report Form) either directly or via the **sample source site**
- Sites must be able to provide data in regards to their CDI testing policy and methodology, CDI surveillance practices, knowledge of national CDI guidelines and their local compliance, and CDI treatment (including according to patient level risk e.g. CDI severity) (based on a provided questionnaire).

If you agree to take part, you would be required to collect all unformed faecal samples (after the completion of all routine microbiology tests) received on two selected sampling days in the year (May/June and October) and send them to the European Coordinating Laboratory in Leeds, UK. A one-off fee of €500 Euros is payable to yourselves, to cover the labour and consumables costs incurred in sending the samples to Leeds. You will receive shipping packaging and shipping instructions to ship the samples to Leeds using DHL. The cost of shipping is covered by the European Coordinating Laboratory.

The European Coordinating Laboratory will test each sample included in the study for CDI using the recommended 'optimal' testing methodology (ESCMID guidelines). Results will be compared to those obtained from your submitting hospital/laboratory, including if the sample was even tested.

All *C. difficile* isolates collected from the study samples will then be typed by various methods including PCR-ribotyping, toxinotyping and whole genome sequencing.

All CDI positive cases (as determined by the European Coordinating Laboratory), and a proportion of negative cases (at the ratio of 1 case to 3 controls) will have clinical data collected (6 months after each sampling date) on patient outcomes and risk factors for disease Data will be collected on a Case Report Form and sent to the European Coordinating Laboratory for analysis. A fee of €200 Euros is payable to yourselves, to cover the labour costs incurred in completion of each CRF.

Each participating site (and their associated community practices) will also be asked to complete a web-based questionnaire on guideline knowledge, compliance, testing policy and methodology, surveillance practices, and stratification of 'severe' patients. Data will be compared to identify differences between countries and healthcare providers. A one-off fee of €100 Euros is payable to yourselves to cover the labour costs incurred in completion of the questionnaire and a further one-off fee of €100 Euros is payable to yourselves to cover the administration in distributing the questionnaire to your associated community practices.

If you wish to participate in this study please confirm your participation via email to XXXXXXXXX by the 16th February 2018. You will then be provided with a study pack for the initial sampling and copy of the full study protocol and study manual. You will also receive an email from our COMBACTE partner, LAB-NET combacte.lab-net@uantwerpen.be requesting you to complete a questionnaire so that you can be added to the COMBACTE LAB-NET system. We ask that you complete this at your earliest opportunity.

We hope that you understand the value of this project and agree to participate in this study.

Yours sincerely

Name

National Coordinator of Country for COMBACTE-CDI

Contact details

This Research project receives support from the Innovative Medicines Initiative Joint Undertaking under grant agreement n° 115523 | 115620 | 115737 | 777362 resources of which are composed of financial contribution from the European Union Seventh Framework Programme (FP7/2007-2013) and EFPIA companies in kind contribution.