Clostridium difficile PCR ribotypes 001 and 176 – the common denominator of *C. difficile* infection epidemiology in the Czech Republic, 2014

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In 2014, 18 hospitals in the Czech Republic participated in a survey of the incidence of *Clostridium difficile* infections (CDI) in the country. The mean CDI incidence was 6.1 (standard deviation (SD):7.2) cases per 10,000 patient bed-days and 37.8 cases (SD: 41.4) per 10,000 admissions. The mean CDI testing frequency was 39.5 tests (SD: 25.4) per 10,000 patient bed-days and 255.8 tests (SD: 164.0) per 10,000 admissions. A total of 774 *C. difficile* isolates were investigated, of which 225 (29%) belonged to PCR ribotype 176, and 184 isolates (24%) belonged to PCR ribotype 001. Multilocus variable-number tandem repeat analysis (MLVA) revealed 27 clonal complexes formed by 84% (190/225) of PCR ribotype 176 isolates, and 14 clonal complexes formed by 77% (141/184) of PCR ribotype 001 isolates. Clonal clusters of PCR ribotypes 176 and 001 were observed in 11 and 7 hospitals, respectively. Our data demonstrate the spread of two *C. difficile* PCR ribotypes within 18 hospitals in the Czech Republic, stressing the importance of standardising CDI testing protocols and implementing mandatory CDI surveillance in the country.

Introduction

Clostridium difficile is the most important bacterial cause of hospital-acquired diarrhoea. Two large studies have been carried out to map and update data on *C. difficile* infection (CDI) in Europe [1,2]. CDI incidence showed an increasing trend: in the first study in 2008, the mean incidence in the participating countries was 4.1 cases per 10,000 patient bed-days [1], while in the second, in 2011–13, it was 7.0 CDI cases per 10,000 patient bed-days in the countries involved [2].

Results of the 2008 study – a hospital-based survey involving 34 European countries – showed that the Czech Republic had a low incidence of CDI (1.1/10,000 patient bed-days), without the presence of *C. difficile* PCR ribotypes 027 and 176 [1]. Spread of PCR ribotype 027 has been seen worldwide [3] and is known to be associated with hospital CDI outbreaks [4] and severe course of disease and increased mortality [5].

Ribotype 176 is closely related to 027 [6,7] and can be misidentified by commercial tests targeting a single-base-pair deletion at nucleotide 117 in the *C. difficile* *letcdC* gene [8]. In 2009, shortly afterward the 2008 study, the occurrence of ribotype 176 was reported in certain areas of the country (Eastern Bohemia and Moravia) [9]. This ribotype has persisted in the Czech Republic [8] and was also reported in Poland in 2008–13, which borders the country [10,11].

Results from the second study – involving 20 European countries – revealed an increasing CDI incidence rate in the Czech Republic (4.4 cases in 2011–12/10,000 patient bed-days and 6.2 cases/10,000 patient bed-days in 2012–13) [2].

This observation prompted us to determine the CDI incidence in 2014 in a number of hospitals distributed across the Czech Republic (n = 18) and to gain an insight into the prevailing *C. difficile* ribotypes.

Methods

A CDI case was defined as a hospitalised patient (more than two years-old) with both diarrhoea and laboratory confirmation of CDI by a positive test result for the presence of GDH and toxin A/B and/or the detection

FIGURE 1

Location of hospitals participating in survey of incidence of *Clostridium difficile* infection, Czech Republic, 2014 (n = 18)

of a toxin-producing *C. difficile* strain using toxigenic culture or nucleic acid amplification test (NAAT) in the stool sample.

Testing for CDI was requested by the attending physician based on clinical symptoms indicating CDI (primarily diarrhoea). Hospital-associated and community-associated CDI cases were included in the analysis.

During 2014, hospital microbiology departments of the 18 selected hospitals were asked to send *C. difficile* isolates cultured from stool samples from hospitalised CDI patients to the Department of Medical Microbiology of the University Hospital Motol in Prague.

Mean CDI incidence and CDI testing frequency for all participating hospitals was calculated using the total number of admissions, total number of patient bed-days, number of non-duplicated glutamate dehydrogenase (GDH) and toxin A/B positive tests performed in 2014, using information obtained from the participating hospitals. The hospitals also provided information about their CDI laboratory diagnostic algorithms.

C. difficile isolates were further characterised using PCR ribotyping, detection of the presence of genes for toxin production (*tcdA* (A), *tcdB* (B), *cdtA* and *cdtB* (binary)) by a multiplex PCR [12] and multilocus variable-number tandem repeat analysis (MLVA).

PCR ribotyping based on capillary electrophoresis was performed according to the method described by Stubbs et al. [13]. The results were compared with data in WEBRIBO, a web-based database containing a broad spectrum of uploaded capillary electrophoresis-ribotyping profiles [14], and profiles from an international capillary electrophoresis-ribotyping validation study [15]. The diversity of ribotypes for each hospital

was calculated using the Shannon index [16], for which a higher value is an indicator of greater diversity.

For MLVA, five regions with short tandem repeats were sequenced: A6Cd, B7Cd, C6Cd, G8Cd [17] and CDR6o [18], with a change of reverse primer for G8Cd, as described elsewhere [19]. The number of tandem repeats was counted manually after software processing (Sequencing Analysis Software, Applied Biosystems). The sum of tandem repeat differences (STRD) in five loci determines the genetic relatedness of isolates. Minimum spanning trees were created using Bionumerics v5.1 (Applied Maths). A clonal complex was defined as an $\text{STRD} \leq 2$, a genetically related cluster as an $\text{STRD} \geq 3$ to ≤ 10 [17].

Results

Participating hospitals

A total of 18 hospitals, covering the country's major regions, voluntarily participated in the survey: seven tertiary care institutions, 10 secondary care facilities and one specialised centre. The size of hospital is indicated by the number of beds in 2014 (Table 1). These 18 hospitals represented about 30% of hospital-bed capacity in the Czech Republic in 2013 [20] (2014 data unavailable). Their location is shown in Figure 1.

Incidence of *C. difficile* infection and testing frequency

The incidence of CDI in 2014 varied from 1.5 to 34.7 (median: 3.9) cases per 10,000 patient bed-days (mean: 6.1 cases (standard deviation (SD): 7.2)/10,000 patient bed-days), and from 11.8 to 201.2 (median: 26.5) cases per 10,000 admissions (mean: 37.8 cases (SD: 41.4)/10,000 admissions).

The frequency of testing for CDI in the hospital laboratories varied from 6.0 to 116.3 tests (median: 28.9) per 10,000 patient bed-days (mean: 39.5 (SD: 25.4) tests per 10,000 patient bed-days), and from 36.4 to 673.5 tests (median: 216.7) per 10,000 admissions (mean: 255.8 tests (SD: 164.0)/10,000 admissions) (Table 1).

C. difficile infection testing algorithms

Four different CDI testing algorithms were used during the study period (Table 1). All hospitals in the study used the detection of GDH and toxins A/B as the first (screening) part of their testing algorithm: 14 used lateral flow immunoassay (LFIA), three used a chemiluminescent immunoassay (CLIA) and one a chromatographic immunoassay (CIA).

A total of 16 hospitals performed anaerobic culture of GDH-positive and toxin-positive or toxin-negative samples, but only two of these tested toxin production or detected the presence of genes for toxin production of isolated *C. difficile* strains (one by LFIA and one by toxin gene multiplex PCR). The remaining two hospitals, which did not routinely perform anaerobic culture, used PCR detection of the presence of *C. difficile* toxin

FIGURE 2

Distribution of the six most prevalent PCR ribotypes of *Clostridium difficile* in 18 hospitals participating in survey of incidence of *C. difficile* infection, Czech Republic, 2014 (n = 774)

genes in GDH-positive and toxin A/B-negative stool samples.

Of the 18 hospitals, 10 used a commercial PCR test, eight for rapid diagnosis if requested by the physician. In total, 774 *C. difficile* isolates were available for further analysis in our study: 378 were from male patients (49%) and 396 from female patients (51%). The mean age was 68 years (SD: 20); the median was 72 years (range: 2–101). Of the 774 patients, 537 (69%) were aged 65 years or older.

PCR ribotypes of *C. difficile* isolates

Of the 774 *C. difficile* isolates, 737 (95%) belonged to 33 different ribotypes, and 37 (5%) were defined as new ribotypes, as their electrophoretic profiles differed from each other and did not match any in the WEBRIBO database.

The most frequent PCR ribotype, 176, was found in 225 isolates (29%) in 17 hospitals. The second most frequent, PCR ribotype 001, was identified in 184 isolates (24%) in 14 hospitals. Other frequently found PCR ribotypes were: 014 (n = 70 (9%); 16 hospitals), 012 (n = 41 (5%); 12 hospitals), 020 (n = 31 (4%); 14 hospitals), 017 (n = 30 (4%); 10 hospitals). The distribution of the six most prevalent PCR ribotypes (581 isolates, 75%) within the participating hospitals is shown in Figure 2. Other less frequent ribotypes found were as follows, with the number of isolates per ribotype shown in parentheses: 002 (n=20), 005 (n=14), 081 (n=11), 029 (n=10), 015 (n=10), 070 (n = 9), 023 (n = 8), 078 (n = 7),

003 (n = 6), 503 (n = 5), 449 (n = 5), 046 (n = 5), 018 (n = 5), 087 (n = 5), 049 (n = 5), 126 (n = 4), AI-75 (n = 4), AI-9-1 (n = 4), 054 (n = 4), 446 (n = 3), AI-82/1 (n = 3), 053 (n = 2), 027 (n = 2), AI-60 (n = 2), 043 (n = 1), 236 (n = 1) and AI-12 (n = 1).

The Shannon index, used to determine the diversity of the ribotypes, varied from 0.54 to 2.56. The Shannon index of all the *C. difficile* ribotypes in the study was 2.58, indicating a highly diverse set of *C. difficile* isolates.

Further characterisation of *C. difficile* isolates

Genes for production of three *C. difficile* toxins (A, B and binary) were detected in 246 (32%) of the isolates belonging to the following PCR ribotypes: 176 (n = 225 (29%)), 023 (n = 8 (1%)), 078 (n = 7 (0.9%)), 126 (n = 4 (0.5%)) and 027 (n = 2 (0.3%)). For the other 528 isolates (68%), only genes for production of toxins A and B were detected.

MLVA of five variable-number tandem repeat loci was performed for the 225 isolates of ribotype 176 and 184 isolates of ribotype 001, and two minimum spanning trees were generated.

In total, 27 clonal complexes comprising 190 isolates (84%) were found in the minimum spanning tree of PCR ribotype 176 isolates (Table 2). For each clonal complex, the number of isolates/number of hospitals in which they were found are shown in parentheses: CC1(52/10); CC2(19/4); CC3(19/1); CC4(11/3); CC5(10/3); CC6(11/1);

FIGURE 3

Minimum spanning tree of *Clostridium difficile* PCR ribotype 176 isolates from 17 of 18 hospitals participating in survey of incidence of *C. difficile* infection, Czech Republic, 2014 (n = 225)

Each hospital is represented by a different colour (see key). The numbers in the circles represent the number of *C. difficile* PCR ribotype 176 isolates. If the number is greater than one, it represents the number of isolates with a sum of tandem repeat differences (STRD) = 0 (i.e. 100% identical in five variable-number tandem repeat loci). The numbers on the lines represent the STRD between isolates.

FIGURE 4

Minimum spanning tree of *Clostridium difficile* PCR ribotype 001 isolates from 14 of 18 hospitals participating in survey of incidence of *C. difficile* infection, Czech Republic, 2014 (n = 184)

Each hospital is represented by a different colour (see key). The numbers in the circles represent the number of *C. difficile* PCR ribotype 001 isolates. If the number is greater than one, it represents the number of isolates with a sum of tandem repeat differences (STRD) = 0 (i.e. 100% identical in five variable-number tandem repeat loci). The numbers on the lines represent the STRD between isolates.

TABLE 1

Characteristics of hospitals participating in survey of the incidence of *Clostridium difficile* infection, Czech Republic, 2014 (n = 18)

Hospital	Number of beds	Care type	CDI testing algorithm	CDI incidence		Testing frequency per 10,000 bed-days	Testing frequency per 10,000 admissions	Number of isolates (n = 774)	Ribotype diversity ^a
				CDI cases per 10,000 patient bed-days	CDI cases per 10,000 admissions				
A	913	T	LFIA, ANAE	2.7	23.1	29.1	251.3	63	1.60
B	1,001	S	LFIA, TC, NAAT	8.9	53.0	52.5	312.1	49	2.11
C	1,913	T	LFIA, ANAE, NAAT	7.5	45.9	66.3	403.7	59	2.45
D	1,063	T	LFIA, NAAT	1.5	11.8	18.3	139.7	28	2.26
E	550	S	LFIA, ANAE, NAAT	3.4	21.9	6.0	36.4	50	2.22
F	1,368	T	LFIA, ANAE, NAAT	3.5	31.4	55.6	494.2	28	2.31
G	305	SC	LFIA, NAAT	6.2	45.6	71.1	519.4	15	1.96
H	342	S	CLIA, ANAE	6.8	39.3	27.7	159.2	28	0.54
I	531	S	LFIA, ANAE	5.6	29.8	25.8	137.2	39	0.78
J	950	S	LFIA, ANAE	4.1	22.2	28.7	154.6	45	1.90
K	247	S	CLIA, ANAE, NAAT	34.7	201.2	116.3	673.5	17	1.43
L	2,189	T	CLIA, TC, NAAT	2.6	15.7	18.3	111.0	167	2.56
M	1,184	T	LFIA, ANAE	2.5	14.9	17.8	106.6	29	1.47
N	938	S	LFIA, ANAE, NAAT	6.2	39.2	45.4	287.7	36	1.15
O	1,689	T	LFIA, ANAE, NAAT	2.2	14.7	28.7	194.8	38	2.27
P	455	S	CIA, ANAE	2.6	16.5	20.9	134.4	17	2.15
Q	664	S	LFIA, ANAE	3.6	22.3	40.6	250.2	14	1.97
R	962	S	LFIA, ANAE	5.5	31.2	42.0	238.6	52	0.87
Mean (SD)	–	–	–	6.1 (7.2)	37.8 (41.4)	39.5 (25.4)	255.8 (164.0)	–	–
Median (range)	–	–	–	3.9 (1.5–34.7)	26.5 (11.8–201.2)	28.9 (6.0–116.3)	216.7 (36.4–673.5)	–	–

ANAE: anaerobic culture on selective media; CDI: *Clostridium difficile* infection; CIA: chromatographic immunoassay (two separate tests for GDH and toxins A/B); CLIA: chemiluminescent immunoassay (two separate tests for GDH and toxins A/B); GDH: glutamate dehydrogenase; LFIA: lateral flow immunoassay (simultaneous tests to detect GDH and toxins A/B; NAAT: nucleic acid amplification test; S: secondary care hospital; SC: specialised centre; SD: standard deviation; T: tertiary care hospital; TC: anaerobic culture on selective media followed by LFIA (Hospital B) or NAAT (Hospital L).

^a Calculated using the Shannon index [16].

CC7(7/2); CC8(7/3); CC9(5/2); CC10(4/1); CC11(4/2); CC12(4/3); CC13(4/1); CC14, 16 and 17(3/1); CC15 and 18(3/2); CC19, 20, 23 and 27(2/1); CC21, 22, 24, 25 and 26(2/2) (Figure 3).

MLVA showed an STRD $\geq$ 3 to $\leq$ 10 in 33 isolates and an STRD $>$ 10 in three isolates (Figure 3).

The minimum spanning tree of ribotype 001 isolates revealed 14 clonal complexes of 141 isolates (76.6%) (Table 3). The clonal complexes, with the number of isolates/number of hospitals in which they were found shown in parentheses, were as follows: CC1(67/7);

CC2(21/3); CC3(11/1); CC4(7/4); CC5, 6(6/1); CC7(5/1); CC8(5/5); CC9(3/2); CC10, 11, 12 and 13(2/1); CC14(2/2). MLVA showed an STRD $\geq$ 3 to $\leq$ 10 in 32 isolates, and an STRD $>$ 10 in 15 isolates, including isolates from CC11 and CC13 (Figure 4).

Discussion

In 2008, three Czech tertiary care hospitals participated in the European *C. difficile* infection study (ECDIS) [1]. In 2012–13, 10 Czech hospitals (nine tertiary care, three of which had participated in the 2008 study, and one secondary care) took part in the European, multi-centre, prospective, biannual, point-prevalence study

TABLE 2

MLVA characteristics of *Clostridium difficile* PCR ribotype 176 isolates (n = 225) from 17 of 18 hospitals participating in survey of incidence of *C. difficile* infection, Czech Republic, 2014

Hospital	Total number of isolates	Number of ribotype 176 isolates	Number of ribotype 176 isolates in clonal complexes	Clonal complex number/number of ribotype 176 isolates in the clonal complex	Presence of 100% identical ^a ribotype 176 isolates within a hospital	Presence of 100% identical ^a ribotype 176 isolates between hospitals
A	63	7	6	16/3; 17/3	Yes	No
B	49	16	13	1/1; 2/3; 5/4; 9/1; 22/1; 23/2; 26/1	Yes	Yes (Hospitals L, I)
C	59	4	1	11/1	No	No
D	28	8	7	1/7	Yes	Yes (Hospital L)
E	50	17	16	1/15; 7/1	Yes	Yes (Hospital Q)
F	28	11	6	1/3; 8/2; 18/1	No	Yes (Hospital K)
G	15	3	3	1/1; 21/1; 24/1	No	Yes (Hospital L)
H	28	0	0	–	–	–
I	39	32	28	1/5; 2/11; 5/4; 10/4; 15/2; 19/2	Yes	Yes (Hospitals L, O, B)
J	45	2	1	22/1	No	No
K	17	9	5	4/1; 12/2; 18/2	Yes	Yes (Hospitals F, N)
L	167	50	46	1/13; 2/1; 3/19; 5/2; 8/3; 9/4; 21/1; 25/1; 24/1; 26/1	Yes	Yes (Hospitals B, D, G, I)
M	29	14	13	6/11; 20/2	Yes	No
N	36	26	23	4/9; 7/6; 8/2; 12/1; 13/4; 25/1	Yes	Yes (Hospital K)
O	38	13	12	1/5; 2/4; 4/1; 27/2	Yes	Yes (Hospital I)
P	17	3	2	1/1; 15/1	No	No
Q	14	5	4	1/1; 14/3	Yes	Yes (Hospital E)
R	52	5	4	11/3; 12/1	No	No
Total	774	225	190	–	11 hospitals	11 hospitals

MLVA: multilocus variable-number tandem repeat analysis.

^a Sum of tandem repeat differences (STRD) = 0.

of CDI in hospitalised patients with diarrhoea (EUCLID) [2]. Our current study, which involved 18 hospitals distributed across the Czech Republic (including seven tertiary, 10 secondary and one specialised healthcare facility) reflects better the CDI epidemiological situation in the country. Of these 18 hospitals, eight (seven tertiary care and one secondary care) also participated in EUCLID.

In the Czech Republic, it is mandatory to report cases of CDI to EPI-DAT, the Czech reporting system for infectious diseases, but CDI is reported as 'other bacterial intestinal infections'. Colonisation by *C. difficile* is not mandatorily reportable. An increasing incidence of other bacterial intestinal infections was observed, from 26.4 per 100,000 habitants in 2005 to 64.3 per 100,000 habitants in 2014 [21]. As it is impossible to determine which of these infections are CDIs, however, CDI incidence data among hospitalised patients can only be derived from our study and the European studies mentioned above.

The results of our study showed a mean incidence of CDI per hospital of 6.1 cases per 10,000 patient bed-days and 37.8 cases per 10,000 admissions. Compared

with incidence data for the Czech Republic in the 2008 European study [1], the incidence of CDI in the country has dramatically increased. Our findings are similar to those of EUCLID, which reported an incidence rate of 6.2 CDI cases per 10,000 patient bed-days in 2012–13 for the Czech Republic [2].

Our study also showed that the mean reported testing frequency was 39.5 tests per 10,000 patient bed-days, which is 1.7 times less than the mean testing frequency reported in EUCLID (65.8 tests per 10,000 patient bed-days) and almost three times less than the mean testing frequency reported for the United Kingdom (139 tests per 10,000 patient bed days) [2]. This indicates that CDI in the Czech Republic is most likely under-diagnosed and highlights the need for improvement of clinical awareness and laboratory algorithms (by adding a confirmatory test for GDH positive and toxin A/B-negative stool samples from patients with clinical symptoms of CDI).

It should be noted that considerable variation in CDI incidence was seen between the 18 participating hospitals. The highest incidence seen in Hospital K is probably due to the fact that this hospital also had

TABLE 3

MLVA characteristics of *Clostridium difficile* PCR ribotype 001 isolates (n = 184) from 14 of 18 hospitals participating in survey of incidence of *C. difficile* infection, Czech Republic, 2014

Hospital	Total number of isolates	Number of ribotype 001 isolates	Number of ribotype 001 isolates in clonal complexes	Clonal complex number/ number of ribotype 001 isolates in the clonal complex	Presence of 100% identical ^a ribotype 001 isolates within a hospital	Presence of 100% identical ^a ribotype 001 isolates between hospitals
A	63	38	32	1/19; 6/6; 7/5; 11/2	Yes	Yes (Hospitals C, E, H, R)
B	49	2	0	–	No	No
C	59	21	13	1/11; 2/1; 8/1	Yes	Yes (Hospitals A, E, M, R)
D	28	2	1	4/1	No	No
E	50	7	3	1/1; 4/1; 8/1	No	A, C
F	28	0	0	–	–	–
G	15	3	2	1/1; 8/1	No	Yes (Hospital R)
H	28	24	21	1/4; 3/11; 4/4; 8/1; 9/1	Yes	Yes (Hospital A)
I	39	0	0	–	–	–
J	45	24	21	2/19; 8/1; 14/1	Yes	No
K	17	0	0	–	–	–
L	167	9	4	2/1; 13/2; 14/1	Yes	No
M	29	8	5	1/3; 12/2	Yes	Yes (Hospitals C, R)
N	36	1	0	–	–	–
O	38	3	2	10/2	No	No
P	17	0	0	–	–	–
Q	14	1	1	4/1	No	No
R	52	41	36	1/28; 5/6; 9/2	Yes	Yes (Hospitals A, C, G, M)
Total	774	184	141	–	7 hospitals	7 hospitals

MLVA: multilocus variable-number tandem repeat analysis.

^a Sum of tandem repeat differences (STRD) = 0.

the highest testing frequency, as there were sufficient local financial sources for extensive CDI testing. The high incidence in this hospital had a considerable impact on the SD of the mean incidence for all 18 hospitals. Despite the high incidence, the number of isolates submitted during the study was small. The sending of strains was voluntary and this hospital was unable to send a representative number of isolates.

All 18 participating hospitals used GDH testing, as a recommended screening step [22]. A total of 14 used a lateral flow immunoassay for a single GDH and toxin A/B test as the first step of their testing algorithm; the other four used these tests separately. The use of two separate tests is more economical because testing can be stopped when samples are GDH negative, as a GDH-negative result has a high predictive value for the absence of CDI [23].

Of the 18 hospitals, eight did not confirm toxin production in GDH-positive and toxin A/B-negative stool samples, although they performed anaerobic culture; thus their testing algorithm were suboptimal. Diagnostic uncertainty of diarrhoeal patients with a positive GDH

test and negative toxin A/B tests because of a lack of a confirmatory test may have also contributed to the spread of CDI in the Czech Republic.

It is clear that CDI diagnostic testing in the Czech Republic is very variable. A web-questionnaire, completed by 61 laboratories in 2014 showed that 21% (n = 13) used only GDH and toxin A/B test, and 8% (n = 5) used toxin A/B test as a screening test [24].

Ribotyping of *C. difficile* isolates in our study revealed the presence of PCR ribotype 176 in 29% and PCR ribotype 001 in 24% of isolates. The frequent occurrence of PCR ribotype 176 simultaneously with PCR ribotype 027 was reported in Poland in 2008–13 [10,11]. In 2013, the first sporadic case of an imported infection caused by PCR ribotype 027 was found in the Czech Republic [25]. In the study presented here, we diagnosed two new CDI cases due to PCR ribotype 027 infection: one was a man in his 30s, the other a man in his 70s.

Whereas PCR ribotype 176 has been only reported from two countries (Czech Republic and Poland) [10,11,25],

which neighbour each other, PCR ribotype 001 has been problematic for a long time in many European countries [1,2,26]. It has dominated, as in Slovakia in 2012 [27], or has occurred together with PCR ribotype 027, as reported from Germany (Hesse region) in 2011–13 [28], the north-east of England in July 2009 to December 2010 [29] and Scotland in November 2007 to December 2009 [30]. It has also occurred together with other PCR ribotypes, such as 014/020 and 126/078, in a single-day study in Spain [31].

MLVA of the two predominant ribotypes identified in our study revealed close genetic relatedness between isolates of each ribotype. The occurrence of 100%-identical (STRD = 0) PCR ribotype 176 isolates in 11 hospitals and PCR ribotype 001 isolates in seven hospitals, suggests clonal clusters within and between healthcare facilities, probably due to ineffective hospital infection control measures and transfer of patients between healthcare facilities who were in fact CDI cases but had not been diagnosed. This is supported by the observation of clonal complexes in tertiary and secondary hospitals in the same region. The question remains as to which specific molecular characteristics of PCR ribotypes 176 and 001 allow them to spread rapidly within healthcare facilities in contrast to the other less frequent PCR ribotypes identified in the study.

Antibiotic susceptibility testing of *C. difficile* isolates was not performed in this study but multiresistance of PCR ribotype 176 isolates [32,33], as well as PCR ribotype 001 isolates, has been reported [26,34]. The results of a recently published European study on antibiotic resistance among prevalent *C. difficile* ribotypes showed the Czech Republic as a country with a high cumulative resistance score (4–5), calculated based on susceptibility to nine antimicrobials tested [26].

An important limitation of our study is the lack of clinical patient data. The Czech national reference centre for healthcare-associated infections is currently organising the implementation of CDI surveillance based on the recent CDI surveillance protocol from the European Centre for Disease Prevention and Control [35]. The first national CDI incidence data, including clinical data on CDI patients and data on antibiotic susceptibility to metronidazole, vancomycin and moxifloxacin of *C. difficile* isolates, should be available in 2016 (CDI surveillance started in April 2016 in the Czech Republic).

Conclusion

The results of our study showed an unfavourable CDI epidemiological situation in the Czech Republic in 2014 caused by the occurrence of epidemic PCR ribotypes 176 and 001. The absence of national surveillance at that time, the low frequency of testing and variability in testing algorithms probably contributed to the spread of these PCR ribotypes.

A Czech standardised CDI testing protocol and the implementation of CDI surveillance in a large number

of hospitals is urgently needed for monitoring, management and reduction of these infections in the Czech Republic.

Members of the Czech *Clostridium difficile* study group (in no particular order)

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Conflict of interest

None declared.

Authors' contributions

ON, PD, MK, JM designed the study. ON, JM supervised CDI diagnostics. MK performed molecular characterisation of *C. difficile* isolates. Study group members provided *C. difficile* isolates, data on diagnostics algorithm and annual incidence and testing data. MK, ON, EK analysed the collected data. MK wrote the first draft of the manuscript. EK, ON, PD critically revised the subsequent drafts of the manuscript. All

authors approved the final version of the manuscript and agreed with its submission.

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