Advances in Medical Sciences 63 (2018) 290–295

Contents lists available at ScienceDirect

Advances in Medical Sciences

journal homepage: www.elsevier.com/locate/advms

Original research article

Prevalence of *Clostridium difficile* infection in hospitalized patients with diarrhoea: Results of a Polish multicenter, prospective, biannual point-prevalence study

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ARTICLE INFO

Keywords:
Clostridium difficile infection (CDI)
Surveillance of CDI
Incidence rates
Diagnosis
PCR-ribotypes

ABSTRACT

Purpose: We aimed to measure the underdiagnosis of *Clostridium difficile* infection across Poland and the distribution of PCR-ribotypes of *C. difficile*.

Material and methods: Twenty seven Polish healthcare facilities (HCFs) participated in this prospective study. Each HCF systematically sent all diarrhoeal stools received from inpatients at their laboratories on two days (one in January 2013 and one in July 2013), independently of CDI test request, to the National Coordinating Laboratory (NCL) for standardized testing of CDI. Positive samples (using two-stage algorithm), had CDI, confirmed by qPCR and toxigenic culture. *C. difficile* isolates were characterized by PCR-ribotyping. Hospitals were questioned about their methods and testing policy for CDI during the study period: September 2011 to August 2013.

Results: During the study period, participating hospitals reported a mean of 33.2 tests for CDI per 10 000 patient-days and a mean of 8.4 cases of CDI per 10 000 patient-days. The overall prevalence of positive CDI patients at NCL was 16.5%. Due to absence of clinical suspicion, 19.1% of these patients were not diagnosed by the local diagnostic laboratory. We identified 23 different PCR-ribotypes among 87*C. difficile* strains isolated from patients. PCR-ribotype 027 (48%) was the most prevalent.

Conclusions: The incidence of CDI in Poland in study period was very high. It should be noted however, that there is a lack of clinical suspicion and underestimation of the need to perform diagnostic tests for CDI in hospitalized patients. This will have an impact on the reported epidemiological status of CDI in Poland.

1. Introduction

Clostridium difficile is a leading cause of nosocomial diarrhoea [1]. Since 2003, a rising incidence of *C. difficile* infection (CDI) in North America and Europe has coincided with outbreaks of *C. difficile* North American Pulsotype 1/BI/PCR-ribotype 027 [2,3]. Epidemic PCR-ribotype 027 strains carry a nonsense mutation within the *tcdC* gene, leading to the derepression of the toxin genes *tcdA* and *tcdB*, which potentially leads to an increase in the level of virulence of these strains [4,5]. The first Polish isolate of *C. difficile* PCR-ribotype 027 was found in 2005 [6]. The main predisposing factors to CDI include increasing age, use of broad spectrum antibiotics and dysbiosis of the gut microbiota [7,8,9]. A pilot study supported by the European Centre for Disease Prevention and Control (ECDC) using standardized CDI surveillance performed in 14 European countries demonstrated that the incidence of healthcare-associated CDI in European hospitals was 4.2–131.8 per 10 000 discharges (median 16.4 per 10 000 discharges) and 0.6–18.5 per 10 000 patient-days (median 3.7 per 10 000 patient-days). The incidence of healthcare-associated CDI in the Polish hospitals in the same pilot study, was higher and reached 40.7–44.6 per 10

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https://doi.org/10.1016/j.advms.2018.03.003
Received 11 August 2017; Accepted 15 March 2018
Available online 16 April 2018

000 discharges (median 42.6 per 10 000 discharges) and 7.0–8.2 per 10 000 patient-days (median 7.6 per 10 000 patient-days) [10]. Within the Polish network the mean annual hospital CDI incidence rates were 6.1, 8.6 and 9.6 cases per 10 000 patient-days in 2011, 2012, and 2013, respectively [11]. Rapid and accurate diagnosis is important to optimize the patient's care and, as a result, to decrease further CDI transmission. A wide variety of testing strategies for CDI are used across Europe and in Poland [12,13,14,15], and the European guidelines have been recently updated [16].

We aimed to investigate the underdiagnosis of CDI in Polish hospitals by asking the participating hospitals to forward inpatients diarrhoeal faecal samples to a national coordinating laboratory for CDI testing, irrespective of whether microbiology tests had been requested or done locally. The participating hospitals were also asked to complete a study questionnaire about CDI testing and reporting.

2. Material and methods

2.1. Study design

Poland participated in the European, multicentre, prospective, biannual, point-prevalence study of *Clostridium difficile* infection in hospitalized patients with diarrhoea (EUCLID). The study was strictly non-interventional. The Polish national coordinator selected the participating hospitals; the number of the participating healthcare facilities (HCFs) was set to a minimum of 1 HCF per 1 million inhabitants. In Poland, 40 hospitals were invited but only 27 agreed to participate. Of the 27 hospitals that responded favourably, 24 provided secondary (n = 16) or tertiary care (n = 8), and 3 were specialized centres: 1 in pediatrics, 1 in pulmonology/thoracic surgery and 1 in oncology. We reported the summarised results back to the participating hospitals.

2.2. Outcomes

The primary outcome measures were: mean reported rates of testing for and cases of CDI per 10 000 patient-days, using the data reported in the questionnaires by the 27 participating Polish HCFs; and EUCLID study measured rates of testing for and cases of CDI per 10 000 patient-days as determined by NCL (to calculate the proportion of missed diagnosis of CDIs). The secondary outcomes were the testing methods used by the local laboratories participating in the Polish part of the EUCLID study to establish a diagnosis of CDI, and the PCR-ribotype of strains causing infections in the study period.

2.3. Samples

On two sampling days (one in January 2013 and one in July 2013, to reflect possible seasonal variations in CDI), all inpatient diarrhoeal faecal samples were sent to the microbiology departments in the 27 participating HCFs in Poland, regardless of the test requested, and further transferred to a national coordinating laboratory (NCL) located in the Department of Medical Microbiology in Warsaw. Samples were transported at ambient temperature during winter and at 2–8 °C during summer. Each HCF also completed a single standardized questionnaire about the patient, including age, sex, the service in which the patient was hospitalized, sample collection date, information whether the stool was tested for *C. difficile* and, if appropriate, the result reported. A second more general questionnaire asked each laboratory to describe the methods and strategies for CDI diagnosis, the number of *C. difficile* tests performed, the number of CDI positive results and the number of patient bed days for 2012. We did not exclude patients by age, but we included only one sample per patient on each sampling day. The participating hospitals anonymised samples with a EUCLID study number and sent the anonymous sample and data capture form to the NCL in Warsaw.

2.4. Procedure

At the NCL all faecal samples were tested with a three-stage algorithm, a membrane EIA for glutamate dehydrogenase (GDH) and *C. difficile* toxins A/B (*C. DIFF QIIK CHEK COMPLETE*, TechLab, Orlando, FL, USA) following the manufacturer instructions. We did confirmatory testing on all samples that were positive for either GDH or *C.difficile* toxins (GDH + TOX+, GDH + TOX- and GDH-TOX +), which consisted of two combinations of tests: *The Xpert kit* (Cepheid, Sunnyvale, CA, USA; qPCR) and culture for *C difficile* on selective medium (ChromID *C. difficile*, bioMérieux SA; Marcy l'Etoile, France). We sent all *C. difficile* isolates identified at our NCL to the European coordinating laboratory in Leeds, United Kingdom, to confirm the identification and for PCR-ribotyping.

2.5. Toxigenicity and PCR ribotyping analysis

C. difficile isolates were frozen and stored at −70 °C in a Microbank™ bacterial storage system (Pro-Lab Diagnostics, UK) before sending to University of Leeds, Leeds, UK. The presence of *tcdB*, and binary toxin genes (*cdtA* and *cdtB*) and deletions in the *tcdC* gene were determined by qPCR and PCR ribotyping was performed on all *C. difficile* isolates using the previously published method [17].

3. Results

3.1. Reported incidence of CDI and testing frequency

Twenty seven hospitals spread over Poland participated in two days of prevalence survey: 8 centres were teaching hospitals (29.6%), 16 provincial hospitals (59.3%) and 3 specialized hospitals (11.1%). The total number of annual admissions in the study period was 888 628.0 (average 34 178.0; range 4 404.0–77 279.0).

Of the 27 hospitals c.a 100% (92.6%–96.3%; average 94.5%) reported (via questionnaires) their rates of testing and cases of CDI per 10 000 patient-days for the 1st September 2011 to 31st August 2013. The average number of CDI tests performed for the study period per HCF was 532.4 (range 59.0–1 945.0). The average number of positive CDI cases per HCF was 110.7 (10.0–545.0). The average number of CDI tests per 10 000 patient-days per HCF was 33.2 (3.6–97.1/10 000 patient-days). The mean reported incidence was 8.4/10 000 patient-days (range from 0.4 to 35/10 000 patient-days). The full hospital data is presented in Table 1.

3.2. Diagnostic tests used for CDI and decision criteria for testing at the participating hospitals

During the first study period, from September 2011 to August 2012, 6 (22%) of the 27 laboratories used optimised diagnostic tests for CDI (a two-step or three-step algorithm to diagnose CDI). In the second study period, from September 2012 to August 2013, 15 (65%) of the 27 laboratories used optimised diagnostic tests for CDI. In addition, one laboratory used the Illumigene® *C. difficile* – Meridian Bioscience, Inc. test for confirmation. None of the Polish laboratories used stand-alone molecular diagnosis of CDI in the study period. Different decision criteria were applied to perform the diagnostic tests for CDI on faecal specimens. Five (19%) of the 27 laboratories tested all faecal samples submitted to the laboratory, 16 (59%) tested specimens only on the request of a physician and 6 (22%) applied additional criteria for CDI diagnosis (e.g. testing all samples where there is a suspicion of antibiotic associated diarrhoea and testing all diarhoeal samples from patients who developed diarrhoea more than 48 h after admission (Fig. 1).

Among all the patients tested in the 27 HCF laboratories in the two prevalence surveys: 184 (57.3%) were male and 137 (42.7%) were female (in January: male n = 104 and female n = 69; in July: male n = 80 and female n = 68). The median age of patients was 43.5 years

Table 1
Characteristics of all 27 hospitals/laboratories participating in the EUCLID study period in Poland.

	No. of hospitals with available data (%)	
Academic level of care	8 (29.6.%)	
Availability of diagnostic tests for CDI	27 (100%)	
Data of hospitals		**Mean value (range)**
Admission of serviced hospitals	26 (96.3%)	34 178.0 (4 404–77 279)
The number of patients bed days	25 (92.6%)	150 611.5 (21 803–294 814)
The number of patients- days	25 (92.6%)	148 639.2 (24 634–285 410)
Average number of CDI tests performed per HCF	26 (96.3%)	532.4 (59.0–1 945.0)
Average number of positive CDI cases per HCF	26 (96.3%)	110.7 (10.0–545.0)
Average number of CDI tests performed per 10 000 patient-days per HCF	25 (92.6%)	35.9/10 000 patient-days (3.6–97.1/10 000 patient-days)
Average number of positive CDI cases per 10 000 patient-days per HCF	26 (96.3%)	8.4/10 000 patient-days (0.4–35.0/10 000 patient-days)

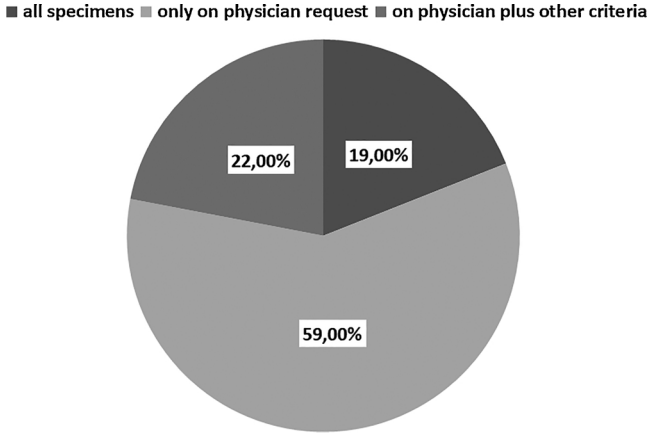

Fig. 1. Criteria used for performing CD diagnostic tests in Polish hospitals in 2012.

in January 2013 and 45.9 years in July 2013. Most samples (n = 108) originated from medical wards or from wards defined as 'other' (n = 114). The remaining were from pediatric (n = 72) and surgical (n = 27) wards. In the two prevalence surveys, 320 faecal samples and one swab with faecal material (173 in January 2013 and 148 in July 2013) were received for testing. Of the 321 samples received, 316 were analyzed by the NCL using (C. DIFF QIIK CHEK COMPLETE, TechLab, Orlando, FL, USA); four samples (because they were insufficient) and one swab was excluded. The results of screening and confirmatory tests are presented in Tables 2 and 3.

Of the 321 (173 in winter and 148 in summer) faecal samples (one sent as a swab), submitted to the NCL, 57.6% (n = 99) were tested for CDI in winter period and 60.8% (n = 90) in the summer period at the participating hospitals, with 89/321 (27.7%) CDI positive and 72.3% CDI negative. The overall prevalence of positive CDI patients at NCL was 16.5% and negative 83.5% what was confirmed using qPCR test.

Twenty six patients (13.2%) had a false-positive result (i.e. were diagnosed with CDI at the participating hospitals but did not have demonstrable *C. difficile* toxin in their faecal sample when tested with the reference method at NCL). In addition, 5 (2.6%) faecal samples had a false negative result at the local laboratories. The proportion of false positive diagnoses on the first study sampling day (in the winter) did not differ on the second study sampling day in the summer (13.1% vs. 13.3%). The number of patients with CDI not diagnosed by the local laboratory was 27 (8.45%) due an absence of clinical suspicion.

3.3. *C. difficile* PCR-ribotypes diversity in Poland

Among 87*C. difficile* isolates tested by PCR-ribotyping 77 were toxigenic and 10 non-toxigenic by using qPCR. Among 87 isolates of *C. difficile* we identified 23 different PCR-ribotypes across the 27 hospitals, 16 toxigenic: 027 (n = 37), 014/020 (n = 9), 176 (n = 5), 001/072 (n = 4), 023 (n = 4), 002 (n = 3), 046 (n = 2), 017 (n = 1), 018 (n = 1), 026 (n = 1), 029 (n = 3), 035 (n = 1), 045 (n = 1), 107 (n = 1), 115 (n = 1), 144 (n = 1). Among 10 nontoxigenic strains we detected 7 PCR-ribotypes: 010 (n = 4), 011 (n = 2), 031 (n = 1), 071 (n = 1), 085 (n = 1), 140 (n = 1), 147 (n = 1). The 5 most commonly isolated PCR-ribotypes received by the local laboratories are shown in Fig. 2. PCR-ribotype 027 was the most prevalent (48.0%); 014/020 (11.7%), and 176 (6.5%), and were the second and third most prevalent, respectively. The ribotype of one isolate was not identified.

4. Discussion

We aimed to investigate (as part of the EUCLID study), the underdiagnosis of CDI in hospitals in Poland by asking 27 HCFs to forward inpatiens faecal samples to our NCL for CDI testing by the reference method (two-stage algorithm using a membrane EIA for glutamate dehydrogenase (GDH) and *C. difficile* toxins A/B). The standard diagnostic method used in this study (C DIFF QUIK CHEK COMPLETE) was chosen because toxin detection in faecal samples correlates with clinical outcome. The next step was to confirm CDI positive faecal samples by using qPCR and toxigenicity culture (TC). The participating hospitals

Table 2
Underdiagnosis and misdiagnosis of *Clostridium difficile* infection.

	Period 1 January 2013	Period 2 July 2013
Number of specimens send to NCL	172	148
Average number of specimens per hospital	6.4	5.5
Number (percentage) of specimens tested by the participating hospital	99 (57.6)	90 (60.8)
Number (percentage) of samples reported as CDI positive at hospital (of those tested)	30 (30.3)	32 (35.6)
Number of samples reported as CDI negative at hospital	69	58
Number of samples tested at the NCL	169	147
Number (percentage) of samples CDI positive at NCL	27 (16.0)	25 (17.0)
Number (percentage) of CDI positive samples (at the NCL) with no original hospital test (missed diagnosis)	6 (22.2)	4 (16.0)
Number (percentage) of false positive results at the original hospital	13 (13.1)	12 (13.3)
Number (percentage) of false negative results at the original hospital	4 (5.8)	1 (1.1)

Abbreviations: NCL- the National Coordinating Laboratory, CDI − *Clostridium difficile* infection.

Table 3
Results of investigation of the faecal samples which were diagnosed by the National Coordinating Laboratory (NCL).

Results of C. DIFF QIIK CHEK COMPLETE test	January 2013 No. of faecal samples	July 2013 No. of faecal samples	Total
GDH (−) TOX (−)	115	101	216
GDH (+) TOX (+)	27	25	52
GDH (+) TOX (−)	20	21	41
GDH (−) TOX (+)	7	0	7
Total	**169**[a]	**147**[b]	**316**

Results of C. DIFF Quick CHEK COMPLETE test and culture	January 2013 No. of faecal samples	July 2013 No. of faecal samples	Total
GDH (+) TOX (+) culture (+)	25 tox plus	25 tox plus	50
GDH (+) TOX (−) culture (+)	17 (11 tox and 6 non tox)	19 (15 tox and 4 non tox)	36
GDH (+) TOX (−) culture (−)	3	2	5
GDH (−) TOX (+) culture (+)	1 (PCR ribotype 027)	0	1
Total *C. difficile* strains	43 (37 tox and 6 non tox)	44 (40 tox and 4 non tox)	77 toxigenic and 10 non tox

Abbreviations: GDH – glutamate dehydrogenase; Tox (+) − toxins A and B or A or B *C. difficile* were detected
[a] Total number of faecal samples analyzed by the NCL in January.
[b] Total number of faecal samples analyzed by the NCL in July.

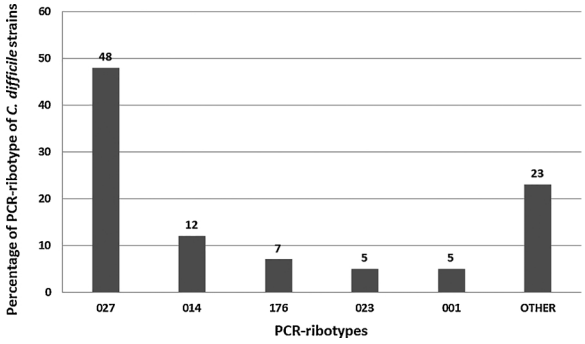

Fig. 2. Distribution of the 5 most commonly isolated *Clostridium difficile* PCR ribotypes from all *C. difficile* isolates from samples submitted by the participating hospitals.

were also asked to complete a study questionnaire about *C. difficile* testing. During the EUCLID study, the participating Polish hospitals reported a mean of 33.2 tests for CDI/10 000 patient-days. In the same study period in Europe (data were available for 19 countries; neither of the participating hospitals in Slovenia provided this information) testing rates for *C difficile* infection in both study periods was overall mean 65.8 tests per 10 000 patient-days (range in two point of study 62.3–69.2 per test per 10 000 patient-days).

Under the EUCLID study, in the 27 Polish HCFs, the reported mean incidence rate of CDI in our country was 8.4 per 10 000 patients-days in 2013. The mean incidence rate of CDI in Poland was higher compared to the mean incidence rate of CDI in the 482 participating hospitals across 20 European countries who reported a mean of 7.0 cases (country range 0.7–28.7) of *C difficile* infection per 10 000 patient-bed days [15]. Mean incidence of CDI reported under the EUCLID study period, by the HCFs in Poland was 8.4 per 10 000 patient-days and was correlated to the density testing (defined by the number of tests per 10 000 patient-days) (data not shown).

Under another study, conducted in Poland over a three year-period in 13 hospitals, we found an annual mean incidence rate of 8.17 CDI per 10, 000 patient-bed days in 2011–2013 [11].

Of the 320 faecal samples (plus one swab), submitted in two days from the 27 Polish HCFs to the NCL, 59.1% (n = 189) were tested in local laboratories for CDI. Of the 7297 samples submitted to the NCLs across Europe, 62.8% (n = 4582) were tested for *C difficile* infection at the participating hospitals [15]. In Poland, out of the 27 participating laboratories, 22% in the first study period and 65% in the second study period used optimised methods for laboratory diagnosis of CDI. During the first period under the EUCLID study, 152 (32%) of 468 participating hospitals reported using optimised methods for laboratory diagnosis of CDI. In the second study period the number of the European hospitals using optimised methods for CDI diagnosis increased significantly to 205 (48%). We observed an increase in the optimisation of methods in Poland.

The average reported CDI positivity rate, diagnosed by the 27 HCFs in 2013, was 27.8%. However, among the faecal samples diagnosed by the local laboratories mean 13.2% had false-positive results and 2.6% faecal samples had false-negative results, when compared to the results at the NCL. The overall positive CDI in the NCL the proportion of false positive diagnoses on the first study sampling day (in the winter) was not different to the second study sampling day in the summer (13.1% vs 13.3%, respectively). Under the EUCLID study, 237 (5%) of all samples tested had false-positive results. The highest false-positive rates were noted in the countries, where the diagnosis of *C difficile* infection was based on detection of *C. difficile* toxins by an EIA only. In the EUCLID study organized in 20 European countries the highest false-positive results were found in: Romania (20.6%), Slovenia (14.3%), Poland (13.2%) Greece (12.9%) and Czech Republic (12.8%) [15]. Under the EUCLID study the United Kingdom had the highest proportion of participating hospitals using an optimised method for *C. difficile* infection diagnosis and had low proportions of both false-positive and false-negative results. Under the EUCLID study organized in Poland, 5 (2.6%) faecal samples had a false negative result at the local laboratories. The participating European laboratories in 20 countries reported 68 (2.0%) false-negative results among all samples. Refrigerated transport of samples was used across Europe and in Poland, during the summer testing period but not during the winter. It could have influenced the degradation of *C. difficile* toxins at room temperature. Some faecal samples were reported as toxins-positive by participating laboratories.

Across the 482 participating hospitals located in 20 European countries on the two sampling days, 148 (23%) of 641 positive samples were undiagnosed. They had a positive result by the standard reference method at an NCL but were not tested for CDI at HCFs located in different countries. Of these 148 samples 125 were confirmed as positive by using toxigenic culture or PCR. In Poland, 10 (19.1%) of all CDI cases were not diagnosed due to the lack of clinical suspicion. Alcala et al. showed that two-thirds of CDI cases were not diagnosed by local laboratories, due to the lack of clinical suspicion in 48.9% (versus 30.2% in French study), or due to the lack of sensitivity of the diagnostic methods used in 20.0% of cases [18,19].

In our study, *C. difficile* PCR-ribotype 027 (48%) was the most prevalent ribotype overall. This is consistent with the main EUCLID study, where PCR-rib type 027 was the most prevalent ribotype (n = 222; 19%) isolated from 1.211*C. difficile* isolates from across the 20 European countries [17]. Among the 222*C. difficile* isolates of 027 in the EUCLID study, 88% of occurrences of this PCR-ribotype were

recorded in only four countries: Germany (43%), Hungary (17%), Poland (16%), and Romania (12%). The second and third most prevalent PCR-ribotypes in the main EUCLID study were: 001/072 (11%, n = 134) and 014/020 (10%, n = 119). In the Polish part of the EUCLID study these PCR-ribotypes made up 11.7% (014/020) and 4.6% (001/002) of our isolates. PCR-ribotype 176 was the third most prevalent in Polish diarrhoeal patients (6.5%). Endemicity for PCR-ribotype 176 was observed in the EUCLID study in the Czech Republic (38% of all ribotypes). Whole-genome sequencing studies have revealed that *C. difficile* isolates belonging to PCR-ribotype 176 are closely related to those of PCR-ribotype 027 and it has been suggested that this type may often be misdiagnosed as a PCR-ribotype 027 infection [11,20].

Overall, we found 23 PCR-ribotypes in the 27 Polish hospitals in the EUCLID study, demonstrating with less diversity than in our own surveillance in Poland organized in 2011–2013 in 13 hospitals, when we found 27 ribotypes [11].

We have clearly shown that the lack of clinical suspicion and the use of tests with lower sensitivity (immunoassay to detect only toxins *C. difficile*) for the diagnosis of CDI in Poland, may facilitate the transmission of *C. difficile.* Five (19%) of the 27 Polish laboratories tested all faecal samples submitted to the laboratory versus 33 (8%) of the 428 laboratories across Europe.

False-positive results may cause unnecessary administration of antibiotics for treatment of patients with CDI. In addition, it can increase the cost of hospitalization due to increasing the length of patient hospital stay, changing treatment, or implementing costly contact precaution. Our findings are similar to that of the EUCLID study and show that CDI is underdiagnosed across Poland, driven mainly by the absence of clinical suspicion and, hence, no local testing for CDI. The findings of the EUCLID study were that the absence of clinical suspicion and/or suboptimum diagnostic methods results in an estimated ca. 40 000 hospitalized patients with CDI to be undiagnosed every year in the hospitals participating in the EUCLID study.

5. Conclusions

The findings of this analysis from a Polish study, as a part of the EUCLID study, emphasize the importance of national surveillance programmes to monitor the epidemiology of *C. difficile*, including the use of optimal diagnostic methods to identify true CDI cases.

Conflict of interest

KD received grants from Astellas Pharma Europe Ltd, Alere ltd and Cepheid; and honorarium from Astellas Pharma Europe Ltd. HP received remuneration for lectures from Astellas Pharma, Poland.

The authors report no other potential conflict of interest relevant to this article.

Financial disclosure

The EUCLID study was initiated and wholly supported financially by Astellas Pharma EMEA.

Acknowledgments

We would like to thank the Polish EUCLID *Clostridium difficile* Study Group:

A. Guzek, Department of Microbiology, Military Institute of Medicine, Warsaw (H1), G. Nurzyńska, Department of Microbiology, Central Public Hospital of Medical University of Warsaw, Warsaw (H2), K. Dzierżanowska-Fangrat, Department of Clinical Microbiology and Immunology, The Children's Memorial Health Institute, Warsaw (H3), A. Mikucka, Department of Microbiology, Nicolaus Copernicus University, Ludwik Rydygier Collegium Medicum, Bydgoszcz, Dr A. Jurasz University Hospital, Bydgoszcz (H4), H. Kołakowska, Department of Microbiology, Hospital in Wyszków (H5), K. Jermakow, Department of Medical Microbiology, Wrocław Medical University, Wrocław (H6), M. Kesler, Deartment of Microbiology, Hospital in Ostrów Mazowiecka (H7), M. Kozak, Department of Microbiology, Hospital in Inowrocław (H8), K. Golec, Department of Microbiology, Hospital in Rzeszów (H9), D. Olszańska, Department of Microbiological Diagnosis and Infection Immunology, Clinical Hospital of Medical University in Bialystok (H10), D. Pawlik, Department of Microbiology, Provincial Hospital, Maków Mazowiecki (H11), J. Bakiera, Department of Microbiology, Hospital in Lublin (H12), M. Musz-Kawecka, Department of Microbiology, the Medical Center in Łańcut, Łańcut (H13), G. Woch, Department Laboratory Diagnostics, Microbiology, Military Teaching Hospital Veterans (H14), M. Kostkiewicz, Department of Microbiology, Voivodship Hospital, Płock (H15), G. Szulencka, Department of Microbiology, Provincial Hospital, Włocławek (H16), A. Byś, Department of Microbiology, Voivodship Hospital, Biała Podlaska (H17), L. Guzik, Voivodship Hospital, in Krosno (H18), K. Sztych, Department of Microbiology, Provincial Hospital, Piła (H19), E. Waker and K. Hass, Department of Microbiology, Institute of Oncology, Warsaw (H20), G. Dubiel, Center of Pulmonology and Thoracic Surgery in Bystra, Bystra (H21), H. Drzewiczak, Department Microbiology, Voivodship Hospital,Siedlce (H22), A. Schneider and A. Mól, Department of Microbiology, Hospital of the Transfiguration, Poznań (H23), J. Kędzierska, Department of Microbiology, University Hospital, Cracow (H24), R. Kuthan and A. Sawicka-Grzelak, Department of Medical Microbiology, Jesus Teaching Hospital, The Medical University of Warsaw (H25), G. Lubszczyk, Department of Microbiology, Hospital in Rybnik (H26), M. Cichoń, Department of Microbiology, Hospital in Bielsko-Biała (H27).

We would like to acknowledge the EUCLID European Coordinator (Univeristy of Leeds, UK) and all Polish microbiologists taking part in this study.

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