Prevalent *Clostridium difficile* genotypes among human and animal isolates other than ribotype 078

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INTRODUCTION

Clostridium difficile is one of the most important nosocomial pathogens (Bartlet, 1994). However, in recent years the incidence of community-acquired *C. difficile* infection has increased and *C. difficile* has also emerged as a pathogen in animals (Chernak *et al*., 2005; Gould and Limbago, 2010; Songer and Anderson, 2006).

PCR ribotype 078 is currently the most frequently isolated strain from pigs and calves and is also increasingly reported in human population particulary in those with community associated infections (Keel *et al*., 2007; Limbago *et al*., 2009) In Slovenia strains other than ribotype 078 are prevalent in humans and animals.

The objective of this study was to compare prevalent PCR ribotypes isolated from humans and different animals with pulsed-field gel electrophoresis and antimicrobial susceptibility testing to determine their relatedness.

MATERIALS AND METHODS

Strains: Altogether 50 representative *C. difficile* isolates from 7 most frequently isolated PCR ribotypes (014/020, SLO 010, SLO 055, 023, 029, 002 and SLO 011) were analyzed (Table 1).
- 32 human strains isolated as a part of routine *C. difficile* testing from three different Slovenian hospitals,
- 18 animal strains isolated from pigs (n=3), poultry (n= 8), cat (n=1), calves (n=2) and dogs (n=4) isolated from different Slovenian farms and households.

Selected strains had been isolated between 2006 and 2009.

Toxinotyping was performed as described by Rupnik *et al*., 1998. PaLoc-negative strains were confirmed by PCR using Lok1/Lok3 primers (Braun *et al*., 1996).

PCR ribotyping: Intergenic spacer regions were amplified with primers described by Bidet *et al*. (1999). Amplification products were separated by electrophoresis in 3 % agarose gel (Bio-Rad, California, USA) for 5 hours at 2.5 V/cm. PCR ribotypes for which the reference strains were available are designated by standard Cardiff nomenclature, while others are designated by internal nomenclature (SLO and number).

Pulsed field-gel electrophoresis was performed as described by Janezic and Rupnik (2010), using two different restriction enzymes *Sma*I and *Sac*II. Dendrograms were constructed using BioNumerics software version 6.1 (Applied Maths), with UPGMA, using Dice coefficient with position tolerance and optimization of 1.10 %.

Antimicrobial susceptibility testing: Minimum inhibitory concentrations (MICs) for 6 antimicrobial agents (rifampicin (RI), moxifloxacin (MX), erythromycin (EM), piperacilin/tazobactam (PTC), tetracycline (TC) and clindamycin (CM)) were determined by E-test method.

Table 2. MICs for 6 antimicrobial agents of human and animal *C. difficile* isolates.

Host		EM [mg/L]	MX [mg/L]	TC [mg/L]	CM [mg/L]	PTC [mg/L]	RI [mg/L]
Human (n=32)	MIC_{50}	1,5	1,0	0,094	3,0	6,0	NA
	MIC_{90}	3,0	>256	0,19	>256	12,0	NA
	Range	0,38->256	0,50->256	0,025-8,0	1,0->256	1,5-64,0	<0,002
Animals (n=18)	MIC_{50}	1,0	0,75	0,125	3,0	6,0	NA
	MIC_{90}	2,0	1,0	0,19	5,0	8,0	NA
	Range	0,38-3,0	0,19-1,0	0,047-4,0	0,023-6,0	1,5-16,0	<0,002

RESULTS AND DISCUSSION

- There is a considerable overlap between *C. difficile* ribotypes isolated from humans and animals (Table 1).
- Two most prevalent PCR ribotypes in humans and animals were 014/020 and 002 accounting for 22,9 % and 8,9 % (humans), and 14,2 and 8,2 % (animals), respectively. These two ribotypes are also among the most prevalent ribotypes in Europe (Bauer *et al*., 2010).

Table 1. *C. difficile* PCR ribotypes isolated from humans and animals between 2006 and 2009.

Source	Nr. of strains	Nr. of different ribotypes	014/020 n (%)	002 n (%)	SLO 011 n (%)	023 n (%)	SLO 010 n (%)	029 n (%)	Other prevalent PCR ribotypes (%)
humans	464	59	106 (22,9)	42 (9,0)	13 (2,8)	25 (5,4)	14 (3,0)	12 (2,6)	012 (5,8 %) SLO 009 (5,4 %) 070 (3,4 %) 015 (3,0 %)
animals	134	15	19 (14,2)	11 (8,2)	31 (23,1)	3 (2,3)	12 (9,0)	3 (2,3)	066 (26,9 %) SLO 025 (7,5 %)
pigs	70	4	4 (5,7)	-	31 (44,3)	-	1 (1,4)	2 (2,9)	066 (51,4 %)
calves	4	2	3 (75,0)	1 (25,0)	-	-	-	-	NA
poultry	43	12	10 (23,3)	10 (23,3)	-	3 (7,0)	-	1 (2,3)	SLO 026 (23,3 %) SLO 016 (7,0 %)
pets	17	2	6 (35,3)	-	-	-	11 (64,7)	-	NA

NA – not applicable; n – number of isolates

- Majority of strains of a single PCR ribotype grouped together with PFGE regardless which restriction enzyme was used (*Sma*I or *Sac*II) (data not shown).
- Human and animal isolates of the same PCR ribotype clustered together with PFGE and had also similar MIC values for all antibiotics tested.
- In five PCR ribotypes (clusters 1-5 on the Figure 1) animal isolates had indistinguishable banding pattern (100 % similar) when restriction was performed with *Sma*I. However, when restriction was performed with *Sac*II, only one pig isolate was 100 % similar to the human one (Figure 1).

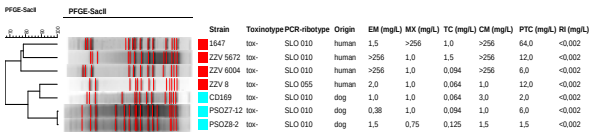

Figure 2. PFGE dendrogram (*Sac*II restriction digest) of PCR ribotypes SLO 010 and SLO 055.

- In general, human isolates had higher MIC_{90} and MIC_{50} values than animal isolates for all antibiotics tested (Table 2).
- With a few exceptions all strains within a single ribotype also had similar MICs for all antibiotics tested.
- Within ribotype 014/020 three strains had MICs for MX >256 mg/L and one had MICs for EM and CM >256 mg/l. All four strains grouped together with other 014/020 strains (Figure 1).
- Two out of seven PaLoc negative strains (SLO 010 and SLO 055) had MICs for EM and CM >256 mg/L and one had MICs for MX and CM >256 mg/L. In contrast to 014/020 strains this three strains grouped together but not with other PaLoc negative strains (Figure 2).

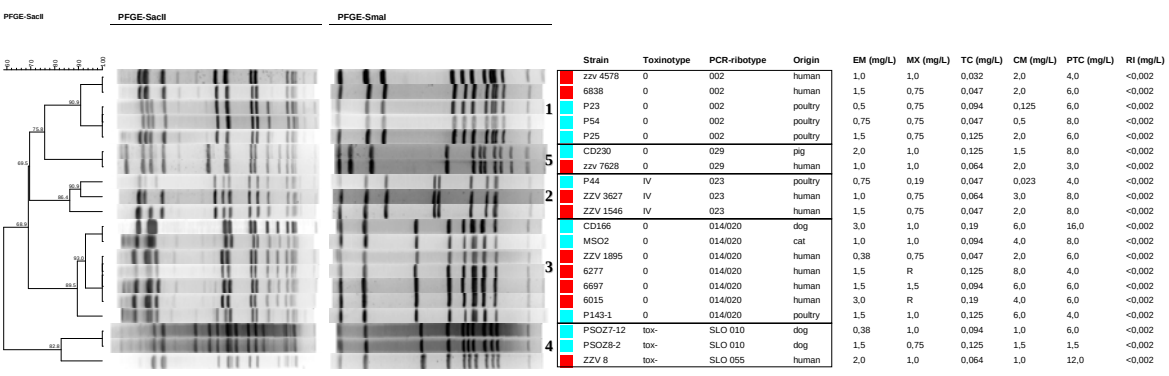

Figure 1. PFGE dendrogram of human and animal isolates (*Sac*II restriction digest) and their association with PFGE patterns of *Sma*I restriction digest, toxinotype, PCR ribotype, origin and antibiotic susceptibility testing. Dendrogram is color coded according to origin of the isolate (humans/animals). Five clusters (1-5) of animal and human isolates had indistinguishable banding patterns (100 % similar) when digestion was performed with *Sma*I. R - MIC > 256 mg/L

CONCLUSIONS

Our results show that isolates of the same PCR ribotype also group together with PFGE and that animal and human isolates of the same PCR ribotype are genetically similar which is in agreement with previous studies on ribotype 078 strains (Baker *et al*., 2010; Jhung *et al*., 2008).

Currently most laboratories uses *Sma*I restriction enzyme for PFGE typing of *C. difficile*, but our results show that use of two different enzymes simultaneously could improve discriminatory power of PFGE.

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