Technical University of Denmark

Clostridium difficile PCR Ribotypes from Different Animal Hosts and Different Geographic Regions

Zidaric, V.; Janezic, S.; Indra, A.; Kokotovic, Branko; Blanco, J. L. ; Seyboldt, C.; Diaz, C. Rodriguez; Poxton, I. R.; Perreten, V.; Drigo, I.; Jiraskova, A.; Ocepek, M.; Weese, J. S.; Songer, J. G.; Rupnik, M.

Publication date:
2013

Citation (APA):
Zidaric, V., Janezic, S., Indra, A., Kokotovic, B., Blanco, J. L., Seyboldt, C., ... Rupnik, M. (2013). Clostridium difficile PCR Ribotypes from Different Animal Hosts and Different Geographic Regions. Poster session presented at 5th Congress of European Microbiologists, Leipzig, Germany.

Clostridium difficile PCR RIBOTYPES FROM DIFFERENT ANIMAL HOSTS AND DIFFERENT GEOGRAPHIC REGIONS

V. Zidaric[1], S. Janezic[1,16], B. Pardon[2], A. Indra[3], B. Kokotovic[4], J.L. Blanco[5], C. Seyboldt[6], C. Rodriguez Diaz[7], I.R. Poxton[8], V. Perreten[9], I. Drigo[10], A. Jiraskova[11], M. Ocepek[12], J.S. Weese[13], J.G. Songer[14], M. Rupnik[1, 15,16]

[1]Institute for Public Health Maribor, Maribor, Slovenia; [2]Ghent University, Faculty of Veterinary Medicine, Merelbeke, Belgium; [3]AGES, Vienna, Austria; [4]Technical University of Denmark, National Veterinary Institute, Copenhagen, Denmark; [5]Complutense University, Madrid, Spain; [6]Friedrich-Loeffler Institute, Jena ,Germany; [7]University of Liege, Faculty of Veterinary Medicine, Liege, Belgium; [8]University of Edinburgh, Edinburgh, UK; [9]University of Bern, Institute of Veterinary Bacteriology, Bern, Switzerland; [10]IZSVe, Treviso, Italy; [11]Charles University in Prague, 1[st] Faculty of Medicine, Prague, Czech Republic; [12]University of Ljubljana, Veterinary Faculty, Ljubljana, Slovenia; [13]University of Guelph, Ontario Veterinary College, Ontario, Canada; [14]Iowa State University, Ames, U.S.A.; [15]University of Maribor, Medical Faculty, Maribor, Slovenia; [16]Centre of excellence for integrated approaches in chemistry and biology of proteins, Ljubljana, Slovenia

Background

Clostridium difficile is an anaerobic sporogenic bacterium traditionally associated with human nosocomial infections, and animals have been recognized as an important potential reservoir for human infections (Rodriguez-Palacios *et al.*, 2013).

Ribotype 078 is often reported in animals but according to recent studies the overlap between PCR ribotypes found in humans and animals seems to be increasing (Bakker *et al.*, 2010; Gould and Limbago, 2010; Janezic *et al.*, 2012; Keel *et al.*, 2007; Koene *et al.*, 2011). However, genetic diversity among animal strains remains poorly understood.

The aim of our work was to establish an international *C. difficile* animal collection with one PCR ribotype per species per country/laboratory and to compare PCR ribotypes across animal hosts and countries.

Materials and methods

***C. difficile* strains:**

Altogether 112 strains from 12 different countries were contributed (Figure 1). Collected strains originate from 13 different animal species, including pets, horses wild animals and food animals. Approximately half (55,4 %) of the strains are from cattle and pigs (Table 1).

Molecular characterization of *C. difficile* strains:

All collected strains were characterized by toxinotyping (Rupnik *et al.*, 1998; http://www.mf.uni-mb.si/tox/). In adition, binary toxin genes were detected by PCR as described in Stubbs *et al.* (2000).

Standard agarose gel-based PCR ribotyping was used as described by Bidet *et al.* (1999) and results analyzed by BioNumerics software 5.10 (Applied Maths).

Strains were also typed by capillary gel electrophoresis-based ribotyping using primers for standard agarose gel-based PCR ribotyping with flourescein labelled 16S primer (Indra *et al.*, 2008). PCR ribotype patterns were analyzed and identified on a web-based database Webribo (http://webribo.ages.at).

Results and Discussion

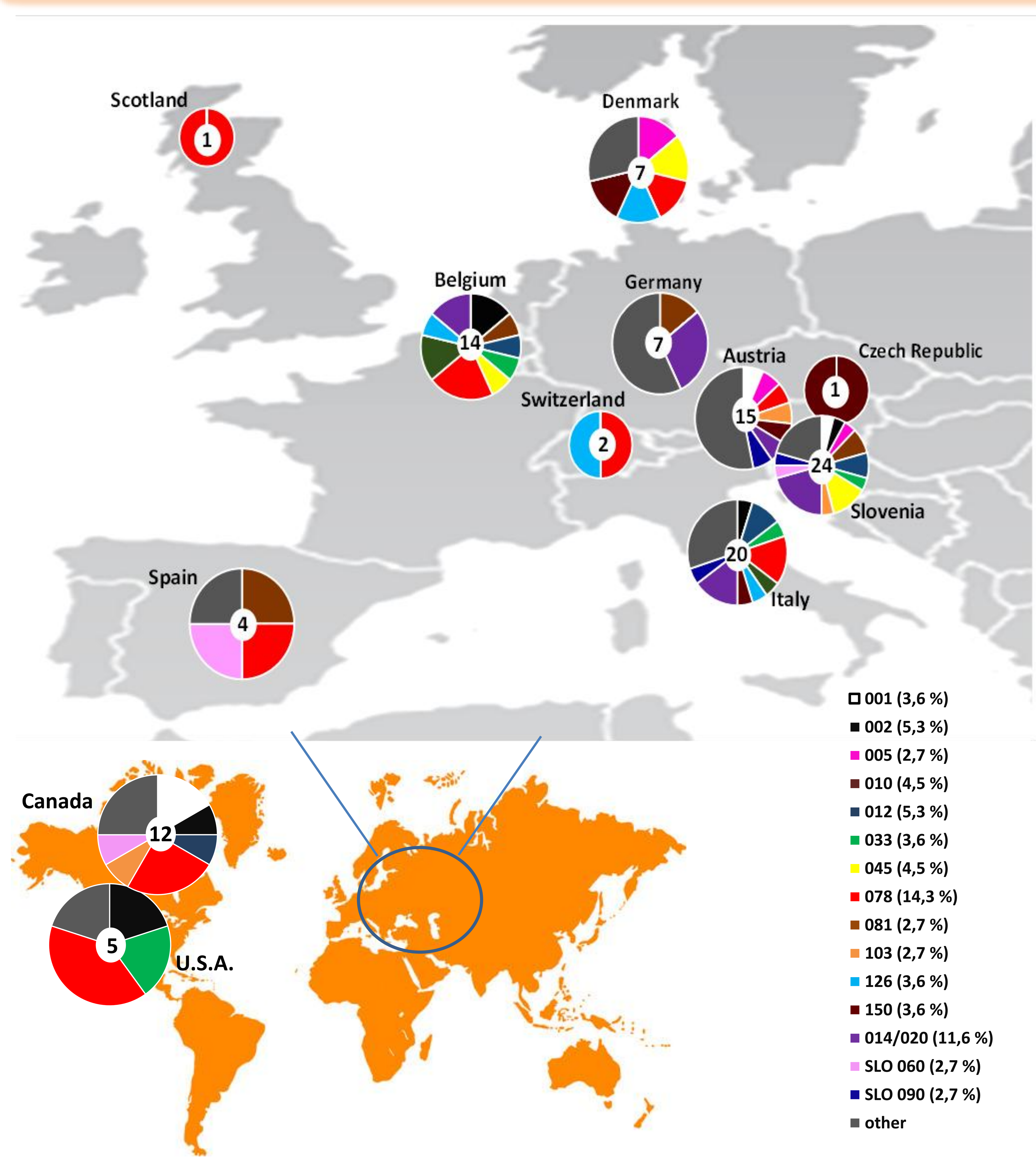

Figure 1. Distribution of animal *C. difficile* PCR ribotypes from participating countries.
Pie charts show proportion of PCR ribotypes (the most prevalent in the collection) per country. The number in the centre of pie charts is the number of isolates received from the country. The proportions in parentheses show number of isolates of specific PCR ribotype out of all collected isolates.

Table 1. Overview of *C. difficile* PCR ribotypes found in different animal hosts (number of isolates/number of countries)

PCR ribotype \ Host	Cattle	Horses	Pigs	Poultry	Cats and dogs	Others*
001				2/2	2/1	
002	2/2		2/2		1/1	1/1
003						1/1
005	1/1		1/1	1/1		
010	1/1			1/1	2/2	1/1
012	2/2		1/1	1/1	1/1	1/1
015	1/1		1/1			
017						1/1
018				1/1		
023				1/1		
027	1/1	1/1				
029	1/1			1/1		
033	3/3	1/1				
045	1/1		2/2	1/1		1/1
056			1/1		1/1	
078	5/4	2/2	8/7			1/1
081	1/1		2/2			
103	1/1			1/1		1/1
126	2/2	1/1	1/1			
150			4/4			
413			1/1			
011/049			1/1			
014/020	3/3	1/1	2/1	1/1	4/2	2/2
SLO 002					1/1	
SLO 012			1/1			
SLO 024					2/1	
SLO 036	1/1		1/1			
SLO 060	1/1	1/1				1/1
SLO 066					1/1	
SLO 084						1/1
SLO 084						1/1
SLO 090	1/1					2/1
SLO 125	1/1					
SLO 132						1/1
SLO 133			1/1			
SLO 137			1/1			
SLO 143	1/1					
SLO 164	1/1					
SLO 165						1/1
SLO 166						1/1
Total ($N_{isolates}$=112)	31/7	7/4	31/10	11/2	15/4	17/3

Others* : raccoons, wild hare, rabbits, goats, partridges, a goose and a crow.

All **112 collected strains** were distributed into **39 different PCR ribotypes** (Table 1, Figure 1). Up to 20 different PCR ribotypes could be found within a single animal species and up to 16 different PCR ribotypes per country.

PCR ribotypes **078**, **126**, **014/020**, **012** and **002** that are frequently associated with animals (Keel *et al*., 2007; Janezic *et al*., 2012) represent **40.0 %** of all strains in the collection (Figure 1).

With **capillary electrophoresis** based PCR ribotyping **subtypes** were found for PCR ribotypes **001** (001 and 001ecdc), **002** (203 and 209), **014/020** (014/0, 014/5, 020, 449 and 659), **SLO 036** (050 and AI-84), **078** (078 and 251), **045** (045 and 598) and **126** (126 and 078ecdc).

Eight strains are nontoxigenic while toxigenic strains account for 92.9% and belong to **11** different **toxinotypes**: 0, I, III, IV, V, VI, VIII, XIa, XIb, XII and XIX.

References

Bakker D, Corver J, Harmanus C, Goorhuis A, Keessen EC, Fawley WN, Wilcox MH, Kuijper EJ. 2010. Relatedness of human and animal *Clostridium difficile* PCR ribotype 078 isolates determined on the basis of Multilocus Variable-Number Tandem-Repeat Analysis and tetracycline resistance. J. Clin. Microbiol. **48(10)**:3744–3749.

Bidet P, Barbut F, Lalande V, Burghoffer B, Petit JC. 1999. Development of a new PCR-ribotyping method for *Clostridium difficile* based on ribosomal RNA gene sequencing. FEMS Microbiol. Lett. **175**:261-266.

Gould LH, Limbago B. 2010. *Clostridium difficile* in food and domestic animals: a new foodborne pathogen? Clin Infect Dis. **51(5)**:577-582.

Indra A, Huhulescu S, Schneeweis M, Hasenberger P, Kernbichler S, Fiedler A, Wewalka G, Allerberger F, Kuijper EJ. 2008. Characterization of *Clostridium difficile* isolates using capillary gel electrophoresis-based PCR ribotyping. J. Med. Microbiol. **57**: 1377-1382.

Janezic S, Ocepek M, Zidaric V, Rupnik M. 2012. *Clostridium difficile* genotypes other than ribotype 078 that are prevalent among human, animal and environmental isolates. BMC Microbiol. **12**:48 doi:10.1186/1471-2180-12-48

Keel K, Brazier JS, Post KW, Weese S, Songer JG. 2007. Prevalence of PCR ribotypes among Clostridium difficile isolates from pigs, calves, and other species. J.Clin. Microbiol. **45(6)**:1963-1964.

Koene MG, Mevius D, Wagenaar JA, Harmanus C, Hensgens MP, Meetsma AM, Putirulan FF, van Bergen MA, Kuijper EJ. 2011. *Clostridium difficile* in Dutch animals: their presence, characteristics and similarities with human isolates. Clin. Microbiol. Infect. doi:10.1111/j.1469-0691.2011.03651.x

Rodriguez-Palacios A, Borgmann S, Kline TR, Lejeune JT. 2013. *Clostridium difficile* in foods and animals: history and measures to reduce exposure. Anim. Health Res. Rev 14(1):11-29.

Rupnik M, Avesani V, Janc M, Von Eichel-Streiber C, Delmée M. 1998. A novel toxinotyping scheme and correlation of toxinotypes with serogroups of *Clostridium difficile* isolates. J. Clin. Microbiol. **36(8)**:2240-2247.

Stubbs S, Rupnik M, Gibert M, Brazier J, Duerden B, Popoff M. 2000. Production of actin-specific ADP-ribosyltransferase (binary toxin) by strains of *Clostridium difficile*. FEMS Microbiol. Lett. **186**:307-312.