Prevalence and Characterization of *Clostridium difficile* from Dogs and Cats in Korea

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Introduction

Clostridium difficile **has been recognized as an important emerging pathogen in both humans and different animal species. Animals are discussed as potential reservoirs and source of infection because of genetic overlap between animals and humans. This study was conducted to evaluate the prevalence of *C. difficile* in dogs and cats and to characterize the isolates in Korea.**

Materials and methods

In a large-scale survey, we collected 940 fecal samples to evaluate the prevalence and characterize of *C. difficile* in dogs and cats (784 dogs and 156 cats/ 272 shelter pets, 571 companion pets and 97 other dogs) from July 2016 – May 2018. PCR ribotyping, MLST, and PCR detection of toxin genes were used to characterize isolated *C. difficile* strains. In addition, MIC was performed using E-test strips of amoxicillin/clavulanate, clindamycin, daptomycin, erythromycin, levofloxacin, metronidazole, moxifloxacin, rifampicin, tetracycline, tigecycline, and vancomycin.

Results

In total, *C. difficile* was isolated from 198 out of 940 (21.0%) fecal samples; 191 from companion pets, six from shelter pets, and one from other dog. Among them, 7.3% (69/940) was toxigenic and 13.7% (129/940) was non-toxigenic strain (Table 1). Most of the toxigenic strains were from companion pets (98.6%, 68/69). All 69 toxigenic strains were $A^+B^+CDT^-$ toxin gene profile, whereas PCR ribotypes and Sequence types of toxigenic strains were variable. R106 (ST42) was the most prevalent ribotype (23.2%), followed by R014/020 (ST2 or ST110), AB24 (ST129) (Table 2). All of the isolates were susceptible to daptomycin, metronidazole, rifampicin, tigecycline, and vancomycin, whereas they were resistant to levofloxacin(100%) and amoxicillin/clavulanate (98.6%).

Table 1. Pevalence of *C. difficile* in dogs and cats (n=940)

Animal species		No. of sample	tCDIFF ($A^+B^+CDT^-$)	non-tCDIFF ($A^-B^-CDT^-$)	Total (%)	
Dogs	Shelter	240	1 (0.4)	5 (2.1)	6 (2.5)	
(n=784)	Companion	447	57 (12.8)	114 (25.5)	171 (38.3)	178 (22.7)
	Military dog	97	0	1 (1.0)	1 (1.0)	
Cats	Shelter	32	0	0	0	
(n=156)	Companion	124	11 (8.9)	9 (7.3)	20 (16.2)	20 (12.8)
Total (n=940)			69 (7.3)	129 (13.7)	198 (21.0)	

tCDIFF, toxigenic *C. difficile*; non-tCDIFF, non-toxigenic *C. difficile*

Table 2. Ribotypes and sequence types of *C. difficile* strains (n=69)

Ribotype(R)	Sequence type(ST)	No. of isolate (%)	Species (n) [a]
R001	ST3/ST29	3 (4.3)/1 (1.4)	-/Feline (1)
R002	ST8	3 (4.3)	
R005	ST63	1 (1.4)	
R012	ST54	3 (4.3)	Feline (1)
R014/020	ST2/ST110	13 (18.8)/1 (1.4)	Feline (1)/-
R018	ST17	1 (1.4)	
R046	ST35	5 (7.2)	Feline (1)
R106	ST42	16 (23.2)	
AB24	ST129	12 (17.4)	Feline (2)
AB25	ST102	5 (7.2)	
AB38	ST36	5 (7.2)	Feline (5)
Total		69	Feline (11)

[a] All isolates that are not indicated were isolated from dogs.

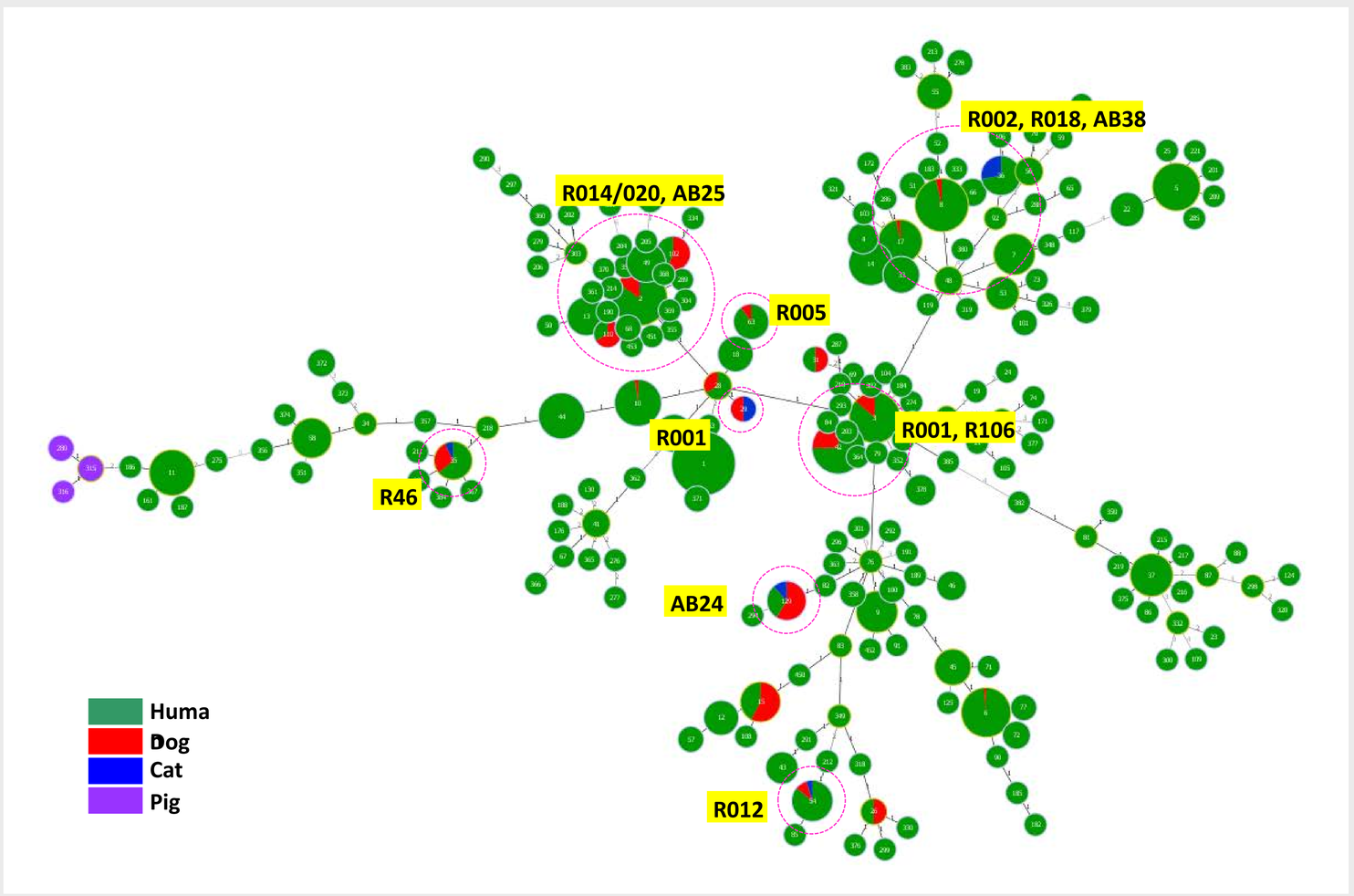

Fig. A goeBURST diagram for MLST data of *Clostridium difficile* isolates from human (green), dog (red), cat (blue), and pig (purple). Each circle in the diagram represents a sequence type (ST), with the size of the circle and its colored segments proportional to the number and origin of isolates, respectively. Numbers on branches represent the number of loci different from that of the founder ST. Dotted pink circles represent ST of isolates from dogs and cats in this study.

Table 3. Antimicrobial susceptibility of *C. difficile* strains (n=69)

Antimicrobials	Break point	% R [a]	MIC_{50} (μg/ml) [b]	MIC_{90} (μg/ml) [b]
Amoxicillin/clavulanate	≥ 16	1.4	0.5	0.75
Clindamycin	≥ 8	58.0	8	> 256
Daptomycin	> 4	0	0.38	0.75
Erythromycin	≥ 8	18.8	1	> 256
Levofloxacin	≥ 8	100	> 32	> 32
Metronidazole	≥ 32	0	0.125	0.19
Moxifloxacin	≥ 8	11.6	0.75	> 32
Rifampicin	≥ 4	0	< 0.002	< 0.002
Tetracycline	≥ 16	8.7	0.047	12
Tigecycline	> 4	0	0.023	0.047
Vancomycin	≥ 32	0	1.5	3

[a] %R, % Resistance; [b] MIC_{50} and MIC_{90}, concentrations at which the growth of 50% and 90%, respectively, of the isolates is inhibited.

Conclusion/Discussion

Based on the similarity between the ribotypes observed in this study and those described in humans in Korea, the zoonotic transmission for *C. difficile* cannot be excluded. In addition, most of toxigenic *C. difficile* (n = 68 out of 69) was isolated from companion pets, not shelter pets, suggests that they might spread from humans to companion pets.

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