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Under-diagnosis of *C. difficile* across Europe: results from the EUropean, multi-centre, prospective bi-annual point prevalence study of *CLostridium difficile* Infection in hospitalised patients with Diarrhoea (EUCLID)

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Abstract

Background: Variations in testing for *Clostridium difficile* infection (CDI) may hinder patient care, increase the risk of transmission and skew epidemiological data. We aimed to measure the under-ascertainment of CDI across Europe.

Methods: We carried out questionnaire-based and point prevalence measurements of CDI in 481 participating hospitals (PHs) across 20 European countries. PHs were questioned about their CDI testing policy and methodology during 2011-2012 and 2012-2013. On one day in winter 2012/2013 and summer 2013 each hospital sent all diarrhoeal samples submitted to their microbiology laboratory to a national co-ordinating laboratory (NCL) for standardised CDI testing. The results of local and NCL CDI testing were compared.

Findings: Mean CDI testing (65.8 tests/10,000 PBD; country range 4.6-223.3) and case rates (7.0 cases/10,000 PBD; country range 0.8-28.7) were markedly higher than in previous studies. However, only 39.9% used optimised methodology (defined by European guidelines). On average 23.1% of all CDI cases, as determined by NCLs, were not diagnosed by PHs due to lack of clinical suspicion, equating to an average of 74 missed diagnoses per day.

Interpretation: A wide variety of testing strategies are used across Europe. Lack of clinical suspicion and sub-optimal laboratory diagnostic methods mean that an estimated 40,000 in-patients with CDI are potentially undiagnosed each year in 481 European hospitals.

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Introduction

Clostridium difficile is the major infective cause of nosocomial diarrhoea in the developed world. Rapid and accurate diagnosis is important to optimise patient care and infection prevention.[1] There has been an increase in the measured incidence of *C. difficile* infection (CDI) in countries with surveillance programmes, and a marked shift in epidemiology over the last decade.[2-4] In Europe *C. difficile* is the most commonly reported pathogen associated with hospital associated gastrointestinal disease, whilst in the US it was the most commonly reported health-care associated microorganism in a survey of 183 hospitals in 2010.[5,6]

In the most recent European epidemiological survey of CDI (2008) the incidence in 97 hospitals across 29 countries varied widely (range 0.0-36.3 per 10,000 patient bed days per hospital (PBH); weighted mean 4.1).[7] The reasons for this large variation are unclear. Predisposing factors to CDI, including increasing age, co-morbidities and use of broad-spectrum antibiotics may be similar across Europe, although exposure to different *C. difficile* strains likely varies.[1,8] Notably, there was a >40-fold variation in CDI testing frequency across European countries, and a correlation between testing rate and reported infection rate was found.[7] Sub-optimal case ascertainment, either due to inadequate laboratory diagnosis or lack of clinical suspicion means that the true burden of CDI is unclear.[9-12] A recent point prevalence study in Spain found that 66% of CDI patients on a single day were undiagnosed or misdiagnosed, due either to lack of clinical suspicion (47%) or inadequate laboratory testing (19%).[9] Optimal laboratory diagnosis of CDI depends on testing the right patients, at the right time with the right tests. Importantly, detection of *C. difficile* toxin in patient faecal samples, as opposed to toxigenic *C. difficile* (strains that produce toxin *in vitro*, or have toxin genes present), correlates with disease severity and mortality.[12,13] To improve the sub-optimal sensitivity of commercially available toxin detection assays, two-stage laboratory diagnosis, involving a sensitive *C. difficile* screening test followed by a *C. difficile* toxin assay is recommended.[1,12] Nevertheless, a questionnaire-based study in 125 European laboratories in 2010 showed wide variation in use of CDI diagnostic methods, with a quarter still using a single assay.[14] Notably, however, such data do not ascertain the true extent of missed CDI diagnoses.

We aimed to determine the under-ascertainment of CDI in hospitals in 20 European countries by asking participating hospitals (PHs) to forward diarrhoeal in-patient faecal samples, regardless of microbiology tests requested or performed locally, received on two days (one in winter 2012/13 and one in summer 2013) to a national coordinating laboratory (NCL) for CDI testing by the study reference method (SRM). PHs were also asked to complete a study questionnaire regarding CDI testing.

Methods

Study design

The study followed the design of a previous point-prevalence study conducted in Spain.[9] Ethical approval was granted in the Netherlands, Sweden and Slovenia; the remainder did not require ethical approval as the study was classed as surveillance. Results were reported back to participating laboratories but (purposely) not in a clinically relevant timescale i.e. (a minimum of 3 weeks after receipt of sample). The study was therefore strictly 'non-interventional'. PHs were recruited at a rate of one per one million population for each of the 20 study countries, and were selected by national co-ordinators to cover all major geographical regions within each country. The full study design is shown in the study flow chart (figure 1.)

Study questionnaires

Details of testing policy and methods and CDI testing and positivity rates for each PH during two 12 month periods (September 2011-August 2012 and September 2012-August 2013) were collected via a questionnaire (see questionnaire in Supplementary materials).

Samples

All in-patient diarrhoeal samples submitted to the PH microbiology laboratory on the study days were eligible for inclusion, regardless of the original test(s) requested. There was no age exclusion but only one sample per patient was included. Samples were anonymised by the PH using a EUCLID study number and sent with a data capture form to the EUCLID National Coordinating Laboratory (NCL) for their country; patient age, gender and specialty, and whether the sample was tested for CDI at the PH and the result of any such test were recorded. Samples were stored at 4°C before transport to the NCL within 7 days. Transport was refrigerated for 6 countries in the winter sampling period and for all 20 in the summer.

Testing at the NCLs

Samples were tested using a 2-test CDI algorithm: membrane enzyme immunoassay (EIA) for glutamate dehydrogenase (GDH) and toxins A & B (*C.DIFF QUIK CHEK COMPLETE*®, Techlab, USA), following manufacturers' instructions; this is the study reference method (SRM). Indeterminate results (GDH-ve/toxin +ve) were repeated; if the second test gave the same result it was recorded as only toxin positive. Samples yielding an invalid result (no control line) were repeated once and the second result recorded, even if still invalid. Confirmatory testing, performed on all samples positive for either GDH or toxin, comprised one of two combinations of tests; either toxigenic culture (culture for *C. difficile* and detection of toxins in culture supernatants*)* or PCR on faeces for *C. difficile* toxin genes and culture for *C. difficile*. Due to differences in availability of culture media and PCR assays within the 20 different countries, it was not possible to use a

single confirmatory method (table 1. in Supplementary materials). All *C. difficile* isolates identified at the NCLs were sent to the European Coordinating laboratory in Leeds, UK for confirmation of identification and PCR-ribotyping.[15]

Quality assurance

Each NCL was asked to process external quality control samples sent by the European Coordinating laboratory. Six blinded 'mock' samples were sent during each testing period to assess each of *C. difficile* assays used by NCLs (table 1. Supplementary materials).

Data analysis

All PH and NCL data were entered onto a secure online system. Using questionnaire data, mean testing and CDI rates/10,000 patient bed days (PBD) (synonymous with occupied bed days) were calculated for each country and Europe. Also, the measured rates on the EUCLID study days were calculated using the number of tests performed, cases detected by NCLs and the PBDs of each PH that year. For testing methodology, an optimised diagnostic algorithm was defined as GDH or NAAT followed by (or simultaneous with) toxin detection.

Patients with samples that were CDI positive at the NCL (GDH positive/toxin positive by SRM), but not tested at the PH, were classified as 'under-diagnosed'; and those samples with positive SRM at the NCL but negative results at the PH (false-negatives) or samples with negative SRM results at the NCL but positive at the PH (false-positives) were classified as 'misdiagnosis'. Results of the confirmatory tests were used to provide additional information on the SRM result but did not change this, as none of the confirmatory assays directly detected toxin in faeces.

All data were analysed on SPSS version 19. Differences in proportions were compared using Mann-Whitney or where data was matched McNemar's. For comparisons across European regions, countries were categorised geographically according to a UN classification.[16] Correlations between testing rates and CDI case rates, and testing rates and the prevalence of ribotype 027 were analysed using Pearson's correlation.

Role of the Funding source

The EUCLID study was initiated and wholly financially supported by Astellas Pharmaceuticals Europe Limited. The funder contributed to the study design but did not contribute to the data collection, analysis or interpretation. Astellas Pharma Europe Ltd reviewed the manuscript before submission in-line with the terms of the funding agreement.

Results

Submitted samples

A total of 3923 (winter) and 3389 (summer) faecal samples were submitted from 481 participating hospitals (94.3% of the target number of PHs required) in 20 European countries in the two sampling periods; 15 samples were excluded due to incomplete data, leaving 3908 and 3389 (mean 7.6 samples per hospital, range 3.5-17.2).

Patient demographics

Data on patient gender, age and speciality were available for between 7293-7 samples (table 1). The ratio of males:females was 1.00 for submitted samples, but there were slightly more females with CDI and confirmed *C. difficile* toxigenic isolates (table 1). The majority of samples (61.8%) originated from medical wards, which included care of the elderly wards (table 1). The median age of CDI cases (confirmed at NCL) with no test at the original PH was 72.0 years, which was significantly lower than the median age (76.0 years) for CDI patients with a PH positive test result (Mann-Whitney p=<0.0001).

Under-diagnosis and misdiagnosis

Across the 481 European hospitals on the two study days, 148 (mean 74) patients with CDI (GDH positive/toxin positive using SRM at NCLs) were not tested for CDI at the PH (table 2,) representing 23.1% of NCL-defined cases. Of these, 125/148 were confirmed to contain toxigenic *C. difficile* (21 by toxigenic culture, 104 by culture/PCR). There was significant variation in the percentage of under-diagnosis across the countries (range 0.0-87.5%; Kruskal-Wallis p = <0.0001).

A further 237 patients (mean 119; 5.2%) were diagnosed with CDI at the PH but did not have demonstrable toxin in their faecal sample when tested using SRM at the NCL (defined here as false-positives). Of these, 136 had a toxigenic strain of *C. difficile* detected in their sample by confirmatory testing. The highest rates of false-positive results in winter occurred in the Czech Republic and Romania (Supplementary materials table 2), where use of stand-alone toxin EIAs (SAT EIAs) for CDI diagnosis was common. The level of false-positives in the Czech Republic decreased from 19.4% to 4.9% after 90% of their PHs changed from SAT EIAs to an optimised diagnostic methodology. On the second sampling day, 87.5% of PHs in Romania still used a SAT EIA and their false-positive rate remained the highest.

C. difficile toxin was detected using SRM in samples from 68 patients (mean 34, 1.6%) who had originally received a negative test result at the PH (false-negatives). Of these, 52/68 were confirmed as toxigenic *C. difficile* (12 by toxigenic culture, 40 by culture/PCR). The UK had the highest proportion of PHs using an optimised method for CDI diagnosis, and had low levels of both false-positive and false-negative results

(table 2). Overall, only 57.2% of the patients tested for CDI across Europe during the study had a diagnosis at their original PH that agreed with the SRM as performed at NCLs.

CDI testing and case rates

Reported versus measured CDI testing and case rates

Of 481 PHs, 458 reported (via questionnaires) their testing and CDI case rates per 10,000 patient bed days (PBD) (table 3), providing data for 19/20 countries; neither PHs in Slovenia provided this information. There was a 48-fold variation in country specific CDI testing rates (4.6-223.3/10,000 PBD). The reported CDI rates also varied markedly (41-fold variation) between countries (mean 6.6-7.3; 0.7-28.7 cases/10,000 PBD). Country reported rates were generally similar comparing 2011/12 with 2012/13, but these approximately doubled in Finland, Ireland and Romania, whilst there was an 80% decrease in Slovakia.

The measured CDI rate during the study (by NCLs) was 2.4-2.9-fold higher than the reported rate (mean 17.2-19 vs 6.6-7.3 cases/10,000 PBD, respectively). The measured testing rate was 1.3-1.5-fold higher than the reported rate (mean 92.4-95.4 vs 62.3-69.2/10,000 PBD, respectively) (table 3). There was a poor correlation between the rate of testing and CDI rate regardless of whether the rates were reported or measured (Pearson's correlation (r) = 0.5741 and 0.2332 respectively, R^2 = 0.2302 and 0.353, respectively) (figures2a and 2b).

Testing policy and methodology

In 2011-12 (questionnaire 1) of 481 hospitals, 468 routinely tested for CDI; the 14 exceptions comprised 3 and 11 hospitals in Bulgaria and Romania, respectively (table 4a). In 2012-13 (questionnaire 2) 427/438 hospitals that replied routinely tested for CDI; again the exceptions were in Bulgaria and Romania (table 4b). Of the PHs routinely testing for CDI, 9.6% (11.3% and 7.5% in questionnaire 1 and 2, respectively) examined all submitted diarrhoeal in-patient samples (empirical testing); 24.5% tested all in-patient diarrhoeal samples if other criteria were met, such as patient >2 years old or in hospital for >3 days (tables 4a and 4b). UK PHs reported the highest level of empirical testing (18%), while 62.4% and 63.2% of PHs and 20% and 30% of European countries only tested for CDI if there was a specific clinician request (questionnaires 1 and 2, respectively).

In 2011-12 there were 152 PHs (32.5%) using optimised methods for CDI laboratory diagnosis, with the UK accounting for 28.9% of these (n = 44) (table 4a); the overall rate increased to 48.0% in 2012-13 (MacNemar's test, for those PHs providing data in both questionnaires, p = <0.00001) (table4b). A toxin

detection method was used by 75.6% and 72.8% of PHs, while few employed stand-alone molecular tests (mean 4.2% across both questionnaires). The largest change in methodology occurred in Czech Republic, where 90% of the PHs changed from SAT assays to an optimised diagnostic method.

PCR-ribotypes

There were 138 different *C. difficile* ribotypes isolated among 1211 isolates; the ten most common are shown in figure 3. *C. difficile* ribotype 027 was the most prevalent, but 88% of these were found in only 4/20 countries: Germany (43.5% of all 027s), Hungary (17.5%), Poland (16.1%) and Romania (11.7%). An inverse correlation was observed between testing rate and prevalence of ribotype 027 across north, south, east and west quadrants of Europe (Pearson's correlation -0.6996) (figure 4).

Quality assurance

EQA results were obtained for all NCLs. All cell-cytotoxicity results were correct; one NCL had an incorrect culture result for 2/12 of their EQA samples (overall NCLs 2/240) and one had an incorrect result using the *C. DIFF QUIK CHEK COMPLETE*® (overall 1/240). PCR results were slightly more variable, with 4 NCLs returning a false-negative result for 1/12 (overall 4/240) EQA samples.

Discussion

In the largest international epidemiological study of CDI diagnosis ever performed, we found that under-reporting across Europe was common, driven primarily by a lack of clinical suspicion (and hence no local testing for CDI), and was compounded by misdiagnosis related to sub-optimal testing. On one day across Europe, a mean of 74 in-patients with CDI were not tested for CDI by their hospital; on average a further 34 patients had a false-negative result at the local hospital (table 2 and supplementary materials table 2). Assuming that our measured daily under- and mis-diagnosis rates were constant, these figures equate to approximately 40,000 missed CDI diagnoses per year at the 481 study hospitals across Europe. The total number of patients with under- or mis-diagnoses in the European Union would be far greater than this estimate, especially considering that there are approximately 8000 hospitals in the region.[17] Notably, the median age of the 148 undiagnosed patients was significantly lower than that of the 426 patients with CDI who were tested at PHs (72 versus 76 years, p= <0.0001). Our data suggest that clinical suspicion of, and therefore testing for CDI is affected by patient age, potentially exaggerating the differences between age-specific diagnosis rates. In the UK, where CDI testing is relatively common, the age specific CDI rate in those aged $\geq$75 years (172.9 per 100 population) is 3.5- and 7.2-fold greater than in those aged 65-74 years and the total population, respectively.[18]

The rate of CDI under-diagnosis (23.1%) we found is similar to that recorded in Spain (25%) in 2009.[9] Unlike the previous study, however, we only collected one sample per patient, thereby reducing the effect of repetitive sampling. We did not determine why a sample was not tested, and so our measured under-diagnosis rate may be inflated. For example, it is the policy in many hospital laboratories not to re-test previously diagnosed patients (e.g. those positive within 14 days), even if a repeat sample is submitted. However, as current CDI laboratory tests are not indicated for treatment monitoring, the number of samples from known CDI positive patients sent to the laboratory is likely to have been small.[1] Conversely, the rate of under-diagnosis could potentially be higher, as we have no indication of the number of patients who had no faecal samples collected due to lack of clinical suspicion of infection, or empirical treatment without attempted laboratory diagnosis.

European guidelines state that if free toxin in faeces is absent but *C. difficile* is detected (bacterium, toxin gene or GDH) then CDI cannot be differentiated from asymptomatic colonisation.[1] Therefore, detection of a *C. difficile* target in the absence of free toxin could be defined as a false-positive result. In our study 237 patients had faecal samples that were designated positive for CDI at the PH (by local definitions) but tested negative by SRM at the NCL (table 2). If, however, we re-designated the 136/237 cases that had a toxigenic *C. difficile* strain isolated (at the NCL) as true-positives for CDI, this would reduce the false-positive rate from 5.2% to 2.2%. Notably, the majority (65%) of these 136 cases were not tested for free toxin at the original PH, even though locally they were considered to represent CDI (68 PCR and 21 cytotoxigenic culture). Whichever is considered the gold-standard testing method for CDI,[12,19] the frequency of misdiagnoses was dwarfed by the undiagnosed rate (20.4%). Patients with a false-positive result may receive unnecessary treatment and/or inappropriate isolation measures (single room or cohorting). Inappropriate isolation can block scarce resources, with unforeseen consequences due to failure to isolate other patients, while cohorting could expose (non-genuine CDI) patients to real CDI cases. It remains unclear whether *C. difficile* positive but toxin-negative cases are a major source of cross-infection. [20]

The diagnostic method used in our study (*QUIK CHEK COMPLETE*, Techlab, US) was chosen as toxin detection in faecal samples correlates with clinical outcome, whilst detection of a toxigenic strain does not.[12,13] Toxin detection is therefore a better indicator of clinically relevant CDI. Our false-negative rate was much lower (1.6% vs. 19.0%) compared with a previous study, but this used toxigenic culture to determine CDI status, which will over-estimate clinically relevant CDI.[9,12] The toxin detection method we used could be criticised as it is not the most sensitive method.[11] It was, however, the only commercial method that could be distributed to all 20 study countries. Refrigerated transport of samples was universal following the summer but not the winter study day (n=6/20 NCLs). It is known that *C. difficile* toxins can degrade at room

temperature, and so some samples reported as toxin-positive by PHs and yet toxin-negative at NCLs could have been wrongly designated as false-positives.[21] However, as this phenomenon could only affect laboratories using a toxin detection method, it may only be relevant for 41/237 (17%) false-positive results. The only time non-refrigerated transport was used during this study was during winter, which may have helped mitigate the potential for toxin degradation.

Other limitations to our study include the possible introduction of bias regarding testing at PH (i.e. we may have unintentionally altered practice on the study days). Notably, the highest testing rate at PHs for samples submitted on the study days was in the Czech Republic (97.8%), even though empirical testing is uncommon here (table 2). This suggests either potential bias, or that the level of clinical suspicion in some countries is relatively high. In support of the latter, no empirical testing was recorded in study hospitals in Bulgaria, and in turn only 35.1% of submitted samples had a CDI test at PHs, which is consistent with a low level of clinical suspicion of CDI. This potential bias of increased testing on the study day may also account for the increased measured incidence of CDI (2.4-2.9-fold) compared with the questionnaire-reported rate (table 3). Interestingly, however, the measured testing incidence was only 1.3-1.5-fold higher than the reported rate, indicating only a moderate level of increased testing. We made several assumptions when calculating the annual measured rates, including that bed occupancy and testing rates were constant throughout the year. Additionally, the measured rate could not be calculated for 14% of NCLs because of missing data.

The reported CDI testing frequency across Europe for 2011-12 has increased from that recorded in 2008 (65.8 vs 52.1 tests/10,000 PBD).[7] There has also been a 70% increase in reported CDI incidence from 4.1 to 7.0 CDI cases/10,000 patent bed days, despite the frequent use of sub-optimal laboratory diagnostics.[7] It should be noted, however, that the 2008 CDI 'European' rate was determined in only 87 hospitals compared with 427-468 in the present study.[7] Importantly, if cases diagnosed at NCLs are used to calculate CDI incidence, the 'true' rate is 2.4-2.9-fold higher than the reported CDI rate (table 3).

The diversity of *C. difficile* ribotypes across Europe was much greater in EUCLID compared with the 2008 study (138 ribotypes from 20 countries vs 65 from 26 countries, respectively).[7] Additionally, the overall prevalence of ribotype 027 has increased more than 3-fold (5% to 18%), although there is marked inter-country variation; high ribotype 027 endemicity has shifted from the UK and Ireland in 2008 to Germany and eastern Europe in 2012/13.[7] Notably, we found an inverse correlation between rate of CDI testing and *C. difficile* 027 prevalence in four regions of Europe (Pearson's correlation (r) = 0.6996, figure 4). This suggests that increased CDI awareness, using optimal testing policies and methodologies, can reduce the

dissemination of epidemic strains. Given the increased morbidity and mortality associated with CDI caused by hypervirulent strains such as ribotype 027, this is an important observation that reinforces the potential clinical and epidemiological value of high CDI testing rates.[2]

Testing policy varied markedly across PHs (tables 2a and 2b). Only two countries (Bulgaria and Romania) had PHs that did not routinely test for CDI, and both had the highest rates of under-diagnosis (table 2). Only 9.6% of PHs routinely tested all diarrhoeal in-patient faecal samples, although 63% of samples submitted to the NCLs did have a previous CDI test at the PH. A wide variety of diagnostic methods for CDI are being used across Europe (tables 4a and 4b). Only 32.5% of PHs used an optimised method for the diagnosis of CDI in 2011-2012, similar to a previous survey (29%).[14] This proportion increased significantly to 48% in the following 12 months ($p = <0.00001$). Three-quarters (74.2%) of PHs employed at least one assay to detect faecal *C. difficile* toxin, but this included use of SAT EIAs, which have low sensitivity and sub-optimal specificity.[11,12] Notably, in those countries where SAT EIAs were commonly used (Czech Republic and Romania), the highest levels of false-positive results were observed (table 2 and Supplementary materials table 2).

It has been reported that CDI testing rates correlate with reported case rates.[7] We found only a week association between these parameters (Figures 2a (reported rates) and 2b (measured rates) R^2=0.2302 and 0.353; Pearson's (r) = 0.5741 and 0.2332 respectively), which is likely due to the much larger study and increased heterogeneity of sampling and testing policies. Changes to sampling and testing policy and methodology clearly affect CDI positivity rates.[22,23] In the UK there has been a national campaign to reduce the incidence of CDI and to standardise laboratory diagnosis.[24] The proportion of UK PHs using methodology consistent with the SRM was the highest of all countries, in line with national guidelines; consequently, under- and misdiagnosis was relatively uncommon. This perhaps serves as an example of how improved monitoring of CDI can help to reduce infection rates. While national surveillance schemes have been associated with reduced incidence of CDI in some countries, this remains a major healthcare burden.[23-27]

Our study highlights the large variation and inconsistencies in the diagnosis of CDI across Europe. Many surveillance systems rely on the reported rate of CDI without taking into consideration the underlying diagnostic methods.[5,29,30] CDI diagnosis should be standardised to ensure that data within and between countries can be meaningfully compared, and so that prevention and control resources are directed appropriately.

Research in context

Systematic review: We searched PubMed for the terms “European”, “*C. difficile* infection”, “prevalence” and “incidence”. There was one multi-centre study that collected data on *C. difficile* infection (CDI) in hospitalised patients, and gave details of methods of diagnosis and incidence.[7] All data were collected via questionnaire with no secondary examination of faecal samples carried out. This study could therefore not evaluate the rate of ‘missed’ CDI diagnosis.

Interpretation: Our study, using a population-adjusted, hospital sample size that is 5 times larger than the 2008 European study,[7] actually measured (using routinely submitted patient faecal samples) the size of the gap between the cases of CDI that are diagnosed and those that are missed. Our data show that on average each hospital in Europe misses about 80 cases of CDI per year (about 40 000 missed cases in total in the 481 hospitals studied). We found that *C. difficile* testing and locally determined CDI incidence had increased by 26% and 70%, respectively, compared with 2008 rates. Additionally, there has been a marked shift in the prevalence of *C. difficile* ribotype 027, particularly in eastern Europe. Thus, despite more *C. difficile* testing and increased recognition, there still remains a large burden of undetected cases that is likely to hamper control measures

Contributers

The study was designed by KD, MHW, EB and CL with support from the EUCLID core group, on behalf of the EUCLID study group. GD was responsible for project management and sample logistics. KD was principal scientific European coordinator. KD, EB, EK, FB, PM, EP, IM, MD, MO, SM, HP, TN, LM, FF, ON, KI, ZsB, DS, MR, EN were national coordinators for each European country. Data were analysed and the manuscript drafted by KD and MHW. All authors reviewed drafts of the manuscript.

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Transparency declaration

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Conflicts of Interest

KD, EB, MD, FF, EK, KI, IM, LvM, PM, SM, EN, TN, MO, EP, HP and DS report grants from Astellas Pharma Europe Ltd during the conduct of the study. GD reports grants from Astellas Pharma Europe Ltd during the conduct of the study and grants from Astellas Pharma Europe Ltd outside the submitted work. CL was a salaried employee of Astellas Pharma Europe Ltd during the conduct of this study. ZsB reports grants, personal fees and non-financial support from Astellas Pharma Europe Ltd during the conduct of this study. MR reports grants from Astellas Pharma Europe Ltd during the conduct of the study and grants and personal fees

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Table 1: Demographic data of in-patients with samples submitted by Participating Hospitals (PHs) during both sample collection periods

	Sample group		
	CDI positive by SRM N (% of total)	CDI confirmation positive N (% of total)	Total N (% of total)
Gender:			
Males	311 (48.5)	478 (49.3)	3646 (50.0)
Total	641	969	7297
Age :			
Median (years)	74.0	73.0	64.0
Inter-quartile range (years)	59.0-81.0	54.8-81.0	35.0-78.0
<20 years	50 (7.8)	104 (10.8)	1310 (18.0)
20-60 years	111 (17.3)	173 (17.9)	1964 (26.9)
60-80 years	275 (43.0)	398 (41.2)	2526 (34.6)
>80 years	204 (31.9)	292 (30.2)	1493 (20.5)
Total	640	967	7293
Ward Location:			
ITU/HDU	21	40	414
Medical	421 (65.7)	603 (62.2)	4506 (61.8)
Obstetrics and gynaecology	0 (0.0)	3 (0.3)	41 (0.6)
Other	93 (14.5)	149 (15.4)	787 (10.8)
Paediatric	44 (6.9)	79 (8.2)	942 (12.9)
Surgery	62 (9.7)	95 (9.8)	605 (8.3)
Total	641	969	7295

Table showing the patient demographics for all samples and samples found positive for CDI at the National Coordinating Laboratory (NCL) using the optimised method and the confirmation method. SRM = Study reference method. ITU = Intensive treatment unit. HDU = High dependency unit.

Table 2: Under-diagnosis and misdiagnosis of CDI in samples from Participating Hospitals (PHs) during the EUCLID Study

Country	Number of samples submitted per hospital (n/N)	Percentage of submitted samples tested at PH (%)	No. of undiagnosed cases N (% of all positives)	No. of false positives at PH N (% of total samples tested)	No. of false negatives at PH N (% of total samples tested)
Austria	77/9	75.3	1 (20.0)	4 (6.9)	0 (0.0)
Belgium	156/10	72.4	0 (0.0)	3 (2.7)	1 (0.9)
Bulgaria	110/8	11.8	7 (87.5)	1 (7.7)	1 (7.7)
Czech Republic	136/10	97.8	1 (6.7)	17 (12.8)	2 (1.5)
Finland	113/5	55.8	2 (33.3)	2 (3.2)	0 (0.0)
France	666/70	61.6	8 (30.8)	13 (3.2)	2 (0.5)
Germany	2146/87	61.6	62 (24.5)	81 (6.1)	32 (2.4)
Greece	118/11	52.5	3 (60.0)	8 (12.9)	0 (0.0)
Hungary	270/10	60.7	6 (15.0)	3 (1.8)	0 (0.0)
Ireland	149/5	83.2	0 (0.0)	6 (4.8)	3 (2.4)
Italy	710/65	64.8	15 (20.0)	21 (4.6)	3 (0.7)
Netherlands	126/14	66.7	0 (0.0)	4 (4.8)	0 (0.0)
Poland	320/27	59.1	10 (19.2)	25 (13.2)	5 (2.6)
Portugal	135/11	64.4	2 (8.7)	3 (3.4)	2 (2.3)
Romania	266/16	12.8	22 (66.7)	7 (20.6)	2 (5.9)
Slovakia	158/6	34.2	2 (20.0)	3 (5.6)	1 (1.9)
Slovenia	28/2	25.0	0 (0.0)	1 (14.3)	0 (0.0)
Spain	431/51	66.1	2 (8.3)	15 (5.3)	5 (1.8)
Sweden	133/9	77.4	0 (0.0)	3 (2.9)	0 (0.0)
UK	1049/56	78.1	5 (14.3)	17 (2.1)	9 (1.1)
Europe	7297/481	62.8	148 (23.1)	237 (5.2)	68 (1.5)

Table shows the number of samples sent from local hospitals to national coordinating laboratories and the percentage of those that had a test at the submitting Participating Hospital (PH) before submission. The percentage of all the samples which were positive for CDI at the National Coordinating

Laboratory (NCL) (using the Study reference method (SRM)) which never received a test are indicated as under-diagnosed. Those which had incorrect results given at the original local hospital when compared with the Study reference method (SRM) result at the NCL are indicated as misdiagnosis. This table shows the combined data from winter and Summer sampling periods. The full data set can be found in Supplementary materials table 2.

Table 3: Mean reported testing and CDI rates/10,000 patient bed days at Participating Hospitals (PH s) for each country in the EUCLID study

	CDI positive rate/10,000 patient bed days				Testing frequency/10,000 patient bed days			
Country	Reported rate (2011-2012) (N = 458)	Measured rate (Winter sampling) (N = 396)	Reported rate (2012-2013) (N = 458)	Measured rate (Summer sampling) (N = 396)	Reported rate (2011-2012) (N = 458)	Measured rate (Winter sampling) (N = 396)	Reported rate (2012-2013) (N = 458)	Measured rate (Summer sampling) (N = 396)
Austria	4.4	8.5	4.1	5.9	49.1	121.7	50.4	82.2
Belgium	5.5	7.6	4.0	3.1	100.2	107.8	108.6	83.0
Bulgaria	0.8	51.5	0.7	12.9	4.6	0.0	7.7	1.9
Czech republic	4.4	33.0	6.2	4.8	49.1	152.2	35.1	127.7
Finland	14.9	16.3	28.7	8.8	124.3	87.7	223.3	116.7
France	3.9	4.6	3.3	2.9	38.2	40.9	37.7	36.7
Germany	10.2	27.9	11.0	21.7	70.0	130.6	78.8	143.7
Greece	3.4	3.1	3.9	3.8	29.5	45.4	26.4	67.4
Hungary	12.3	9.6	15.5	25.8	45.8	67.4	60.8	76.0
Ireland	4.8	12.2	9.1	0.0	129.3	265.7	173.1	283.3
Italy	9.5	9.4	7.2	14.3	67.6	69.8	55.0	65.5
Netherlands	7.4	0.0	5.3	12.1	97.3	96.8	71.1	79.4
Poland	8.6	29.4	8.2	48.3	34.4	127.0	37.4	143.0
Portugal	2.9	19.3	3.0	14.7	28.1	69.4	28.2	75.1
Romania	3.9	92.3	7.4	94.4	12.3	57.7	32.4	13.0
Slovakia	5.3	9.6	1.2	24.1	16.2	80.9	6.5	87.4
Slovenia	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]
Spain	3.5	11.0	3.2	9.8	57.3	82.2	49.2	108.1
Sweden	16.2	9.7	13.3	14.1	98.9	87.5	91.6	56.1
UK	3.8	6.2	3.7	5.1	132.5	122.1	142.2	109.5
Europe	6.6	19.0	7.3	17.2	62.3	95.4	69.2	92.4

[a] No data was supplied by the participating hospitals in Slovenia.

Table showing the CDI testing and CDI case rates per 10,000 patient bed days found in both of the EUCLID study questionnaire periods (2011-2012 and 2012-2013) as reported by the participating hospitals (PHs) from each study. The EUCLID measured rate was calculated using the actual number of cases or tests performed on the study day and the patient bed days supplied for each participating hospital for that year.

Table 4a*: C. difficile* infection testing policy and methodology reported by participating hospitals (PHs) in each country, Sept 2011-Aug 2012

Country	No. participating hospitals that test for CDI N (% of responders)	No. that test all diarrhoeal in-patient samples N (%)	No. that test all diarrhoeal in-patient samples if criteria are met[a] N (%)	No. that test diarrhoeal samples if requested by a physician N (%)	No. using optimised CDI diagnostic tests[b] N (%)	No. using stand-alone molecular diagnosis of CDI N (%)	No. using a method to detect toxins in faecal samples (although not optimal)[c] N (%)
Austria	9 (100)	1 (11.1)	4 (44.4)	4 (44.4)	1 (11.1)	0 (0.0)	6 (66.7)
Belgium	10 (100)	0 (0.0)	2 (20.0)	8 (80.0)	7 (70%)	0 (0.0)	10 (100)
Bulgaria	5 (50.0)	0 (0.0)	3 (60.0)	2 (40.0)	0 (0.0)	0 (0.0)	4 (80.0)
Czech republic	10 (100)	0 (0.0)	4 (40.0)	6 (60.0)	1 (10.0)	0 (0.0)	10 (100)
Finland	5 (100)	0 (0.0)	0 (0.0)	5 (100.0)	0 (0.0)	1 (20.0)	3 (60.0)
France	70 (100)	7 (10.0)	7 (10.0)	55 (78.6)	29 (41.4)	5 (7.1)	45 (64.3)
Germany	87 (100)	12 (13.7)	30 (34.5)	38 (43.7)	25 (28.7)	1 (1.1)	64 (73.6)
Greece	11 (100)	0 (0.0)	3 (27.3)	8 (72.7)	1 (9.1)	0 (0.0)	7 (63.6)
Hungary	10 (100)	0 (0.0)	2 (20.0)	8 (80.0)	9 (90.0)	0 (0.0)	10 (100)
Ireland	5 (100)	1 (20.0)	3 (60.0)	1 (20.0)	0 (0.0)	0 (0.0)	1 (20.0)
Italy	65 (100)	10 (15.4)	7 (10.8)	47 (72.3)	15 (23.1)	6 (9.2)	48 (73.8)
Netherlands	14 (100)	0 (0.0)	1 (7.1)	13 (92.8)	0 (0.0)	2 (14.3)	10 (71.4)
Poland	27 (100)	5 (18.5)	6 (22.2)	16 (59.3)	6 (22.0)	0 (0.0)	27 (100.0)
Portugal	11 (100)	0 (0.0)	1 (9.0)	10 (90.9)	2 (18.2)	1 (9.1)	10 (90.9)
Romania	5 (31.2)	0 (0.0)	0 (0.0)	3 (60.0)	0 (0.0)	0 (0.0)	4 (80.0)
Slovakia	6 (100)	0 (0.0)	0 (0.0)	6 (100.0)	0 (0.0)	0 (0.0)	6 (100)
Slovenia	2 (100)	0 (0.0)	0 (0.0)	2 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
Spain	51 (100)	3 (5.9)	4 (7.8)	44 (86.3)	12 (23.5)	1 (2.0)	32 (62.7)
Sweden	9 (100)	0 (0.0)	0 (0.0)	9 (100.0)	0 (0.0)	2 (22.0)	3 (33.3)
UK	56 (100)	14 (25.0)	38 (67.9)	4 (7.1)	44 (78.6)	0 (0.0)	54 (96.4)
Europe	468 (97.1)	53 (11.3)	115 (24.6)	292 (62.4)	152 (32.5)	18 (3.8)	354 (75.6)

Table 4b: *C. difficile* infection testing policy and methodology reported by participating hospitals (PHs) in each country, Sept 2012-Aug 2013

Country	No. participating hospitals that test for CDI N (% of responders)	No. that test all diarrhoeal in-patient samples N (%)	No. that test all diarrhoeal in-patient samples if criteria are met[a] N (%)	No. that only test on physician request N (%)	No. using optimised CDI diagnostic tests[b] N (%)	No. using stand-alone molecular diagnosis of CDI N (%)	No. using a method to detect toxins in faecal samples (although may not be optimal)c N (%)
Austria	9 (100)	0 (0.0)	2 (22.0)	4 (44.4)	1 (11.1)	1 (11.10)	2 (22.2)
Belgium	10 (100)	0 (0.0)	1 (10.0)	9 (90.0)	6 (60%)	0 (0.0)	9 (90.0)
Bulgaria	8 (80.0)	0 (0.0)	0 (0.0)	8 (100.0)	0 (0.0)	0 (0.0)	7 (87.5)
Czech republic	10 (100)	0 (0.0)	0 (0.0)	8 (80.0)	9 (90.0)	1 (10.0)	9 (90.0)
Finland	5 (100)	0 (0.0)	0 (0.0)	5 (100.0)	0 (0.0)	0 (0.0)	3 (60.0)
France	55 (100)	7 (10.0)	9 (16.4)	38 (69.1)	23 (41.8)	3 (5.4)	26 (47.3)
Germany	71 (100)	12 (13.7)	22 (31.0)	33 (46.5)	30 (42.2)	3 (4.2)	52 (73.2)
Greece	11 (100)	0 (0.0)	2 (18.1)	9 (81.8)	4 (36.4)	0 (0.0)	9 (81.8)
Hungary	9 (100)	0 (0.0)	3 (33.3)	4 (44.4)	9 (90.0)	0 (0.0)	9 (100)
Ireland	4 (100)	1 (20.0)	3 (60.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (20.0)
Italy	65 (100)	1 (1.5)	2 (3.1)	60 (92.3)	20 (30.8)	6 (9.2)	39 (60.0)
Netherlands	13 (100)	2 (15.4)	5 (38.5)	6 (46.1)	2 (15.4)	1 (7.7)	6 (46.1)
Poland	23 (100)	2 (8.7)	3 (13.0)	16 (59.3)	15 (65.2)	0 (0.0)	22 (95.6)
Portugal	11 (100)	0 (0.0)	0 (0.0)	11 (100.0)	2 (18.2)	1 (9.1)	9 (81.8)
Romania	8 (50.0)	0 (0.0)	0 (0.0)	8 (100.0)	0 (0.0)	1 (12.5)	7 (87.5)
Slovakia	6 (100)	0 (0.0)	0 (0.0)	6 (100.0)	0 (0.0)	0 (0.0)	6 (100)
Slovenia	0 (100)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Spain	51 (100)	3 (5.9)	11 (21.6)	36 (70.6)	35 (68.6)	1 (2.0)	42 (82.4)
Sweden	7 (100)	0 (0.0)	0 (0.0)	7 (100.0)	0 (0.0)	2 (22.0)	2 (28.6)
UK	52 (100)	5 (9.6)	44 (84.6)	2 (3.8)	49 (94.2)	0 (0.0)	51 (98.1)
Europe	427 (97.4)	33 (7.5)	107 (25.1)	270 (63.2)	205 (48.0)	20 (4.7)	311 (72.8)

[a] Tested all in-patient samples only if they met certain criteria (which varied at different hospitals), these included patient >2 years old, patient hospitalised >3 days, query antibiotic-associated diarrhoea.

[b] Optimised testing defined as detection of GDH and *C. difficile* toxins directly from a faecal sample, either using a combined method or algorithm approach.

[c] Includes stand-alone toxin EIAs and toxigenic culture

Tables showing numbers of participating hospitals (PH**s)** in each country and the percentage that reported testing all diarrhoeal faecal samples (empirical testing) in the two questionnaires. Testing methodology is indicated as those that use an optimised method, stand-alone molecular method or some form of detection of *C. difficile* toxins from faecal samples.

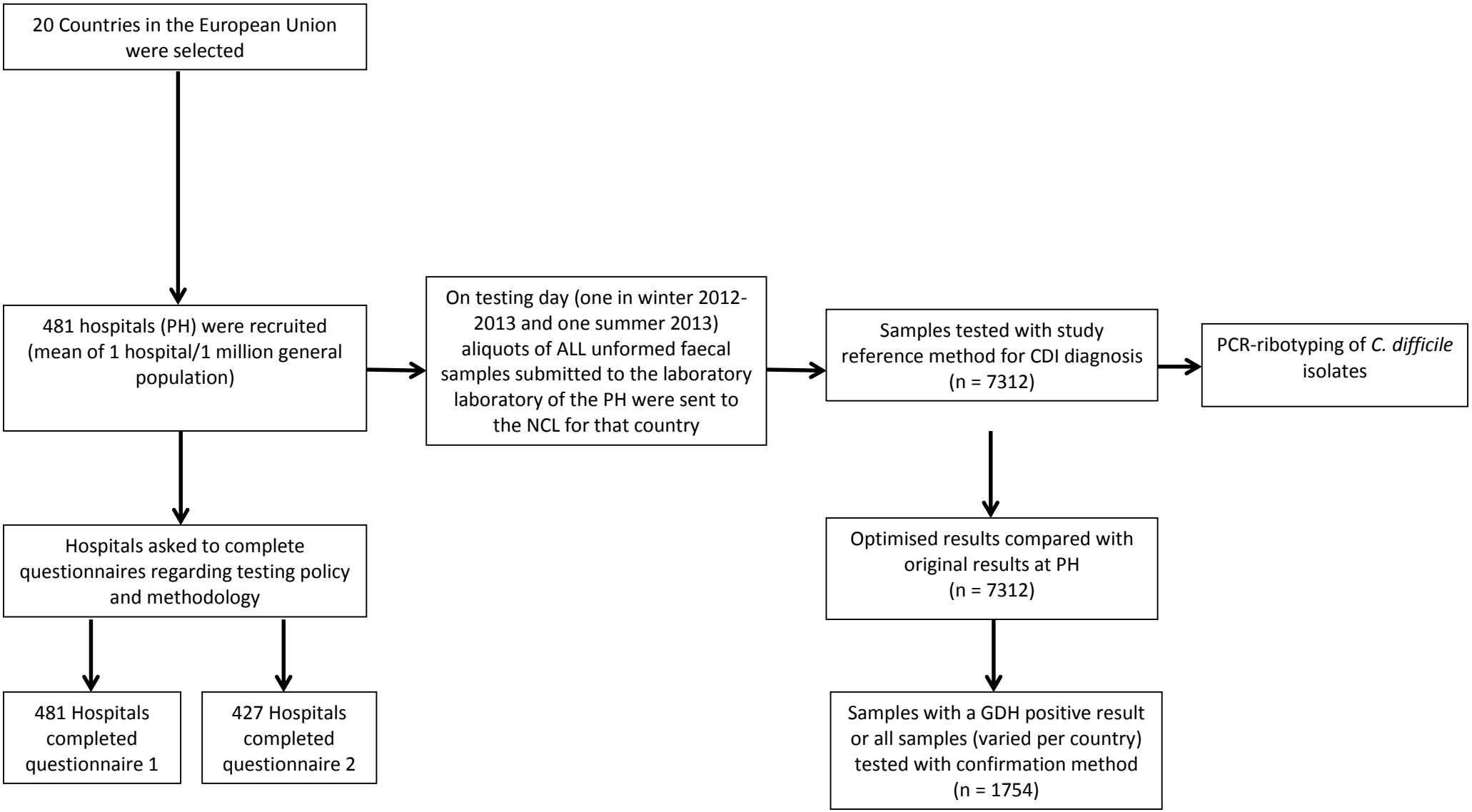
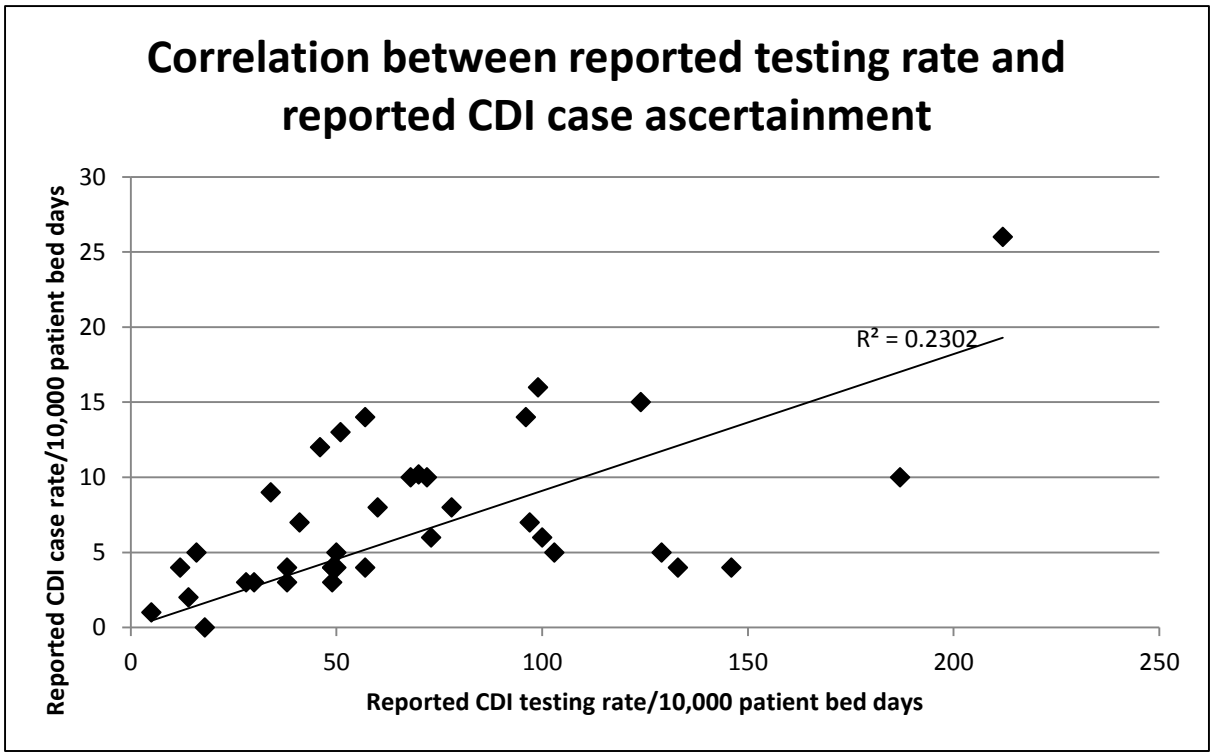
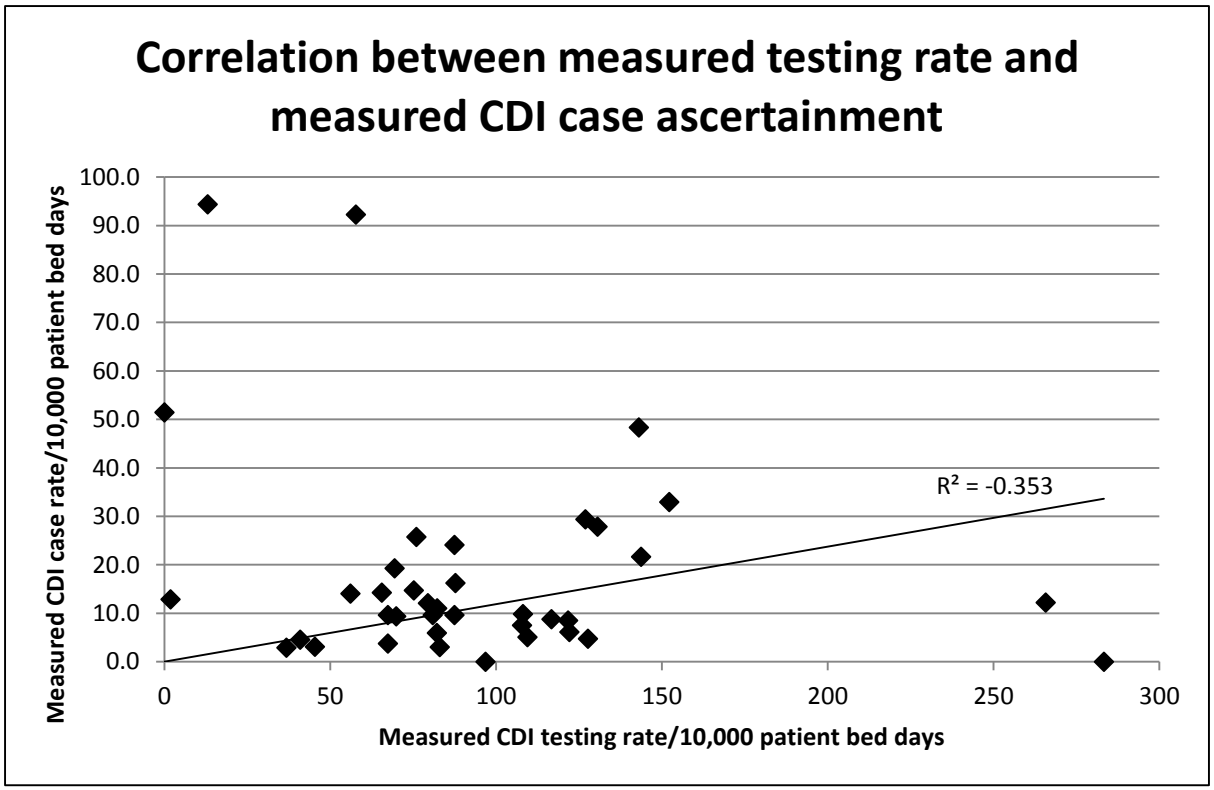


Figure 1. Flow diagram of EUCLID study





Figures 2a and 2b. The correlation between the reported and measured testing rates and CDI case ascertainment across Europe during both sampling periods

Pearson's correlation (r) for reported testing rate and reported CDI cases rate = 0.5741

Pearson's correlation (r) for measured testing rate and measured CDI cases rate = 0.2332

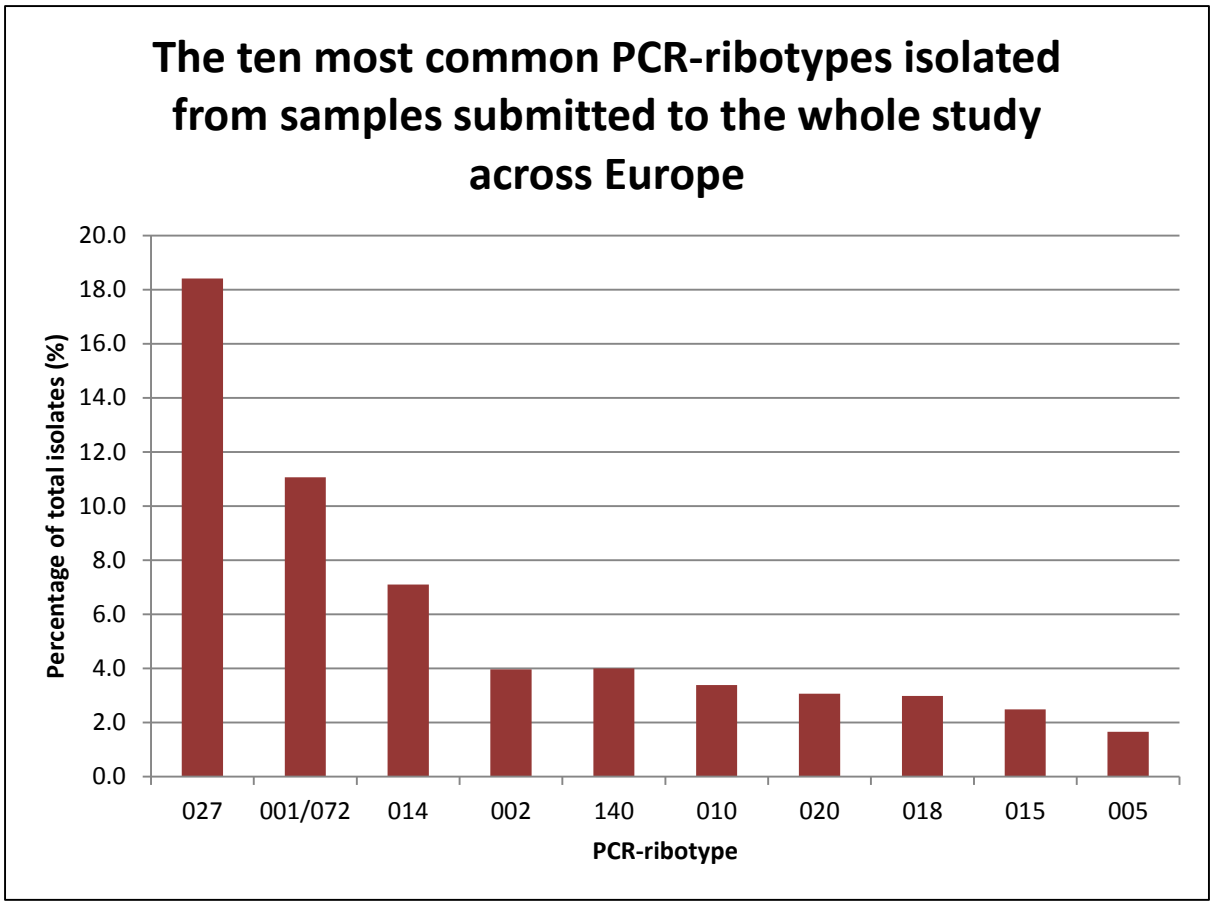
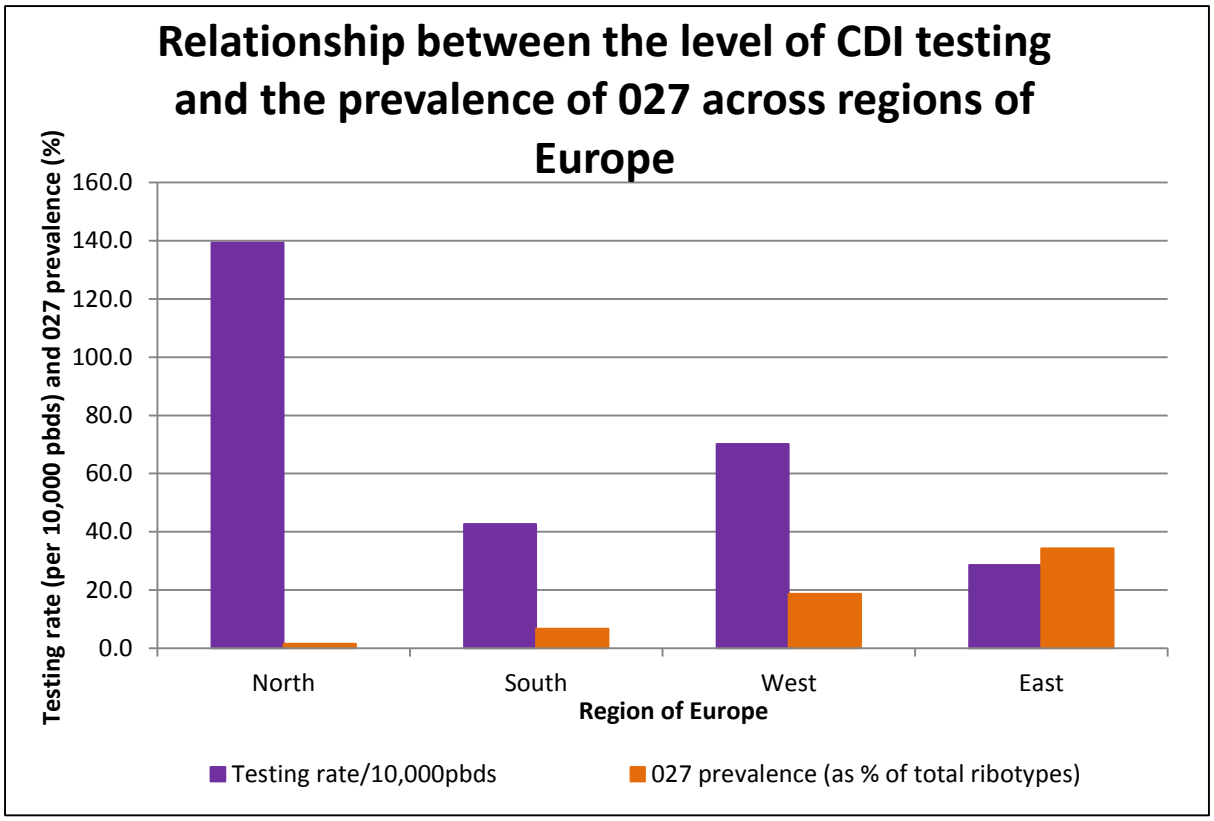


Figure 3. The ten most common PCR-ribotypes 1211 isolates from 7297 samples submitted to the study (winter and summer sampling) from across Europe



Pearson's correlation =-0.6996

Figure 4. The relationship between the level of CDI testing in each of four regions of Europe and the prevalence of 027 in that region.

Key:
North = Finland, Ireland, Sweden, UK , South = Greece, Italy, Portugal, , Spain, West = Austria, Belgium, France, Germany, Netherlands, East = Bulgaria, Czech Republic, Hungary, Poland, Romania, Slovakia. Based on the divisions of Europe according to UN Geoscheme for Europe (https://unstats.un.org/unsd/methods/m49/m49regin.html)