TRENDS IN *CLOSTRIDIUM DIFFICILE* RIBOTYPE EPIDEMIOLOGY IN BELGIUM 2009-2011.

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Introduction

The beginning of the XXIst century was marked by a dramatic change in the epidemiology of *Clostridium difficile* infections (CDI) in North-America and in Western Europe. The incidence significantly increased but also the severity of the disease with a mortality rate that had never been observed before.(2,3,7) Large outbreaks were reported in many hospitals. A specific clone (ribotype 027/NAP1) was rapidly identified as the cause of the outbreaks.

Belgium was one of the first countries in Europe to report cases of CDI linked to ribotype 027. Retrospectively, it was shown that a hospital outbreak involving more than one hundred patients in a geriatric center in Brussels had already been caused by this epidemic isolate in 2003.

The Belgian Scientific Institute of Public Health (IPH) together with the National Reference Center for *C. difficile* (NRC) rapidly organized a national surveillance program that was launched in 2006. Surveillance of CDI became compulsory in Belgian hospitals in 2007.(5,6) All cases have to be recorded online at the IPH. In addition, every six months, every hospital laboratory is invited to send to the NRC the first five strains isolated in the routine bacteriology laboratory. It is mandatory at least once a year (Fig. 1). Since 2009, all strains are ribotyped. Here we report on the major trends in ribotype incidence during the last three years.

Materials and methods

Strains: Hospitals are requested to send 5 consecutive strains per semester following a Protocole of Surveillance of *Clostridium difficile* infections established by IPH (version 4.0. Brussels, 2010). (6)

Stools: Laboratories that cannot perform culture are allowed to send stool samples.

Medium : CCFAT (Cycloserin cefotaxim fructose agar + sodium taurocholate 0,1%) and Columbia agar (Becton Dickinsson) were used for sub-cultures.

CTA: cell cytotoxicity assay was performed on 48 hours culture supernatant on MRC5 cells.

Ribotyping: DNA were extracted with chelex and 16S - 23S rRNA intergenic spacer regions were amplified using primers as described by Barbut et al. (1). Amplicon size were analysed by capillary electrophoresis using an automatic sequencer (ABI 3100 Automated Capillary DNA Sequencer) and GeneMapper Analysis (Applied Biosystems, Inc.). A 35–500 bp ROX ladder (ABI) was used as internal marker. Profiles were analyzed by comparison with those of reference strains from the European collection (Brazier classification) and with our own database.

Genotyping: Toxin genes: *tpi, tcdA, tcdB*, binary genes: *cdtA, cdtB*, deletions in *tcdC* gene and moxifloxacin resistance mutation were tested using GenoType CDiff (Hain Lifescience).

Minimal Inhibitory Concentrations (MIC): MIC were performed using the Liofilchem test (Elitech).

Surveillance

The National Surveillance program IPH-NRC is organized as summarized here below:

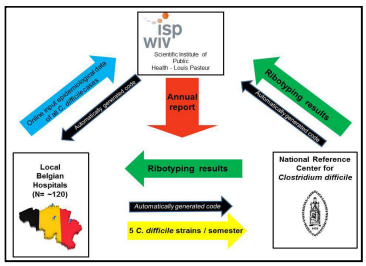

Fig. 1 Organization of the National Surveillance.

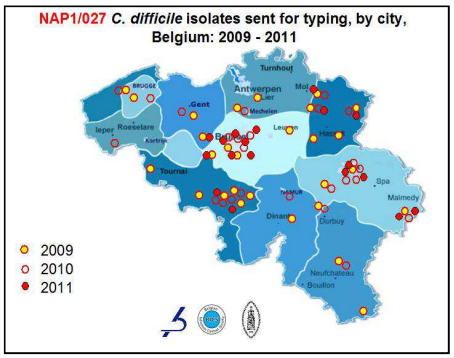

Fig. 2: NAP1/027 spread in Belgium

Results

In 2009, the NRC received 389 isolates from 117 different laboratories (Table 2).
In 2010, 505 isolates from 107 different laboratories (Table 2).
In 2011, 462 isolates from 89 different laboratories (Table 2).
A total of 129 different ribotypes were identified, 92 of them beeing only seen in a single case (results not shown).
Table 2 shows the distribution of the ribotypes in order of frequency every year.
Figure 3 shows the evolution of the 9 most frequent ribotypes.

	tpi gene	gyrA mut 1A	gyrA mut 1B	*cdtA*	*cdtB*	*tcdA*	*tcdB*	18 bp del *tcdC*	39 bp del *tcdC*	mut 117 *tcdC*	toxinotype	CTA MRC5	CMI moxi
BR014	POS	1-	1-	NEG	NEG	POS	POS	NEG	NEG	NEG	ND	POS	20S
BR002	POS	3-	3-	NEG	NEG	POS	POS	NEG	NEG	NEG	ND	POS	12S
BR027	POS	17+	17-	POS	POS	POS	POS	POS	NEG	POS	III	POS	19R/11S
BR078	POS	4-	4-	POS	POS	POS	POS	NEG	POS	NEG	ND	POS	3R/9S
BR020	POS	1-	1-	NEG	NEG	POS	POS	NEG	NEG	NEG	ND	POS	ND
UCL 46	POS	1-	1-	NEG	NEG	POS	POS	NEG	NEG	NEG	0	POS	12S
UCL 18	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	POS	ND
UCL 26	POS	2-	2-	NEG	NEG	POS	POS	NEG	NEG	POS	0	POS	ND
BR001	POS	1+/1-	2-	NEG	NEG	POS	POS	NEG	NEG	NEG	ND	POS	8S
BR023	POS	2-	2-	POS	POS	POS	POS	POS	POS	NEG	ND	POS	ND
UCL 23a	POS	1-	1-	NEG	NEG	POS	POS	NEG	NEG	NEG	0	POS	ND
BR012	POS	3-	3-	NEG	NEG	POS	POS	NEG	NEG	NEG	ND	POS	ND
UCL 16i	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	POS	ND
BR015	POS	2-	2-	NEG	NEG	POS	POS	POS	NEG	NEG	ND	POS	2R/11S
UCL 5a	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	POS	ND
UCL 20a	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	POS	ND
UCL 49	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	POS	ND

Table 1: Caracteristics of the most frequent ribotypes

The 17 most prevalent ribotypes remained the same all over the 3 years and represented 65 % of all strains(Table 2).

Between 2009-2011, a marked decrease of the frequency of ribotype 027 was observed while other ribotypes significantly increased, especially ribotype 002 which went from 15/389 (3.9%) in 2009 to 21/505 (4.2%) in 2010 and 37/462 (8%) in 2011(Table 2).

Binary toxin and deletion in *tcdC* were detected in strains belonging to ribotype 027, 023 and 078(Table 1).

Gyr A mutation was detected in all 17 strains of 027 tested and in one ribotype 001 strain (Table 1).

A mutation at position 117 in the *tcdC* gene was observed in all 17 strains belonging to the 027 ribotype and in one strain belonging to the UCL 26 ribotype (Table 1).

Moxifloxacin resistance mutation was detected using MIC test from Liofilchem in most of ribotype 027 strains and in some of ribotype 078 (Table 1).

Distribution of ribotypes in Belgium 2009 - 2011

2009 N=389			2010 N=505			2011 N=462		
Ribotype	Nbr strains	% strains	Ribotype	Nbr strains	% strains	Ribotype	Nbr strains	% strains
BR027	72	18,50	BR027	65	12,2	BR014	56	12,1
BR014	44	11,30	BR014	51	10,1	BR002	37	8
BR020	26	6,10	BR078	33	6,5	BR027	36	7,8
BR001	16	4,10	BR020	31	6,1	BR078	35	7,6
BR002	15	3,80	BR001	24	4,7	BR020	31	6,7
BR015	14	3,60	BR002	21	4,1	UCL 46	25	4,3
BR078	13	3,30	UCL 46	21	3,96	UCL 16i	15	3,3
UCL 46	11	2,80	BR023	16	3,10	UCL 26	12	2,6
UCL 49	10	2,60	UCL 49	12	2,40	BR001	10	2,1
UCL 49	10	2,60	BR012	12	2,40	BR023	10	2,1
UCL 20a	7	1,90	UCL 18	11	2,10	UCL 23f	9	2
BR012	6	1,50	UCL 5a	10	2,00	BR012	9	2
UCL 5a	6	1,50	BR015	9	1,80	UCL 18	8	1,7
BR023	4	1,00	UCL 20a	6	1,10	BR015	4	0,87
UCL 26	2	0,50	UCL 49	5	0,90	UCL 5a	4	0,87
UCL 16i	2	0,50	UCL 26	3	0,60	UCL 20a	4	0,87
UCL 23f	0	0,00	UCL 23f	0	0,00	UCL 49	2	0,43

Table 2: Distribution of ribotypes

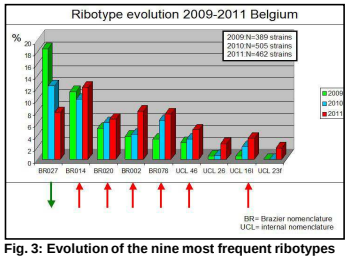

Fig. 3: Evolution of the nine most frequent ribotypes

Discussion

The Belgian CDI surveillance program allows a good monitoring of the epidemiology(5,6). As in surrounding countries like France, the UK and the Netherlands the incidence of the hypervirulent 027 strain has decreased over the last 3 years while other ribotypes (002,014,020 and 078) are on the increase.

At a local level, in our 1000 bed university hospital, the same trend was observed (results not shown).

The main ribotypes are observed in all provinces of the country. Ribotype 078 is currently the fourth most common. This type was recently isolated in animals in Belgium, on carcasses in slaughtery houses and in prepared meat. Possible transfer from animals to humans has to be studied (4).

We ribotyped all strains from our university hospital during the same period and we found almost the same ribotype distribution in the adult and child population, as in the National Surveillance.

The fact that moxifloxacin resistance was not detected in all 078 strains, underlines the necessity of a resistance surveillance of the strains.

Abstract

Surveillance of *Clostridium difficile* infection (CDI) is compulsory in Belgian hospitals since 2007. All cases have to be recorded at the Institute of Public Health. Every six months every hospital laboratory is invited to send to the *C. difficile* National Reference Center the first five strains isolated in the routine bacteriology laboratory. It is mandatory at least once a year. Since 2009, all strains are ribotyped. Here we reported on the major trends in ribotype incidence during the last three years.

Method. For ribotyping, DNA were extracted with chelex and 16S - 23S rRNA intergenic spacer regions were amplified using primers as described by Barbut et al (J. Clin. Microbiol. 2000). Amplicon size were analysed by capillary electrophoresis using an automatic sequencer (ABI 3100 Automated Capillary DNA Sequencer) and GeneMapper Analysis (Applied Biosystems, Inc.). A 35–500 bp ROX ladder (ABI) was used as internal marker. Profiles were analyzed by comparison with those of reference strains from the European collection (Brazier classification) and with our own database.

Toxin genes (A, B and binary), deletion in *tcdC* and moxifloxacin resistance mutation were tested using GenoType CDiff (Hain Lifescience).

Results. We received 389 isolates from 117 different laboratories in 2009, 505 from 107 in 2010 and 462 from 89 in 2011. A total of 129 different ribotypes were identified, 92 of them being seen only in one case. Ribotype 027 was the most frequent in 2009 (72/389, 18.5%) but gradually decreased in 2010 (62/505, 12.3%) and 2011 (56/462, 12.1%). Ribotype 014 was the second most frequent but had an opposite trend going from 44/389 (11.3%) in 2009 to 56/462 (12%) in 2011. Ribotypes 020, 002 and 078 also gradually increased, the most marked increase being observed with ribotype 002 which went from 15/389 (3.9%) in 2009 to 21/505 (4.2%) in 2010 and 37/462 (8%) in 2011. Binary toxin and deletion in tcdC were detected in strains belonging to ribotype 027, 023 and 078. Moxifloxacin resistance mutation was detected in most of ribotype 027 strains and in some of ribotype 078.

Conclusion. Ribotype 027 is decreasing in Belgium while other ribotypes (014, 002, 078 and 020) are on the increase. Our surveillance program allows a good follow-up of the epidemiology.

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