The burden of *Clostridioides difficile* infection in Japan: a prospective multi-center study

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Background:

Despite predisposing factors for *Clostridioides* (*Clostridium*) *difficile* infection (CDI) such as increasing age in Japan, retrospective studies have generally found a lower incidence than those reported from Europe and North America. There are several potential explanations, including testing practices and strain distribution.

We conducted a multicenter study at 12 medical facilities to determine CDI incidence and molecular epidemiology in Japan.

Study design:

1. **From May 12, 2014 to May 11, 2015, all patients 18 years of age or older with clinical significant diarrhea (CSD) were approached to participate in the study.**
2. **CSD was defined as follows: at least 3 diarrheal bowel movement (Bristol stool chart grade 6-7) in the prior 24 hours, or a diarrheal bowel movement (Bristol stool chart grade 6-7) associated with abdominal cramping.**
3. **CDI was defined as CSD with any of positive results of *C. difficile* toxin A/B by enzyme immunoassay (EIA), toxigenic culture (TC) and nucleic acid amplification test (NAAT).**

Laboratory assays and *C. difficile* analysis

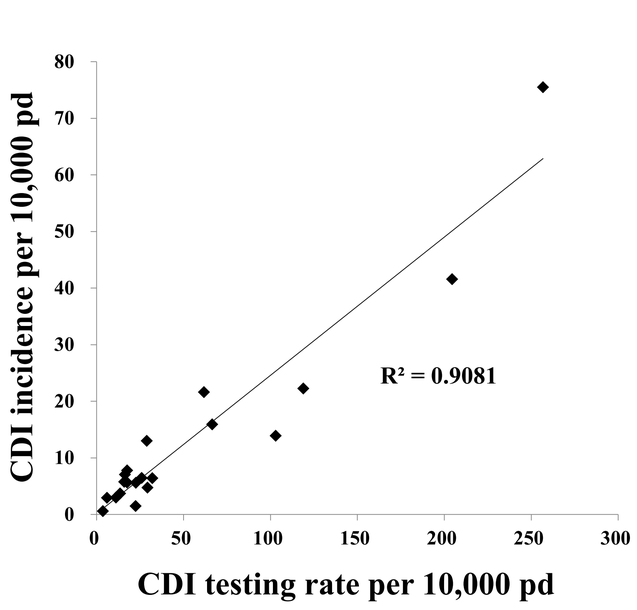
1. **Detection of fecal toxin by EIA was performed at each hospital.**
2. **Stool specimens were cultured on CCMA-EX (Nissui) after treating with alcohol for spore selection. CCMB-TAL (Anaerobe Systems) was also used. The presence of genes encoding toxin A, toxin B and binary toxin (CDT) was examined by PCR.**
3. **NAAT was performed by detection of the toxin B gene from fecal specimens by BD MAX Cdiff Assay kit (BD Diagnostics).**
4. **Typing analysis was performed by PCR-ribotyping (RT) and *slpA* sequence typing (*slpA*-ST).**
5. **Susceptibility testing was carried out by using Etest strips (bioMérieux SA).**

Table 1. Comparison of CDI incidence rate by ward type

Ward (No. of wards examined)	No. of CDI cases	CDI incidence rate (per 10,000 pd)
Medical (n=10)	86	6.0
ICU (n=5)	45	22.2
Medical/surgical (n=3)	13	3.2
Surgical (n=2)	29	9.0
All (n=20)	173	7.4

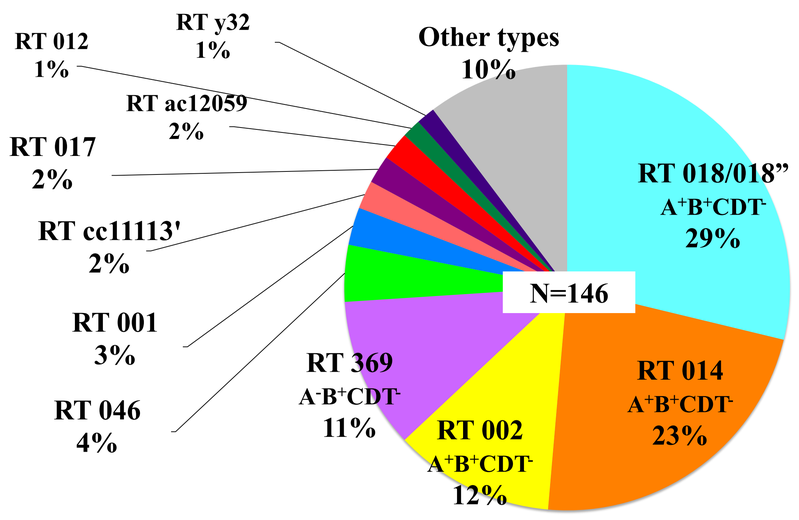
The high incidence in 5 ICU wards indicates that intervention designed to reduce CDI in ICUs may be particularly critical.

Table 2. Correlation of CDI testing rate and CDI incidence rate



A clear correlation between testing frequency and CDI incidence rate indicates that a significant number of CDI cases have been overlooked because of inappropriate diagnostic testing in Japan.

Figure Typing results by PCR-ribotyping



Toxin A-positive, toxin B-positive, binary toxin-positive *C. difficile* was recovered from only three CDI cases. They were typed into distinct RTs and none of them were RT027, RT078, RT126 or RT244.

Table 3. Typing results of recovered isolates and CDI incidence per ward

Hospital-ward		A-1	B-1	C-1	C-2	D-1	E-1	F-1	F-2	F-3	F-4	F-5	F-6	F-7	G-1	G-2	H-1	I-1	J-1	K-1	L-1	Total
Ward-type	ICU	Yes	No	No	Yes	Yes	No	No	No	No	No	No	Yes	No	No	Yes	No	No	No	No	No	
Incidence (/10,000 pd)		13.9	4.8	1.5	22.3	41.6	13.0	5.8	7.1	3.0	3.8	3.0	21.6	7.8	0.6	75.5	6.5	5.7	6.4	5.6	15.9	7.4
RT	*slpA*-ST																					
018	smz-01																		1			1
018	smz-02				1			2			1		1			1	1				7	14
018	smz-09	1																				1
018"	smz-01	2	1			1	7		2		1					1	2	3	1	1	4	26
014	hr-01	2	1	1	1	3	2	1	3			1	2	2			2		3	2	4	30
014	hr-07																			2		2
014	tc-01								1													1
002	yok-01	2	1								1	2	2			1	1	3		2	1	16
002	hr-10																	1				1
369	fr-01					2		1	3		2			2								10
369	fr-06			1																	2	3
369	fr-20																				1	1
369	fr-21														1							1
369	fr-22												1									1
Other types		7	3		4	2	1	3	1	4		1	1			1	3	1	3		3	38
Total No. of isolates		14	6	2	6	8	10	7	10	4	5	4	7	4	1	4	9	8	8	7	22	146

Among the 15 non-ICU wards, two had high CDI incidence rates (13.0 and 15.9), with clusters due to RT 018/*slpA*-ST smz-02 and 018" /smz-01, respectively.

Table 4. Susceptibility of 146 isolates against 8 antimicrobial agents

PCR-ribotype	No. of isolates	Percentage of resistant isolates (%)							
		Moxiflo-xacin	Gatiflo-xacin	Erythro-mycin	Clinda-mycin	Imipenem	Rifampicin	Vanco-mycin	Metro-nidazole
018/018"	42	100	100	100	100	52.3	0	0	0
014	33	33.3	42.4	36.4	39.4	12.1	0	0	0
002	17	82.4	76.5	64.7	58.8	5.9	0	0	0
369	16	100	100	100	100	18.8	6.3	0	0
Other types	38	10.5	10.5	34.2	31.6	2.6	0	0	0

All isolates belonging to RT 018/018" were resistant to moxifloxacin, gatifloxacin, and clindamycin but susceptible to vancomycin, metronidazole and rifampicin.

Conclusions:

1. **A sizable number of patients with CDI are not identified due to low testing rates in Japan.**
2. **It is suggested that selective pressure by overuse of antimicrobial agents leads to the spread of *C. difficile*, especially of RT 018/018" and RT369.**
3. **It is important to raise the awareness of infection control for CDI, including optimal laboratory diagnosis and implementation of antimicrobial stewardship programs in Japan.**

The Clostridium difficile infection Japan Study Group

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