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INTERNATIONAL *Clostridium difficile* ANIMAL STRAIN COLLECTION

V. Zidaric, S. Janezic[1], 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, 1st 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

Animals have been recognized as an important potential reservoir of *Clostridium difficile* and according to recent studies the overlap between PCR ribotypes of human and animal isolates seems to be increasing (Bakker *et al*., 2010; Gould and Limbago, 2010; Janezic *et al*., 2012; Keel *et al*., 2007; Koene *et al*., 2011).

Here we report on an International *Clostridium difficile* animal strain collection that was established to enhance comparative studies on animal-associated strains and contribute to interlaboratory exchange of the strains.

The goal of the collection is to include one PCR ribotype per species per country/laboratory.

Results and Discussion

All 100 included strains were distributed into 39 different PCR ribotypes. Up to 17 different PCR ribotypes can be found within a single animal species and up to 15 different PCR ribotypes per country.

Within one standard (agarose based) PCR ribotype several PCR ribotypes could be distinguished by capillary gel based PCR ribotyping (Table 1; PCR ribotypes 014/020, 078, 002, 045, 056, 033).

Five strains are nontoxigenic while toxigenic strains account for 95.0% and belong to 10 different toxinotypes: 0, I, III, IV, V, VI, VIII, XI, XII and XIX. 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.

Materials and methods

***C. difficile* strains:**

Altogether 100 strains from 12 different countries were contributed. Collected strains originate from 11 different animal species, including pets, horses and food animals. Approximately half (58,0%) of the strains are from cattle and pigs (Table 1).

For every strain aditional availible information on animal host and strain was obtained.

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).

Table 1. Distribution of *C. difficile* genotypes by species and country given as toxinotype/standard PCR ribotype [capillary gel electrophoresis based PCR ribotype].

Country / Animal host	Austria	Belgium	Canada	Czech Republic	Denmark	Germany	Italy	Scotland	Slovenia	Spain	Switzerland	U.S.A.	Total number of strains per animal species	Number of different PCR ribotypes per animal species
Pig	XII/056 [new RT] 0/011/049 [049/1] 0/150 [AI-12]	V/078 [078] 0/002 [002/0] 0/081 [081]	V/078 [078] 0/015 [211]	0/150 [AI-12]	V/078 [new RT] V/045 [598] V/126 [126] VI/066 [413] 0/150 [AI-12] 0/005 [005] VI/SLO 137 [new RT]		V/078 [251] 0/150 [AI-12] XIX/012 [012] 0/014/020 [014 subtype] 0/081 [081] 0/SLO 036 [050/AI-84] XII/SLO 133 [AI-15] VI/SLO 012 [new RT]	V/078 [078]	V/045 [045]	V/078 [078]		V/078 [078] 0/002 [nd]	29	17
Cattle	V/078 [078] 0/014/020 [014/0] 0/029 [029] 0/005 [005] 0/015 [211] 0/SLO 125 [014 subtype] 0/SLO 036 [AI-84/050] 0/SLO 090 [AI-9-1] 0/SLO 143 [610] 0/SLO 164 [nd] 0/SLO 165 [nd]	V/078 [078] V/078 [078] V/126 [078 ecdc] XI/033 [033] 0/012 [012] 0/014/020 [014/0] 0/002 [002/2] 0/081 [081]	0/SLO 074 [new RT] V/078 [078]				V/126 [126] XIb/033 [033]		0/002 [209] 0/014/020 [014/0]	XIb/283 [new RT]		V/078 [078] XIa/033 [033] III/027 [027]	29	15
Dog			0/001/072 [nd] 0/002 [002/0]			0/SLO 066 [AI-60] 0/014/020 [014/0] V/SLO 024 [new RT] Tox-/010 [010]			0/014/020 [new RT] 0/012 [012]	XII/056 [new RT] Tox-/010 [010]			10	8
Cat			0/001/072 [001 ecdc]			V/SLO 024 [new RT] 0/014/020 [449] Tox-/SLO 002 [AI-34]			0/014/020 [new RT]				5	4
Poultry	0/001/072 [001]								0/029 [029] 0/014/020 [014/0] IV/023 [023] 0/103 [AI-82/1] 0/001/072 [001]				6	5
Horse		0/014/020 [020]	XIb/033 [new RT] V/078 [078] III/027 [027]						XI/033 [nd]		V/078 [078] V/126 [126]		7	5
Rabbit/Hare							0/012 [012] V/078 [078] 0/014/020 [014/0] Tox-/SLO 132 [new RT] 0/SLO 090 [AI-9-1] 0/002 [002/0] VIII/017 [017] Tox-/SLO 084 [205]						8	8
Partridge									XII/056 [446] XI/033 [new RT]				2	2
Goose									0/SLO 090 [AI-9-1]				1	1
Crow									0/003 [003]				1	1
Raccoon			I/SLO 166 [nd] 0/103 [AI-82/1]										2	2
Total number of strains per country	15	12	12	1	7	7	18	1	16	4	2	5	100	
Number of different PCR ribotypes per country	15	7	9	1	7	5	15	1	12	4	2	4		

nd- typing not done **; new RT-** PCR ribotype has on webribo database not yet been determined**; subtype-** subtype has on webribo not yet been determined

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