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Title

Multi-institution Case-control and Cohort Study of Risk Factors for the Development and Mortality of *Clostridium difficile* Infections in Japan

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Abstract

Objective: To examine risk factors for *Clostridium difficile* infection (CDI) morbidity and mortality in Japan.

Design: Multi-method investigation including a case–control study and cohort study.

Setting: Forty-seven participating facilities of the National Hospital Organization (NHO).

Participants: One thousand twenty six CDI patients and 878 patients in control group over the age of 18 years admitted to the subject NHO facilities from November 2010 to October 2011.

Main Outcome Measures: In case-control study, we identify risk factors for CDI development. Next, in cohort study, we identify risk factors for all-cause mortality within 30 days following CDI onset.

Results: A total of 1,026 cases of CDI meeting the definitions of this investigation were identified, encompassing 878 patients at 42 of the 47 subject facilities. In the case–control study, we identified, compared with no antibiotics use, use of first- and second-generation cephem antibiotics (odds ratio[OR], 1.44; 95% confidence interval [CI], 1.10 to 1.87), use of third- and fourth-generation cephem antibiotics(OR, 1.86; 95%CI, 1.48 to 2.33), and use of carbapenem antibiotics (OR, 1.87; 95%CI, 1.44 to 2.42) were risk factors for CDI development. However, use of penicillin was not identified as risk factors. In the cohort study, sufficient data for analysis was available for 924 CDI cases; 102 of them (11.0%) resulted in death within 30 days of CDI onset. Compared with no anti-CDI drug use, use of vancomycin was associated with reduced risk of mortality (OR, 0.43; 95%CI, 0.25 to 0.75) whereas metronidazole was not.

Conclusions: The findings mirror those of previous studies from Europe and North America, identifying the administration of broad-spectrum antibiotics as a risk factor for CDI development. The use of vancomycin is associated with a decreased risk of mortality.

Strengths and limitations of this study

- This study is the first large-scale nationwide multi-center CDI investigation in Japan.
- Most of the epidemiological data of CDI has been limited in the North America and Europe. Our data plays a role of completion of the missing data in Asia.
- Use of β-lactam antibiotics except penicillin was the risk factor for CDI development in the first Japanese large-scale investigation. Appropriate antibiotic use is necessary in order to control the incidence of CDI.
- Vancomycin administration for CDI was associated with decreased risk of mortality. Although the cost-effective treatment of CDI may necessitate the appropriate use of less-expensive metronidazole, vancomycin should be administered in case expected to become severe or life-threatening.
- The most salient limitation of the case–control study phase is the existence of many confounding factors. In particular, probiotic use, which was recently shown to be correlated with CDI prevention, was not included in the predictive model of this study.

Introduction

Clostridium difficile is the main causative pathogen of antibiotic-associated colitis. Since 2000, outbreaks of BI/NAP1/027 strain *C. difficile* infections (CDI) have been reported in North American and European hospitals and elder care facilities. The numbers of CDI patients as well as severe and intractable cases have increased simultaneously. Consequently, epidemiological surveillance systems have been set up in several countries. However, very few countries have implemented such national-level measures.

In Japan, the Ministry of Health, Welfare, and Labor's Japan Nosocomial Infection Surveillance program investigates the incidence rates of a variety of drug-resistant bacteria; however, this program does not monitor the incidence rate of *C. difficile* (http://www.nih-janis.jp/index.asp). Therefore, CDI epidemiological studies in Japan to date have been based on scattered data from individual medical facilities. Consequently, the phenomenon of CDI in Japan is not sufficiently understood. Reports of BI/NAP1/027 infections are limited, and conditions in Japan possibly differ from those in Europe and North America.

Previous studies report that antibiotic administration is the largest risk factor for CDI development. Other risk factors include advanced age and proton pump inhibitor use.[1, 2] CDI mortality rates differ depending on the presence or absence of an outbreak as well as the relevant definitions of epidemiological surveillance. Furthermore, it is especially difficult to objectively determine precise CDI-related mortality rates because of factors such as underlying patient conditions.[3]

This report documents a case–control study of CDI in Japan based on data from the National Hospital Organization (NHO), which is Japan's largest group of hospitals and includes facilities located nationwide. In addition, a cohort investigation of mortality among CDI cases was conducted.

Materials and Methods

Research Design

This multicenter study is a collaborative effort of the 47 facilities that met our facility standards from among the 143 NHO facilities in Japan. The study was planned as a part of the NHO's "National Hospital Organization Multi-Center Clinical Research for Evidence-Based Medicine" project. This study was conducted with the approval of the Central Ethics Committee of the NHO. The CDI group in this study included in principal all newly diagnosed CDI cases among patients hospitalized from November 1, 2010 to October 31, 2011; cases were registered continuously.

In the case–control study of CDI development, CDI cases newly diagnosed during the investigation period were registered in the CDI group; meanwhile, age-, sex-, and underlying disease-matched patients in the same facilities were registered to the control group. In addition, a prospective cohort study of CDI group patients who died within 30 days of CDI development was conducted. This investigation is a multi-method study using standard case–control and cohort study designs.

Definition of CDI

CDI was defined as the presence of any gastrointestinal symptoms accompanied by a clinical suspicion of CDI as well as a positive result for *C. difficile* toxins from rapid stool testing or *C. difficile* isolation from stool cultures or both. Final determinations were made by the attending physician or the facility's infection control team.

Enzyme immunoassay testing kits for *C. difficile* toxins A and B were used as the rapid testing method (Immunocard CD toxin A&B, Meridian Bioscience Inc., Cincinnati, OH, USA; C. Diff Quik Chek, Alere Medical Co. Ltd., Tokyo, Japan; Tox A/B Quik Chek, Nissui Pharmaceutical Co., Ltd., Tokyo, Japan; X/pect Toxin A/B, Kanto Chemical Co Ltd., Tokyo, Japan). Cycloserine-cefoxitin mannitol agar (Nissuipure-to CCMA baichi EX, Nissui Pharmaceutical Co. Ltd., Tokyo, Japan), cycloserine-cefoxitin fructose agar (CCFA baichi, Becton, Dickinson and Company Co. Ltd., Tokyo, Japan; Poamedhia® CCFA® kairyoubaichi, Eiken Chemical Co., Ltd., Tokyo, Japan), and brucella HK agar (RS) (brucella HK agar (RS), Kyokuto Pharmaceutical Industrial Co. Ltd., Tokyo, Japan) were used in the *C. difficile* isolation cultures.

Case–Control Study of CDI Development

No additional information besides age, sex, and date of diagnosis was gathered when new patients were registered in the CDI group. After the end of the study registration period, additional patient clinical data were gathered, including clinical department, underlying diseases, dates of hospital admittance and discharge, and medical treatments administered for ≥3 days between admittance and CDI development. Recorded treatments included disruption of feeding, parenteral nutrition, enteral feeding, surgery with general anesthetic, cancer drugs, antibiotics (excluding external-use antibiotics), proton pump inhibitors (oral or intravenous). We also collected data regarding the use of intravenous antibiotics including penicillins, first- and second-generation cephems, third- and fourth-generation cephems, carbapenems, fluoroquinolones, clindamycin/lincomycin, anti–Methicillin-resistant *Staphylococcus aureus* (MRSA) drugs, and anti-fungal drugs, and others. Finally, we collected data regarding the use of oral antibiotics including cephems, fluoroquinolones, and others.

The control group was divided into three subgroups according to age: ≤74, 75–84, and ≥85 years. The control patients were selected from among patients at the same facilities who did not contract CDI and were matched to the CDI patients with respect to age, sex, underlying disease, and hospital stays of ≥5 days within the same month as a counterpart's CDI diagnosis. We strove to ensure that the CDI and control groups were as matched as possible. The same data were collected from both groups. The control patients were registered, and relevant patient data were gathered after the end of the CDI group study registration period.

Cohort Study on Mortality among CDI Patients

The prospective cohort study of registered CDI group patients from the case–control study examined all-cause mortality within 30 days as the primary outcome. The following data were collected: whether the underlying disease was infectious and whether comorbidities were related to malignant tumors (i.e., gastrointestinal, respiratory, blood/lymph, gynecologic, urological, or other tumors including cancers of the ear, nose, and throat), diabetes, renal failure, heart failure, respiratory

failure, or cirrhosis. We also considered patient nutritional status including whether the patient was subjected to parenteral nutrition or enteral feeding as well as serum albumin levels measured within 30 days prior to CDI development (i.e., ≥3.5, 2.7–3.4, or ≤2.6 g/dL). In addition, we examined CDI treatment factors including whether antibiotic use was halted, probiotic use, and the type of anti-CDI drugs used (i.e., vancomycin and metronidazole). All patient data for the cohort investigation were collected after the end of the registration period.

Data Management and Statistical Analysis

The study coordinator established independent data management centers within the NHO facilities for data collection. All input data were verified by a designated study data manager. Data from each facility were entered directly into a web-based case report form and subsequently encrypted for security. The data management center was responsible for confirming any missing data and directly inquiring the relevant facilities as necessary. After the end of the study period, the data were finalized and subsequently transferred to the Research Coordinator's office.

During the case–control phase of the study, CDI development was treated as the outcome and odds ratios (ORs) were calculated from bivariate analysis comparing the use of different types of antibiotics as outcome causes. For each type of antibiotic, those used for ≥3 days were designated "used" while all others were designated "unused." A dummy variable regression was subsequently performed. Statistical significance in the bivariate analysis was tested by the chi-square test. Logistic regression analysis was performed using the individual patient characteristics and other assumed confounding variables as independent variables. The 95% confidence intervals (CIs) for each variable were used to determine the relationships between the various predictive variables and outcomes.

In the cohort study, gastrointestinal perforations, toxic megacolon, CDI-related surgeries, and the all-cause in-hospital mortality of patients within 30 days of CDI development were recorded. The clinical outcome of mortality within 30 days was set as the dependent variable, and the relationships among the underlying diseases, nutritional status, probiotic use, and

types of anti-CDI drugs used were subjected to bivariate and multivariate analyses. Like the case–control phase, bivariate analysis were conducted using the chi-square test, and the multivariate analysis was conducted using logistic regression. The significance level for all analyses was set at $p < 0.05$. We used IBM SPSS Statistics version 20 for statistical analysis.

Ethics Committee Approval and Informed Consent

This study was conducted with the approval of the Central Ethics Committee of the NHO. In principle, individual patients who met the inclusion criteria were not given direct explanations of the study, and no direct consent was sought. Information about the study was made public through postings on facility notice boards and webpages. Patients and their representative agents had the right to refuse study participation.

Results

Participating Facilities

Among the 47 facilities, a total of 1,026 CDI cases were registered at 42 facilities throughout Japan, from Hokkaido in the north to Okinawa in the south. No CDI cases were recorded at the remaining 5 participating facilities, more than 280 patient beds. The regional locations of the 47 facilities were as follows: 5 in Hokkaido and Tohoku, 10 in Kanto and Koshinetsu, 2 in Tokai and Hokuriku, 9 in Kinki, 10 in Chugoku and Shikoku, and 11 in Kyushu and Okinawa (Table 1).

Table 1. Number of registered cases of CDI and characteristics of hospitals included in the surveillance of CDI in the NHO (from november 2010 through october 2011)

Region	No. patient beds	No. patient days	No. patients registered		30-day all-cause mortality	Bacteriological survey	
			CDI group	Control group		EIA detection: toxins A and B	Culture
Hokkaido, tohoku	698	208,388	55	55	3 (5%)	+	+
	500	150,603	42	32	1 (2%)	+	+
	310	82,687	28	19	2 (7%)		+
	310	72,144	17	12	2 (12%)	+	+
	220	76,539	1	1	0 (0%)	+	+
Kanto, koshinetsu	780	238,420	124	121	15 (12%)	+	+
	455	151,622	36	36	3 (8%)	+	
	560	158,921	35	30	4 (11%)	+	+
	243	60,155	34	34	6 (18%)	+	+
	350	109,025	22	22	4 (18%)	+	+
	500	159,432	15	14	1 (7%)	+	
	510	166,668	4	4	0 (0%)	+	
	380	109,482	3	2	0 (0%)	+	+
	455	132,483	3	1	0 (0%)	+	
	429	104,802	0	0	— (—)	+	
Tokai, hokuriku	430	195,209	42	26	10 (24%)	+	+
	280	56,475	0	0	— (—)	+	
Kinki	316	103,677	24	22	1 (4%)	+	
	220	47,354	23	23	1 (4%)	+	+
	600	191,041	20	20	3 (15%)	+	
	494	70,455	15	15	6 (40%)	+	+
	520	145,299	13	9	1 (8%)	+	
	500	142,409	6	6	1 (17%)	+	
	180	55,721	3	3	1 (33%)	+	
	346	118,014	2	2	0 (0%)	+	
	370	94,722	0	0	— (—)	+	
Chugoku, shikoku	388	99,728	54	49	5 (9%)	+	+
	700	211,595	49	48	4 (8%)	+	+
	506	119,356	33	8	1 (3%)	+	+
	400	122,846	30	30	5 (17%)	+	
	401	108,303	26	0	2 (8%)	+	+
	250	80,558	21	21	0 (0%)	+	
	424	128,868	12	10	0 (0%)	+	
	365	125,645	10	10	3 (30%)	+	+
	300	87,061	0	0	– (–)		+
	459	66,454	0	0	– (–)	+	
Kyushu, okinawa	424	137,827	46	22	5 (11%)	+	
	702	239,448	38	37	1 (3%)	+	
	190	54,038	33	31	9 (27%)	+	
	550	189,417	27	26	3 (11%)	+	
	285	58,185	25	25	3 (12%)	+	
	500	140,371	24	23	2 (8%)	+	
	300	90,457	14	14	4 (29%)	+	
	320	103,315	6	5	1 (17%)	+	+
	280	79,580	4	4	2 (50%)	+	
	366	112,906	4	4	0 (0%)	+	
	368	89,195	3	2	2 (67%)	+	
Total	19,486	5,592,077	1,026	878	117 (11%)	45	20

Patient Grouping

A total of 1,026 CDI cases that met the study definitions were recorded at the various institutions. We were unable to collect clinical records regarding medical treatments for 1 case; therefore, this case was excluded from the case–control study, and the remaining 1,025 cases were analyzed. A total of 962 patients (93.9%) developed CDI within 48 hours after hospital admittance. The control group comprised 878 patients who were selected from 41 of the 42 facilities. In the cohort study, we analyzed the data from 924 of the 1,025 CDI group patients, excluding 101 patients with no available recent serum albumin level data (i.e., within 30 days prior to CDI development (Figure 1).

Case–Control Study of CDI Development

The mean ages of the CDI and control groups were 75.8 and 75.4 years, respectively. The majority of the subjects were of advanced age: 64.0% and 62.5% of the CDI and control group patients were aged ≥75 years, respectively. No significant differences were identified between the CDI and control groups in the univariate analysis of age distribution, sex differences, or underlying disease (Table 2). Among the medical treatments administered before CDI development, the following were significantly more prevalent in the CDI group than the control group: disruption of feeding (48.6% vs. 30.4%), parenteral nutrition (24.7% vs. 10.3%), and enteral feeding (24.8% vs. 9.1%). Antibiotics were used prior to CDI development in 85.8% of cases. The use of all types of intravenous antibiotics was significantly more prevalent in the CDI group. No significant differences were identified between the 2 groups with respect to oral antibiotic use. Meanwhile, in the univariate analysis, proton pump inhibitor use was significantly more prevalent in the CDI group than the control group (40.3% vs. 31.2%).

We used logistic regression analysis to determine the risk factors for CDI development. The following medical treatments prior to CDI development were identified as significant risk factors in comparison to the control group: disruption of feeding (odds ratio[OR], 1.31; 95% confidence interval[CI], 1.05 to 1.64), parenteral nutrition (OR, 1.63; 95%CI, 1.21 to

2.20) and enteral feeding (OR, 2.16; 95%CI, 1.60 to 2.92).The following intravenous antibiotics were also identified as statistically significant risk factors for CDI development: first- and second-generation cephems (OR, 1.44; 95%CI, 1.10 to 1.87), third- and fourth-generation cephems (OR, 1.86; 95%CI, 1.48 to 2.33), and carbapenems (OR, 1.87; 95%CI, 1.44 to 2.42). However, penicillin (OR, 1.04; 95%CI, 0.82 to 1.33), fluoroquinolones (OR, 1.16; 95%CI, 0.74 to 1.83), clindamycin/lincomycin (OR, 1.35; 95%CI, 0.81 to 2.26), and proton pump inhibitor use (OR, 1.17; 95%CI, 0.95 to 1.44) were not identified as risk factors.

Table 2. Univariate and multivariate analyses of CDI development-related risk factors

Characteristics	CDI group %	Control group %	Univariate analysis P value	Multivariate analysis Odds ratio (95% CI)	Multivariate analysis P value
All	(1,025)	(878)	—	—	—
Age					
≤74 years	36.0 (369)	37.5 (329)		Ref.	—
75–84 years	37.0 (379)	37.2 (327)	0.67	1.02 (0.81 to 1.28)	0.88
≥85 years	27.0 (277)	25.3 (222)		1.09 (0.84 to 1.41)	0.52
Sex					
Women	43.0 (441)	42.6 (374)	0.85	1.11 (0.91 to 1.36)	0.28
Underlying disease					
Respiratory infections	15.8 (162)	17.5 (154)		—	—
Other infectious conditions	16.9 (173)	14.2 (125)		—	—
Gastrointestinal conditions	8.1 (83)	9.0 (79)		—	—
Malignant tumors	22.6 (232)	24.3 (213)	0.14	—	—
Cardiovascular conditions	7.7 (79)	9.8 (86)		—	—
Other conditions	28.9 (296)	25.2 (221)		—	—
Medical treatment prior to CDIdevelopment					
Disruption of feeding	48.6 (498)	30.4 (267)	<0.001	1.31 (1.05 to 1.64)	<0.05
Parenteral nutrition	24.7 (253)	10.3 (90)	<0.001	1.63 (1.21 to 2.20)	<0.01
Enteral feeding	24.8 (254)	9.1 (80)	<0.001	2.16 (1.60 to 2.92)	<0.001
Surgery with general anesthetic	18.2 (187)	15.6 (137)	0.14	0.89 (0.67 to 1.18)	0.41
Cancer drugs	11.3 (116)	14.2 (125)	0.06	0.86 (0.62 to 1.18)	0.35
Antibiotics use	85.8 (879)	66.5 (584)	<0.001	—	—
Intravenous					
Penicillins	27.6 (283)	21.0 (184)	<0.01	1.04 (0.82 to 1.33)	0.75
First/second-generation cephems	22.7 (233)	15.6 (137)	<0.001	1.44 (1.10 to 1.87)	<0.01
Third/fourth-generation cephems	35.2 (361)	19.9 (175)	<0.001	1.86 (1.48 to 2.33)	<0.001
Carbapenems	31.8 (326)	15.0 (132)	<0.001	1.87 (1.44 to 2.42)	<0.001
fluoroquinolones	7.5 (77)	4.0 (35)	<0.01	1.16 (0.74 to 1.83)	0.52
Clindamycin/lincomycin	6.5 (67)	2.8 (25)	<0.001	1.35 (0.81 to 2.26)	0.25
MRSA drugs	10.7 (110)	4.3 (38)	<0.001	1.10 (0.71 to 1.72)	0.66
Anti-fungal drugs	6.9 (71)	3.2 (28)	<0.001	1.01 (0.60 to 1.70)	0.96
Others(aminoglycosides, monobactam,etc.)	8.5 (87)	5.9 (52)	<0.05	1.19 (0.80 to 1.77)	0.39
Oral					
Cephems	5.6 (57)	4.4 (39)	0.29	1.49 (0.95 to 2.32)	0.08
fluoroquinolones	14.5 (149)	11.5 (101)	0.06	1.11 (0.82 to 1.51)	0.49
Others (macrolides, penicillins, etc.)	14.0 (144)	13.9 (122)	0.95	0.84 (0.63 to 1.13)	0.26
Proton pump inhibitors	40.3 (413)	31.2 (274)	<0.001	1.17 (0.95 to 1.44)	0.14

Cohort Study on Mortality among Patients with CDI

The cohort study examined mortality among the 924 patients from the 1,025 CDI group patients in the case–control study for whom serum albumin level data before CDI development were available.

Among the 924 patients, 102 (11.0%) died within 30 days of developing CDI. Among those cases, the cause of death was attributed to CDI in 11 cases (1.2%). The mean age of the 102 patients who died during the study was 80.1 ± 8.3 years. Patients ≥75 years old were especially prevalent in this subgroup, accounting for 77.5% (79/102) of the cases.

Some patients developed severe complications within 30 days of CDI development, including gastrointestinal perforation in 1 patient (0.1%) and toxic megacolon in 2 patients (0.2%); 1 patient (0.1%) underwent a CDI-related surgery. Among the 714 cases in which CDI was treated directly, recurrence within 30 days was observed in 34 cases (4.8%).

The univariate analysis indicated that comorbidities of heart and respiratory failure were significantly more prevalent among CDI patients. In addition, lower serum albumin levels were significantly associated with mortality. Among CDI treatments, mortality was significantly lower among cases in which probiotics were administered.

A logistic regression analysis of the 102 cases in which the patients died within 30 days of CDI development was performed to identify the factors associated with the risk of mortality. Compared to patients ≤74 years old, the odds ratio of mortality among patients aged 75–84 years was 2.08 (95%CI, 1.19 to 3.62). Among underlying diseases, heart failure (OR, 2.12; 95%CI, 1.26 to 3.55) and respiratory failure (OR, 1.98; 95%CI, 1.19 to 3.32) were identified as risk factors for mortality within 30 days of CDI development. Regarding nutritional status, neither parenteral nutrition nor enteral nutrition was identified as a risk factor for mortality. However, low serum albumin level (i.e., ≤2.6 g/dL) was identified as a significant risk factor for mortality (OR, 3.50; 95%CI, 1.33 to 9.22). Among CDI treatments, probiotic use (OR, 0.66; 95%CI, 0.42 to 1.04) was not identified as a risk factor for mortality. However, compared to cases in which no anti-CDI drugs were administered, vancomycin administration yielded an odds ratio of 0.43 (95%CI, 0.25 to 0.75), indicating a

significantly lowered risk of mortality in the CDI group. Meanwhile, no such lowered mortality was observed in cases treated with metronidazole (OR, 0.85; 95%CI, 0.48 to 1.51).

Table 3. Univariate and multivariate analyses of all-cause mortality in CDI patients

Characteristics	All-cause mortality rate %	Univariate analysis P value	Multivariate analysis Odds ratio (95% CI)	Multivariate analysis P value
All	11.0 (102/924)	—	—	—
Age				
74 years	7.1 (23/326)		Ref.	
75–84 years	13.3 (47/353)	<0.05	2.08 (1.19 to 3.62)	<0.05
85 years	13.1 (32/245)		1.86 (0.98 to 3.55)	0.06
Sex				
Men	12.2 (64/524)		Ref.	
Women	9.5 (38/400)	0.21	0.78 (0.49 to 1.24)	0.29
Underlying disease				
Non-infectious	10.3 (64/619)		Ref.	
Infectious	12.5 (38/305)	0.37	0.99 (0.60 to 1.62)	0.97
Comorbidities				
Malignant tumors				
Not present	10.6 (67/630)		Ref.	
Present	11.9 (35/294)	0.57	1.54 (0.94 to 2.53)	0.09
Diabetes				
Not present	11.6 (89/765)		Ref.	
Present	8.2 (13/159)	0.27	0.71 (0.37 to 1.35)	0.29
Renal failure				
Not present	10.7 (84/784)		Ref.	
Present	12.9 (18/140)	0.46	0.90 (0.49 to 1.65)	0.73
Heart failure				
Not present	9.3 (70/756)		Ref.	
Present	19.0 (32/168)	<0.01	2.12 (1.26 to 3.55)	<0.01
Respiratory failure				
Not present	9.2 (69/754)		Ref.	
Present	19.4 (33/170)	<0.001	1.98 (1.19 to 3.32)	<0.01
Cirrhosis				
Not present	11.2 (100/895)		Ref.	
Present	6.9 (2/29)	0.76	0.61 (0.13 to 2.83)	0.53
Indicators of nutritional status				
Parenteral nutrition or enteral feeding				
Not present	9.4 (53/563)		Ref.	
Present	13.6 (49/361)	0.05	1.16 (0.73 to 1.84)	0.53
Serum albumin (g/dL)				
≥3.5	4.0 (5/124)		Ref.	
2.7–3.4	7.2 (27/376)	<0.001	1.55 (0.57 to 4.21)	0.39
≤2.6	16.5 (70/424)		3.50 (1.33 to 9.22)	<0.05
CDI treatments				
Cessation of antibiotics				
Not present	12.5 (65/519)		Ref.	
Present	9.1 (37/405)	0.11	0.77 (0.48 to 1.22)	0.26
Probiotics (for intestine treatment)				
Not present	13.8 (52/378)		Ref.	
Present	9.2 (50/546)	<0.05	0.66 (0.42 to 1.04)	0.08
Anti-CDI drugs				
Not present	15.2 (32/210)		Ref.	
Vancomycin	7.4 (32/433)	<0.05	0.43 (0.25 to 0.75)	<0.01
Metronidazole	13.5 (32/237)		0.85 (0.48 to 1.51)	0.59
Vancomycin and metronidazole	13.6 (6/44)		0.75 (0.27 to 2.08)	0.57

Discussion

This is the first large-scale clinical study of CDI in Japan. This study examined 1,026 cases of CDI recorded over 1 year at the nationwide facilities of Japan's largest hospital group. The findings of this investigation are similar to those reported in previous studies conducted in Europe, North America, and Australia with respect to the identification of several risk factors for CDI development, including age, severity of the underlying condition, and artificial feeding.[2, 4, 5] Antibiotic use is a known risk factor for CDI development.[6] The present case–control study confirms that intravenous cephems and carbapenems are important risk factors. Some studies report a low risk of CDI development owing to intravenous penicillin administration.[7, 8] Concordantly, penicillin use was not identified as a risk factor in the present study. Although proton pump inhibitor use was indicated as a risk factor for CDI development in previous studies[9, 10] it was not identified as a risk factor in the present logistic regression analysis. This finding might be influenced by the relatively high *Helicobacter pylori* infection rate in elderly Japanese people; proton pump inhibitors might produce smaller changes in pH levels in such patients than American and European patients.[11]

In this study, 11.0 % of CDI patients died within 30days. In comparison, higher 30-day mortality rates have been reported in previous outbreaks: 24.8% in the ribotype 027 strain outbreak in Canada, and 36.7% in an examination of a single intensive care unit in the USA.[12, 13] However, reports of non-outbreak conditions indicate mortality rates of 13%, similar to the findings of the present study.[14] Some reports state that the CDI-associated mortality rate has increased 2.5 fold, possibly indicating that CDI cases are more severe and contribute more significantly to mortality than previously though.[3, 14] The mortality rate of CDI patients is reported to increase with age.[15] Concordantly, the present study also found a significantly elevated risk of death in patients ≥75 years old.

The findings of this study indicate that the mortality risk of CDI patients was not reduced as a result of metronidazole treatment but was reduced with vancomycin treatment, corroborating the existing recommendation.[16] It is worth noting

that metronidazole is less expensive than vancomycin, making it economically advantageous. a patient's condition must be carefully evaluated when selecting anti-CDI drugs. In particular, for patients in the present study who had conditions associated with a greater mortality risk, including advanced age (i.e., ≥75 years), heart or respiratory failure, or malnutrition as determined by low serum albumin levels, the use of vancomycin rather than metronidazole for treatment appears to have provided better outcomes.

Regardless, this study has also several methodological limitations. The most salient limitation of the case–control study phase is the existence of many confounding factors. In particular, probiotic use, which was recently shown to be correlated with CDI prevention, was not included in the predictive model of this study.[17] When interpreting the findings of this study, it is necessary to consider the influence of confounding factors that were not included in the analytical models. Regarding antibiotic use, the present analyses included independent explanatory variables for each antibiotic. However, actual antibiotic use is more complicated. Therefore, it is difficult to clearly determine the roles of individual antibiotics as risk factors for CDI development. In addition, although data for the control group were analyzed during the entire study period until hospital discharge, only data from the period prior to CDI development were analyzed in the CDI group. Therefore, the risks might be underestimated, because the control group had a longer period of exposure risk than the CDI group. Confounding factors that were not included in the present analyses also represent a limitation of the cohort study phase. Furthermore, issues of data quality among the facilities affect all aspects of this study. More than 40 different facilities participated in this study. While some facilities registered nearly all of their CDI patients, other facilities registered smaller proportions of patients. Finally, there might have been differences with regard to individual researchers' understanding of the outcome definitions.

As the Japanese population continues to age, the number of elderly patients suffering from multiple ailments is increasing as well. As the number of patients requiring intravenous administration of broad-spectrum antibiotics has increased, close

and careful monitoring of CDI epidemiology is necessary. In order to ensure appropriate antibiotic use and control the incidence of CDI, it is important to create institutional measures such as infection control teams and to not limit such controls to the efforts of individual doctors. The cost-effective treatment of CDI may necessitate the appropriate use of less-expensive metronidazole. However, in cases expected to become severe or life-threatening, the more expensive drug vancomycin should be administered. In countries facing an aging population, CDI is one of many issues concerning medicine and medical treatment costs. Accordingly, further and more proactive research into CDI epidemiology is needed.

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Contributors

MT conceived the idea for the study, designed the study, developed the protocol, was responsible for study management and data collection, interpreted the findings, and drafted the paper. NM interpreted the findings and drafted the paper. SB designed this study, developed the protocol, performed data analysis, and interpreted findings.

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Competing interests

None.

Ethics approval

The Central Ethics Committee of the NHO.

Provenance and peer review

Not commissioned; externally peer reviewed.

Data sharing statement

No additional data are available.

Contributorship Statement

All authors had full access to all of the data and can take responsibility for the integrity of the data and the accuracy of the data analysis.The lead author affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned have been explained.

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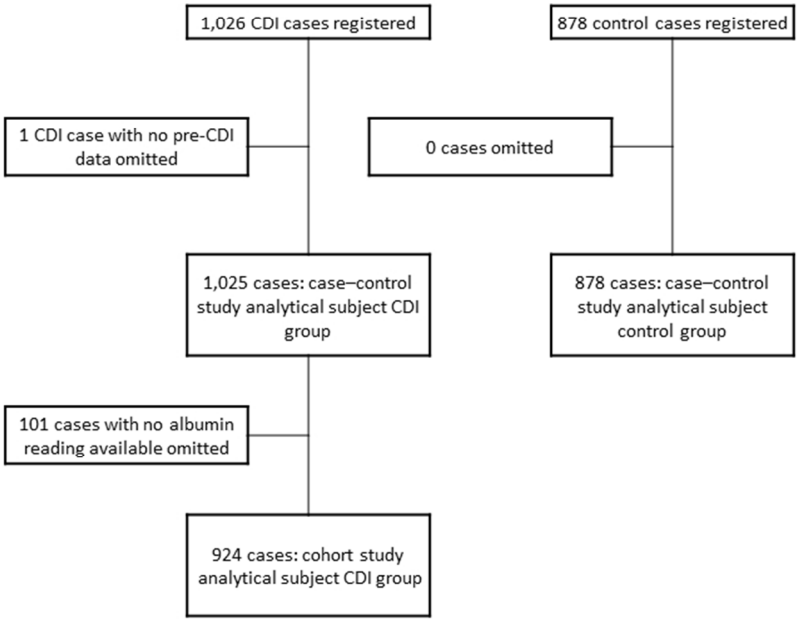

Figure 1. Study populations for the analysis of patients with *Clostridium difficile* infection (CDI) and controls.

190x254mm (96 x 96 DPI)

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STROBE Statement—Checklist of items that should be included in reports of ***case-control studies***

	Item No	Recommendation
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported
Objectives	3	State specific objectives, including any prespecified hypotheses
Methods		
Study design	4	Present key elements of study design early in the paper
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	(a) Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls
		(b) For matched studies, give matching criteria and the number of controls per case
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding
		(b) Describe any methods used to examine subgroups and interactions
		(c) Explain how missing data were addressed
		(d) If applicable, explain how matching of cases and controls was addressed
		(e) Describe any sensitivity analyses
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
Outcome data	15*	Report numbers in each exposure category, or summary measures of exposure
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(b) Report category boundaries when continuous variables were categorized
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

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STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

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Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found
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Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
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		(b) For matched studies, give matching criteria and number of exposed and unexposed
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
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Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding
		(b) Describe any methods used to examine subgroups and interactions
		(c) Explain how missing data were addressed
		(d) If applicable, explain how loss to follow-up was addressed
		(e) Describe any sensitivity analyses
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
		(c) Summarise follow-up time (eg, average and total amount)
Outcome data	15*	Report numbers of outcome events or summary measures over time
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
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Title

Multi-institution Case-control and Cohort Study of Risk Factors for the Development and Mortality of *Clostridium difficile* Infections in Japan

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Abstract

Objective: To examine risk factors for *Clostridium difficile* infection (CDI) morbidity and mortality in Japan.

Design: Multi-method investigation including a case–control study and cohort study.

Setting: Forty-seven participating facilities of the National Hospital Organization (NHO).

Participants: One thousand twenty six CDI patients and 878 patients in control group over the age of 18 years admitted to the subject NHO facilities from November 2010 to October 2011.

Main Outcome Measures: In case-control study, we identify risk factors for CDI development. Next, in cohort study, we identify risk factors for all-cause mortality within 30 days following CDI onset.

Results: A total of 1,026 cases of CDI meeting the definitions of this investigation were identified, encompassing 878 patients at 42 of the 47 subject facilities. In the case–control study, we identified, compared with no antibiotics use, use of first- and second-generation cephem antibiotics (odds ratio[OR], 1.44; 95% confidence interval [CI], 1.10 to 1.87), use of third- and fourth-generation cephem antibiotics(OR, 1.86; 95%CI, 1.48 to 2.33), and use of carbapenem antibiotics (OR, 1.87; 95%CI, 1.44 to 2.42) were risk factors for CDI development. However, use of penicillin was not identified as risk factors. In the cohort study, sufficient data for analysis was available for 924 CDI cases; 102 of them (11.0%) resulted in death within 30 days of CDI onset. Compared with no anti-CDI drug use, use of vancomycin was associated with reduced risk of mortality (OR, 0.43; 95%CI, 0.25 to 0.75) whereas metronidazole was not.

Conclusions: The findings mirror those of previous studies from Europe and North America, identifying the administration of broad-spectrum antibiotics as a risk factor for CDI development. The use of vancomycin is associated with a decreased risk of mortality.

Strengths and limitations of this study

- This study is the first large-scale nationwide multi-center CDI investigation in Japan.
- Most of the epidemiological data of CDI has been limited in the North America and Europe. Our data plays a role of completion of the missing data in Asia.
- Use of β-lactam antibiotics except penicillin was the risk factor for CDI development in the first Japanese large-scale investigation. Appropriate antibiotic use is necessary in order to control the incidence of CDI.
- Vancomycin administration for CDI was associated with decreased risk of mortality. Although the cost-effective treatment of CDI may necessitate the appropriate use of less-expensive metronidazole, vancomycin should be administered in case expected to become severe or life-threatening.
- The limitation of this study is the low number of registered CDI cases from quite a few participants and the existence of many confounding factors.

Introduction

Clostridium difficile is the main causative pathogen of antibiotic-associated colitis. Since 2000, outbreaks of BI/NAP1/027 strain *C. difficile* infections (CDI) have been reported in North American and European hospitals and elder care facilities. The numbers of CDI patients as well as severe and intractable cases have increased simultaneously. Consequently, epidemiological surveillance systems have been set up in several countries. However, very few countries have implemented such national-level measures.

CDI epidemiological studies in Japan to date have been based on scattered data from individual medical facilities. Consequently, the phenomenon of CDI in Japan is not sufficiently understood, including *C. difficile* typing.[1, 2, 3, 4, 5, 6, 7, 8, 9]

Previous studies report that antibiotic administration is the largest risk factor for CDI development. Other risk factors include advanced age and proton pump inhibitor use. [10, 11] CDI mortality rates differ depending on the presence or absence of an outbreak as well as the relevant definitions of epidemiological surveillance. Furthermore, it is especially difficult to objectively determine precise CDI-related mortality rates because of factors such as underlying patient conditions. [12]

This report documents a case–control study of CDI in Japan based on data from the National Hospital Organization (NHO), which is Japan's largest group of hospitals and includes facilities located nationwide. In addition, a cohort investigation of mortality among CDI cases was conducted.

Materials and Methods

Research Design

This multicenter study is a collaborative effort of the 47 facilities that met our facility standards from among the 143 NHO facilities in Japan. The study was planned as a part of the NHO's "National Hospital Organization Multi-Center

Clinical Research for Evidence-Based Medicine" project. This study was conducted with the approval of the Central Ethics Committee of the NHO. The CDI group in this study included in principal all newly diagnosed CDI cases among patients hospitalized from November 1, 2010 to October 31, 2011; cases were registered continuously.

In the case–control study of CDI development, CDI cases newly diagnosed during the investigation period were registered in the CDI group; meanwhile, age-, sex-, and underlying disease-matched patients in the same facilities were registered to the control group. In addition, a prospective cohort study of CDI group patients who died within 30 days of CDI development was conducted. This investigation is a multi-method study using standard case–control and cohort study designs.

Definition of CDI

CDI was defined as the presence of any gastrointestinal symptoms accompanied by a clinical suspicion of CDI as well as a positive result for *C. difficile* toxins from rapid stool testing or *C. difficile* isolation from stool cultures or both. Final determinations were made by the attending physician or the facility's infection control team.

Enzyme immunoassay testing kits for *C. difficile* toxins A and B were used as the rapid testing method (Immunocard CD toxin A&B, Meridian Bioscience Inc., Cincinnati, OH, USA; C. Diff Quik Chek, Alere Medical Co. Ltd., Tokyo, Japan; Tox A/B Quik Chek, Nissui Pharmaceutical Co., Ltd., Tokyo, Japan; X/pect Toxin A/B, Kanto Chemical Co Ltd., Tokyo, Japan). Cycloserine-cefoxitin mannitol agar (Nissuipure-to CCMA baichi EX, Nissui Pharmaceutical Co. Ltd., Tokyo, Japan), cycloserine-cefoxitin fructose agar (CCFA baichi, Becton, Dickinson and Company Co. Ltd., Tokyo, Japan; Poamedhia® CCFA® kairyoubaichi, Eiken Chemical Co., Ltd., Tokyo, Japan), and brucella HK agar (RS) (brucella HK agar (RS), Kyokuto Pharmaceutical Industrial Co. Ltd., Tokyo, Japan) were used in the *C. difficile* isolation cultures.

Case–Control Study of CDI Development

No additional information besides age, sex, and date of diagnosis was gathered when new patients were registered in the

CDI group. After the end of the study registration period, additional patient clinical data were gathered, including clinical department, underlying diseases, dates of hospital admittance and discharge, and medical treatments administered for ≥3 days between admittance and CDI development. Recorded treatments included disruption of feeding, parenteral nutrition, enteral feeding, surgery with general anesthetic, cancer drugs, antibiotics (excluding external-use antibiotics), proton pump inhibitors (oral or intravenous). We also collected data regarding the use of intravenous antibiotics including penicillins, first- and second-generation cephems, third- and fourth-generation cephems, carbapenems, fluoroquinolones, clindamycin/lincomycin, anti–Methicillin-resistant *Staphylococcus aureus* (MRSA) drugs, and anti-fungal drugs, and others. Finally, we collected data regarding the use of oral antibiotics including cephems, fluoroquinolones, and others.

The control group was divided into three subgroups according to age: ≤74, 75–84, and ≥85 years. The control patients were selected from among patients at the same facilities who did not contract CDI and were matched to the CDI patients with respect to age, sex, underlying disease, and hospital stays of ≥5 days within the same month as a counterpart's CDI diagnosis. The control group cases were selected regardless of gastrointestinal symptoms such as diarrhea. We strove to ensure that the CDI and control groups were as matched as possible. The same data were collected from both groups. The control patients were registered, and relevant patient data were gathered after the end of the CDI group study registration period.

Cohort Study on Mortality among CDI Patients

The prospective cohort study of registered CDI group patients from the case–control study examined all-cause mortality within 30 days as the primary outcome. Clinical outcomes of patients who discharged within 30 days of CDI development were not investigated in this study. The following data were collected: whether the underlying disease was infectious and whether comorbidities were related to malignant tumors (i.e., gastrointestinal, respiratory, blood/lymph, gynecologic, urological, or other tumors including cancers of the ear, nose, and throat), diabetes, renal failure, heart failure,

respiratory failure, or cirrhosis. We also considered patient nutritional status including whether the patient was subjected to parenteral nutrition or enteral feeding as well as serum albumin levels measured within 30 days prior to CDI development (i.e., ≥3.5, 2.7–3.4, or ≤2.6 g/dL). In addition, we examined CDI treatment factors including whether antibiotic use was halted, probiotic use, and the type of anti-CDI drugs used (i.e., vancomycin and metronidazole). All patient data for the cohort investigation were collected after the end of the registration period.

Data Management and Statistical Analysis

All input data were verified by a designated study data manager. Data from each facility were entered directly into a web-based case report form and subsequently encrypted for security. The data management center was responsible for confirming any missing data and directly inquiring the relevant facilities as necessary.

During the case–control phase of the study, CDI development was treated as the outcome and odds ratios (ORs) were calculated from bivariate analysis comparing the use of different types of antibiotics as outcome causes. For each type of antibiotic, those used for ≥3 days were designated "used" while all others were designated "unused." A dummy variable regression was subsequently performed. Statistical significance in the bivariate analysis was tested by the chi-square test. Logistic regression analysis was performed using the individual patient characteristics and other assumed confounding variables as independent variables. The 95% confidence intervals (CIs) for each variable were used to determine the relationships between the various predictive variables and outcomes.

In the cohort study, gastrointestinal perforations, toxic megacolon, CDI-related surgeries, and the all-cause in-hospital mortality of patients within 30 days of CDI development were recorded. The clinical outcome of mortality within 30 days was set as the dependent variable, and the relationships among the underlying diseases, nutritional status, probiotic use, and types of anti-CDI drugs used were subjected to bivariate and multivariate analyses. Like the case–control phase, bivariate analysis were conducted using the chi-square test, and the multivariate analysis was conducted using logistic regression.

The significance level for all analyses was set at $p < 0.05$. We used IBM SPSS Statistics version 20 for statistical analysis.

Ethics Committee Approval and Informed Consent

This study was conducted with the approval of the Central Ethics Committee of the NHO. In principle, individual patients who met the inclusion criteria were not given direct explanations of the study, and no direct consent was sought. Information about the study was made public through postings on facility notice boards and webpages. Patients and their representative agents had the right to refuse study participation.

Results

Participating Facilities

Among the 47 facilities, a total of 1,026 CDI cases were registered at 42 facilities throughout Japan, from Hokkaido in the north to Okinawa in the south. No CDI cases were recorded at the remaining 5 participating facilities, more than 280 patient beds (Table 1).

Table 1. Number of registered cases of CDI and characteristics of hospitals included in the surveillance of CDI in the NHO (from november 2010 through october 2011)

Region	No. patient beds	No. patient days	No. patients registered		30-day all-cause mortality in CDI group	Laboratory tests used	
			CDI group	Control group		EIA for toxins A and B	Culture
Hokkaido, tohoku	698	208,388	55	55	3 (5%)	+	+
	500	150,603	42	32	1 (2%)	+	+
	310	82,687	28	19	2 (7%)		+
	310	72,144	17	12	2 (12%)	+	+
	220	76,539	1	1	0 (0%)	+	+
Kanto, koshinetsu	780	238,420	124	121	15 (12%)	+	+
	455	151,622	36	36	3 (8%)	+	
	560	158,921	35	30	4 (11%)	+	+
	243	60,155	34	34	6 (18%)	+	+
	350	109,025	22	22	4 (18%)	+	+
	500	159,432	15	14	1 (7%)	+	
	510	166,668	4	4	0 (0%)	+	
	380	109,482	3	2	0 (0%)	+	+
	455	132,483	3	1	0 (0%)	+	
	429	104,802	0	0	– (–)	+	
Tokai, hokuriku	430	195,209	42	26	10 (24%)	+	+
	280	56,475	0	0	– (–)	+	
Kinki	316	103,677	24	22	1 (4%)	+	
	220	47,354	23	23	1 (4%)	+	+
	600	191,041	20	20	3 (15%)	+	
	494	70,455	15	15	6 (40%)	+	+
	520	145,299	13	9	1 (8%)	+	
	500	142,409	6	6	1 (17%)	+	
	180	55,721	3	3	1 (33%)	+	
	346	118,014	2	2	0 (0%)	+	
	370	94,722	0	0	– (–)	+	
Chugoku, shikoku	388	99,728	54	49	5 (9%)	+	+
	700	211,595	49	48	4 (8%)	+	+
	506	119,356	33	8	1 (3%)	+	+
	400	122,846	30	30	5 (17%)	+	
	401	108,303	26	0	2 (8%)	+	+
	250	80,558	21	21	0 (0%)	+	
	424	128,868	12	10	0 (0%)	+	
	365	125,645	10	10	3 (30%)	+	+
	300	87,061	0	0	– (–)		+
	459	66,454	0	0	– (–)	+	
Kyushu, okinawa	424	137,827	46	22	5 (11%)	+	
	702	239,448	38	37	1 (3%)	+	
	190	54,038	33	31	9 (27%)	+	
	550	189,417	27	26	3 (11%)	+	
	285	58,185	25	25	3 (12%)	+	
	500	140,371	24	23	2 (8%)	+	
	300	90,457	14	14	4 (29%)	+	
	320	103,315	6	5	1 (17%)	+	+
	280	79,580	4	4	2 (50%)	+	
	366	112,906	4	4	0 (0%)	+	
	368	89,195	3	2	2 (67%)	+	
Total	19,486	5,592,077	1,026	878	117 (11%)	45	20

Patient Grouping

A total of 1,026 CDI cases that met the study definitions were recorded at the various institutions. We were unable to collect clinical records regarding medical treatments for 1 case; therefore, this case was excluded from the case–control study, and the remaining 1,025 cases were analyzed. A total of 962 patients (93.9%) developed CDI within 48 hours after hospital admittance. The control group comprised 878 patients who were selected from 41 of the 42 facilities. In the cohort study, we analyzed the data from 924 of the 1,025 CDI group patients, excluding 101 patients with no available recent serum albumin level data (i.e., within 30 days prior to CDI development (Figure 1).

Case–Control Study of CDI Development

The mean ages of the CDI and control groups were 75.8 and 75.4 years, respectively. The majority of the subjects were of advanced age: 64.0% and 62.5% of the CDI and control group patients were aged ≥75 years, respectively. No significant differences were identified between the CDI and control groups in the univariate analysis of age distribution, sex differences, or underlying disease (Table 2). Among the medical treatments administered before CDI development, the following were significantly more prevalent in the CDI group than the control group: disruption of feeding (48.6% vs. 30.4%), parenteral nutrition (24.7% vs. 10.3%), and enteral feeding (24.8% vs. 9.1%). Antibiotics were used prior to CDI development in 85.8% of cases. The use of all types of intravenous antibiotics was significantly more prevalent in the CDI group. No significant differences were identified between the 2 groups with respect to oral antibiotic use. Meanwhile, in the univariate analysis, proton pump inhibitor use was significantly more prevalent in the CDI group than the control group (40.3% vs. 31.2%).

We used logistic regression analysis to determine the risk factors for CDI development. The following medical treatments prior to CDI development were identified as significant risk factors in comparison to the control group: disruption of feeding (odds ratio[OR], 1.31; 95% confidence interval[CI], 1.05 to 1.64), parenteral nutrition (OR, 1.63; 95%CI, 1.21 to

2.20) and enteral feeding (OR, 2.16; 95%CI, 1.60 to 2.92).The following intravenous antibiotics were also identified as statistically significant risk factors for CDI development: first- and second-generation cephems (OR, 1.44; 95%CI, 1.10 to 1.87), third- and fourth-generation cephems (OR, 1.86; 95%CI, 1.48 to 2.33), and carbapenems (OR, 1.87; 95%CI, 1.44 to 2.42). However, penicillin (OR, 1.04; 95%CI, 0.82 to 1.33), fluoroquinolones (OR, 1.16; 95%CI, 0.74 to 1.83), clindamycin/lincomycin (OR, 1.35; 95%CI, 0.81 to 2.26), and proton pump inhibitor use (OR, 1.17; 95%CI, 0.95 to 1.44) were not identified as risk factors.

Table 2. Univariate and multivariate analyses of CDI development-related risk factors

Characteristics	CDI group %	Control group %	Univariate analysis P value	Multivariate analysis Odds ratio (95% CI)	Multivariate analysis P value
All	(1,025)	(878)	—	—	—
Age					
≤74 years	36.0 (369)	37.5 (329)	0.67	Ref.	—
75–84 years	37.0 (379)	37.2 (327)		1.02 (0.81 to 1.28)	0.88
≥85 years	27.0 (277)	25.3 (222)		1.09 (0.84 to 1.41)	0.52
Sex					
Women	43.0 (441)	42.6 (374)	0.85	1.11 (0.91 to 1.36)	0.28
Underlying disease					
Respiratory infections	15.8 (162)	17.5 (154)	0.14	—	—
Other infectious conditions	16.9 (173)	14.2 (125)		—	—
Gastrointestinal conditions	8.1 (83)	9.0 (79)		—	—
Malignant tumors	22.6 (232)	24.3 (213)		—	—
Cardiovascular conditions	7.7 (79)	9.8 (86)		—	—
Other conditions	28.9 (296)	25.2 (221)		—	—
Medical treatment prior to CDIdevelopment					
Disruption of feeding	48.6 (498)	30.4 (267)	<0.001	1.31 (1.05 to 1.64)	<0.05
Parenteral nutrition	24.7 (253)	10.3 (90)	<0.001	1.63 (1.21 to 2.20)	<0.01
Enteral feeding	24.8 (254)	9.1 (80)	<0.001	2.16 (1.60 to 2.92)	<0.001
Surgery with general anesthetic	18.2 (187)	15.6 (137)	0.14	0.89 (0.67 to 1.18)	0.41
Cancer drugs	11.3 (116)	14.2 (125)	0.06	0.86 (0.62 to 1.18)	0.35
Antibiotics use	85.8 (879)	66.5 (584)	<0.001	—	—
Intravenous					
Penicillins	27.6 (283)	21.0 (184)	<0.01	1.04 (0.82 to 1.33)	0.75
First/second-generation cephems	22.7 (233)	15.6 (137)	<0.001	1.44 (1.10 to 1.87)	<0.01
Third/fourth-generation cephems	35.2 (361)	19.9 (175)	<0.001	1.86 (1.48 to 2.33)	<0.001
Carbapenems	31.8 (326)	15.0 (132)	<0.001	1.87 (1.44 to 2.42)	<0.001
fluoroquinolones	7.5 (77)	4.0 (35)	<0.01	1.16 (0.74 to 1.83)	0.52
Clindamycin/lincomycin	6.5 (67)	2.8 (25)	<0.001	1.35 (0.81 to 2.26)	0.25
MRSA drugs	10.7 (110)	4.3 (38)	<0.001	1.10 (0.71 to 1.72)	0.66
Anti-fungal drugs	6.9 (71)	3.2 (28)	<0.001	1.01 (0.60 to 1.70)	0.96
Others(aminoglycosides, monobactam,etc.)	8.5 (87)	5.9 (52)	<0.05	1.19 (0.80 to 1.77)	0.39
Oral					
Cephems	5.6 (57)	4.4 (39)	0.29	1.49 (0.95 to 2.32)	0.08
fluoroquinolones	14.5 (149)	11.5 (101)	0.06	1.11 (0.82 to 1.51)	0.49
Others (macrolides, penicillins, etc.)	14.0 (144)	13.9 (122)	0.95	0.84 (0.63 to 1.13)	0.26
Proton pump inhibitors	40.3 (413)	31.2 (274)	<0.001	1.17 (0.95 to 1.44)	0.14

Cohort Study on Mortality among Patients with CDI

The cohort study examined mortality among the 924 patients from the 1,025 CDI group patients in the case–control study for whom serum albumin level data before CDI development were available.

Among the 924 patients, 102 (11.0%) died within 30 days of developing CDI. Among those cases, the cause of death was attributed to CDI in 11 cases (1.2%). Of 11 patients, a patient had gastrointestinal perforation, another patient had CDI-related surgery, and the others were not reported as severe complications. The toxic megacolon was reported in 2 patients however, they were not died within 30 days of CDI development. The mean age of the 102 patients who died during the study was 80.1 ± 8.3 years. Patients ≥75 years old were especially prevalent in this subgroup, accounting for 77.5% (79/102) of the cases.

Among the 714 cases in which CDI was treated directly, recurrence within 30 days was observed in 34 cases (4.8%).

The univariate analysis indicated that comorbidities of heart and respiratory failure were significantly more prevalent among CDI patients. In addition, lower serum albumin levels were significantly associated with mortality. Among CDI treatments, mortality was significantly lower among cases in which probiotics were administered.

A logistic regression analysis of the 102 cases in which the patients died within 30 days of CDI development was performed to identify the factors associated with the risk of mortality. Compared to patients ≤74 years old, the odds ratio of mortality among patients aged 75–84 years was 2.08 (95%CI, 1.19 to 3.62). Among underlying diseases, heart failure (OR, 2.12; 95%CI, 1.26 to 3.55) and respiratory failure (OR, 1.98; 95%CI, 1.19 to 3.32) were identified as risk factors for mortality within 30 days of CDI development. Regarding nutritional status, neither parenteral nutrition nor enteral nutrition was identified as a risk factor for mortality. However, low serum albumin level (i.e., ≤2.6 g/dL) was identified as a significant risk factor for mortality (OR, 3.50; 95%CI, 1.33 to 9.22). Among CDI treatments, probiotic use (OR, 0.66; 95%CI, 0.42 to 1.04) was not identified as a risk factor for mortality. However, compared to cases in which no anti-CDI drugs were administered, vancomycin administration yielded an odds ratio of 0.43 (95%CI, 0.25 to 0.75), indicating a

significantly lowered risk of mortality in the CDI group. Meanwhile, no such lowered mortality was observed in cases treated with metronidazole (OR, 0.85; 95%CI, 0.48 to 1.51).

Table 3. Univariate and multivariate analyses of all-cause mortality in CDI patients

Characteristics	All-cause mortality rate %	Univariate analysis P value	Multivariate analysis Odds ratio (95% CI)	Multivariate analysis P value
All	11.0 (102/924)	—	—	—
Age				
≤74 years	7.1 (23/326)		Ref.	
75–84 years	13.3 (47/353)	<0.05	2.08 (1.19 to 3.62)	<0.05
≥85 years	13.1 (32/245)		1.86 (0.98 to 3.55)	0.06
Sex				
Men	12.2 (64/524)		Ref.	
Women	9.5 (38/400)	0.21	0.78 (0.49 to 1.24)	0.29
Underlying disease				
Non-infectious	10.3 (64/619)		Ref.	
Infectious	12.5 (38/305)	0.37	0.99 (0.60 to 1.62)	0.97
Comorbidities				
Malignant tumors				
Not present	10.6 (67/630)		Ref.	
Present	11.9 (35/294)	0.57	1.54 (0.94 to 2.53)	0.09
Diabetes				
Not present	11.6 (89/765)		Ref.	
Present	8.2 (13/159)	0.27	0.71 (0.37 to 1.35)	0.29
Renal failure				
Not present	10.7 (84/784)		Ref.	
Present	12.9 (18/140)	0.46	0.90 (0.49 to 1.65)	0.73
Heart failure				
Not present	9.3 (70/756)		Ref.	
Present	19.0 (32/168)	<0.01	2.12 (1.26 to 3.55)	<0.01
Respiratory failure				
Not present	9.2 (69/754)		Ref.	
Present	19.4 (33/170)	<0.001	1.98 (1.19 to 3.32)	<0.01
Cirrhosis				
Not present	11.2 (100/895)		Ref.	
Present	6.9 (2/29)	0.76	0.61 (0.13 to 2.83)	0.53
Indicators of nutritional status				
Parenteral nutrition or enteral feeding				
Not present	9.4 (53/563)		Ref.	
Present	13.6 (49/361)	0.05	1.16 (0.73 to 1.84)	0.53
Serum albumin (g/dL)				
≥3.5	4.0 (5/124)		Ref.	
2.7–3.4	7.2 (27/376)	<0.001	1.55 (0.57 to 4.21)	0.39
≤2.6	16.5 (70/424)		3.50 (1.33 to 9.22)	<0.05
CDI treatments				
Cessation of antibiotics				
Not present	12.5 (65/519)		Ref.	
Present	9.1 (37/405)	0.11	0.77 (0.48 to 1.22)	0.26
Probiotics (for intestine treatment)				
Not present	13.8 (52/378)		Ref.	
Present	9.2 (50/546)	<0.05	0.66 (0.42 to 1.04)	0.08
Anti-CDI drugs				
Not present	15.2 (32/210)		Ref.	
Vancomycin alone	7.4 (32/433)	<0.05	0.43 (0.25 to 0.75)	<0.01
Metronidazole alone	13.5 (32/237)		0.85 (0.48 to 1.51)	0.59
Vancomycin and metronidazole	13.6 (6/44)		0.75 (0.27 to 2.08)	0.57

Discussion

This is the first large-scale clinical study of CDI in Japan. This study examined 1,026 cases of CDI recorded over 1 year at the nationwide facilities of Japan's largest hospital group. The findings of this investigation are similar to those reported in previous studies conducted in Europe, North America, and Australia with respect to the identification of several risk factors for CDI development, including age, severity of the underlying condition, artificial feeding and mortality. Antibiotic use is a known risk factor for CDI development. [15] The present case–control study confirms that intravenous cephems and carbapenems are important risk factors. Some studies report a low risk of CDI development owing to intravenous penicillin administration. [16, 17] Concordantly, penicillin use was not identified as a risk factor in the present study. The proton pump inhibitor use was discussed as a risk factor for CDI development in the previous studies..[18, 19, 20] In the present logistic regression analysis, it was not identified as a risk factor.

In this study, 11.0 % of CDI patients died within 30days. In comparison, higher 30-day mortality rates have been reported in previous outbreaks: 24.8% in the ribotype 027 strain outbreak in Canada, and 36.7% in an examination of a single intensive care unit in the USA. [21, 22] However, reports of non-outbreak conditions indicate mortality rates of 13%, similar to the findings of the present study. [23] Some reports state that the CDI-associated mortality rate has increased 2.5 fold, possibly indicating that CDI cases are more severe and contribute more significantly to mortality than previously thought. [12, 23] The mortality rate of CDI patients is reported to increase with age. [24] Concordantly, the present study also found a significantly elevated risk of death in patients ≥75 years old.

The findings of this study indicate that the mortality risk of CDI patients was not reduced as a result of metronidazole treatment but was reduced with vancomycin treatment, corroborating the existing recommendation. [25] It is worth noting that metronidazole is less expensive than vancomycin, making it economically advantageous. a patient's condition must be carefully evaluated when selecting anti-CDI drugs. In particular, for patients in the present study who had conditions associated with a greater mortality risk, including advanced age (i.e., ≥75 years), heart or respiratory failure, or malnutrition

as determined by low serum albumin levels, the use of vancomycin rather than metronidazole for treatment appears to have provided better outcomes. The recurrence rate was low (4.8%) in this study compared to the previous studies. [11, 26] We did not investigate the patients neither after 30 days of CDI development nor the patients who discharge even if within 30 days of CDI development. Therefore, the recurrence rate might be underestimated.

Regardless, this study has also several methodological limitations. The most salient limitation is the low number of registered CDI cases from quite a few participants. In the definition of CDI, the times of diarrhea were not investigated. Another limitation of the case–control study phase is the existence of many confounding factors. In particular, probiotic use, which was recently discussed to be correlated with CDI prevention, was not included in the predictive model of this study. [10, 11, 27] When interpreting the findings of this study, it is necessary to consider the influence of confounding factors that were not included in the analytical models. Regarding antibiotic use, the present analyses included independent explanatory variables for each antibiotic. However, actual antibiotic use is more complicated. Therefore, it is difficult to clearly determine the roles of individual antibiotics as risk factors for CDI development. Concerning matching process, we tried to adopt 1 to 1 pair sampling matched with sex, age group and main diagnosis. Some hospital could not find appropriate control sample well matched with case sample. So total number of the control group was less than that of the case sample. In addition, although data for the control group were analyzed during the entire study period until hospital discharge, only data from the period prior to CDI development were analyzed in the CDI group. Therefore, the risks might be underestimated, because the control group had a longer period of exposure risk than the CDI group. Confounding factors that were not included in the present analyses also represent a limitation of the cohort study phase. Furthermore, issues of data quality among the facilities affect all aspects of this study. More than 40 different facilities participated in this study. While some facilities registered nearly all of their CDI patients, other facilities registered smaller proportions of patients. Only C. *difficile* culture but not toxin test was used for the laboratory test in two facilities.

Finally, there might have been differences with regard to individual researchers' understanding of the outcome definitions.

In order to ensure appropriate antibiotic use and control the incidence of CDI, it is important to create institutional measures such as infection control teams The cost-effective treatment of CDI may necessitate the appropriate use of less-expensive metronidazole. However, in cases expected to become severe or life-threatening, the more expensive drug vancomycin should be administered. CDI is one of many issues concerning medicine and medical treatment costs. Accordingly, further and more proactive research into CDI epidemiology is needed.

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Contributors

MT conceived the idea for the study, designed the study, developed the protocol, was responsible for study management and data collection, interpreted the findings, and drafted the paper. NM contributed to data analysis and interpretation of findings and drafted the paper. SB designed this study, developed the protocol, performed data analysis, and interpreted findings. and drafted the paper. All authors read and approved the final manuscript.

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Competing interests

None.

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Data sharing statement

No additional data are available.

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Title

Multi-institution Case-control and Cohort Study of Risk Factors for the Development and Mortality of *Clostridium difficile* Infections in Japan

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Abstract

Objective: To examine risk factors for *Clostridium difficile* infection (CDI) morbidity and mortality in Japan.

Design: Multi-method investigation including a case–control study and cohort study.

Setting: Forty-seven participating facilities of the National Hospital Organization (NHO).

Participants: One thousand twenty six CDI patients and 878 patients in control group over the age of 18 years admitted to the subject NHO facilities from November 2010 to October 2011.

Main Outcome Measures: In case-control study, we identify risk factors for CDI development. Next, in cohort study, we identify risk factors for all-cause mortality within 30 days following CDI onset.

Results: A total of 1,026 cases of CDI meeting the definitions of this investigation were identified, encompassing 878 patients at 42 of the 47 subject facilities. In the case–control study, we identified, compared with no antibiotics use, use of first- and second-generation cephem antibiotics (odds ratio[OR], 1.44; 95% confidence interval [CI], 1.10 to 1.87), use of third- and fourth-generation cephem antibiotics(OR, 1.86; 95%CI, 1.48 to 2.33), and use of carbapenem antibiotics (OR, 1.87; 95%CI, 1.44 to 2.42) were risk factors for CDI development. However, use of penicillin was not identified as risk factors. In the cohort study, sufficient data for analysis was available for 924 CDI cases; 102 of them (11.0%) resulted in death within 30 days of CDI onset. Compared with no anti-CDI drug use, use of vancomycin was associated with reduced risk of mortality (OR, 0.43; 95%CI, 0.25 to 0.75) whereas metronidazole was not.

Conclusions: The findings mirror those of previous studies from Europe and North America, identifying the administration of broad-spectrum antibiotics as a risk factor for CDI development. The use of vancomycin is associated with a decreased risk of mortality.

Strengths and limitations of this study

- This study is the first large-scale nationwide multi-center CDI investigation in Japan.
- Most of the epidemiological data of CDI has been limited in the North America and Europe. Our data plays a role of completion of the missing data in Asia.
- Use of β-lactam antibiotics except penicillin was the risk factor for CDI development in the first Japanese large-scale investigation. Appropriate antibiotic use is necessary in order to control the incidence of CDI.
- Vancomycin administration for CDI was associated with decreased risk of mortality. Although the cost-effective treatment of CDI may necessitate the appropriate use of less-expensive metronidazole, vancomycin should be administered in case expected to become severe or life-threatening.
- ~~The most salient limitation of the case–control study phase is the existence of many confounding factors. In particular, probiotic use, which was recently shown to be correlated with CDI prevention, was not included in the predictive model of this study.~~
- The limitation of this study is the low number of registered CDI cases from quite a few participants and the existence of many confounding factors.

Introduction

Clostridium difficile is the main causative pathogen of antibiotic-associated colitis. Since 2000, outbreaks of BI/NAP1/027 strain *C. difficile* infections (CDI) have been reported in North American and European hospitals and elder care facilities. The numbers of CDI patients as well as severe and intractable cases have increased simultaneously. Consequently, epidemiological surveillance systems have been set up in several countries. However, very few countries have implemented such national-level measures.

~~In Japan, the Ministry of Health, Welfare, and Labor's Japan Nosocomial Infection Surveillance program investigates the incidence rates of a variety of drug-resistant bacteria; however, this program does not monitor the incidence rate of *C. difficile* (http://www.nih-janis.jp/index.asp). Therefore,~~ CDI epidemiological studies in Japan to date have been based on scattered data from individual medical facilities. Consequently, the phenomenon of CDI in Japan is not sufficiently understood, including *C. difficile* typing.[1, 2, 3, 4, 5, 6, 7, 8, 9] ~~Reports of BI/NAP1/027 infections are limited, and conditions in Japan possibly differ from those in Europe and North America.~~

Previous studies report that antibiotic administration is the largest risk factor for CDI development. Other risk factors include advanced age and proton pump inhibitor use. ~~[1, 2]~~[10, 11] CDI mortality rates differ depending on the presence or absence of an outbreak as well as the relevant definitions of epidemiological surveillance. Furthermore, it is especially difficult to objectively determine precise CDI-related mortality rates because of factors such as underlying patient conditions. ~~[3]~~[12]

This report documents a case–control study of CDI in Japan based on data from the National Hospital Organization (NHO), which is Japan's largest group of hospitals and includes facilities located nationwide. In addition, a cohort investigation of mortality among CDI cases was conducted.

Materials and Methods

Research Design

This multicenter study is a collaborative effort of the 47 facilities that met our facility standards from among the 143 NHO facilities in Japan. The study was planned as a part of the NHO's "National Hospital Organization Multi-Center Clinical Research for Evidence-Based Medicine" project. This study was conducted with the approval of the Central Ethics Committee of the NHO. The CDI group in this study included in principal all newly diagnosed CDI cases among patients hospitalized from November 1, 2010 to October 31, 2011; cases were registered continuously.

In the case–control study of CDI development, CDI cases newly diagnosed during the investigation period were registered in the CDI group; meanwhile, age-, sex-, and underlying disease-matched patients in the same facilities were registered to the control group. In addition, a prospective cohort study of CDI group patients who died within 30 days of CDI development was conducted. This investigation is a multi-method study using standard case–control and cohort study designs.

Definition of CDI

CDI was defined as the presence of any gastrointestinal symptoms accompanied by a clinical suspicion of CDI as well as a positive result for *C. difficile* toxins from rapid stool testing or *C. difficile* isolation from stool cultures or both. Final determinations were made by the attending physician or the facility's infection control team.

Enzyme immunoassay testing kits for *C. difficile* toxins A and B were used as the rapid testing method (Immunocard CD toxin A&B, Meridian Bioscience Inc., Cincinnati, OH, USA; C. Diff Quik Chek, Alere Medical Co. Ltd., Tokyo, Japan; Tox A/B Quik Chek, Nissui Pharmaceutical Co., Ltd., Tokyo, Japan; X/pect Toxin A/B, Kanto Chemical Co Ltd., Tokyo, Japan). Cycloserine-cefoxitin mannitol agar (Nissuipure-to CCMA baichi EX, Nissui Pharmaceutical Co. Ltd., Tokyo, Japan), cycloserine-cefoxitin fructose agar (CCFA baichi, Becton, Dickinson and Company Co. Ltd., Tokyo, Japan; Poamedhia® CCFA® kairyoubaichi, Eiken Chemical Co., Ltd., Tokyo, Japan), and brucella HK agar (RS) (brucella HK agar (RS),

Kyokuto Pharmaceutical Industrial Co. Ltd., Tokyo, Japan) were used in the *C. difficile* isolation cultures.

Case–Control Study of CDI Development

No additional information besides age, sex, and date of diagnosis was gathered when new patients were registered in the CDI group. After the end of the study registration period, additional patient clinical data were gathered, including clinical department, underlying diseases, dates of hospital admittance and discharge, and medical treatments administered for ≥3 days between admittance and CDI development. Recorded treatments included disruption of feeding, parenteral nutrition, enteral feeding, surgery with general anesthetic, cancer drugs, antibiotics (excluding external-use antibiotics), proton pump inhibitors (oral or intravenous). We also collected data regarding the use of intravenous antibiotics including penicillins, first- and second-generation cephems, third- and fourth-generation cephems, carbapenems, fluoroquinolones, clindamycin/lincomycin, anti–Methicillin-resistant *Staphylococcus aureus* (MRSA) drugs, and anti-fungal drugs, and others. Finally, we collected data regarding the use of oral antibiotics including cephems, fluoroquinolones, and others.

The control group was divided into three subgroups according to age: ≤74, 75–84, and ≥85 years. The control patients were selected from among patients at the same facilities who did not contract CDI and were matched to the CDI patients with respect to age, sex, underlying disease, and hospital stays of ≥5 days within the same month as a counterpart's CDI diagnosis. The control group cases were selected regardless of gastrointestinal symptoms such as diarrhea. We strove to ensure that the CDI and control groups were as matched as possible. The same data were collected from both groups. The control patients were registered, and relevant patient data were gathered after the end of the CDI group study registration period.

Cohort Study on Mortality among CDI Patients

The prospective cohort study of registered CDI group patients from the case–control study examined all-cause mortality within 30 days as the primary outcome. Clinical outcomes of patients who discharged within 30 days of CDI

development were not investigated in this study. The following data were collected: whether the underlying disease was infectious and whether comorbidities were related to malignant tumors (i.e., gastrointestinal, respiratory, blood/lymph, gynecologic, urological, or other tumors including cancers of the ear, nose, and throat), diabetes, renal failure, heart failure, respiratory failure, or cirrhosis. We also considered patient nutritional status including whether the patient was subjected to parenteral nutrition or enteral feeding as well as serum albumin levels measured within 30 days prior to CDI development (i.e., ≥3.5, 2.7–3.4, or ≤2.6 g/dL). In addition, we examined CDI treatment factors including whether antibiotic use was halted, probiotic use, and the type of anti-CDI drugs used (i.e., vancomycin and metronidazole). All patient data for the cohort investigation were collected after the end of the registration period.

Data Management and Statistical Analysis

~~The study coordinator established independent data management centers within the NHO facilities for data collection.~~ All input data were verified by a designated study data manager. Data from each facility were entered directly into a web-based case report form and subsequently encrypted for security. The data management center was responsible for confirming any missing data and directly inquiring the relevant facilities as necessary. ~~After the end of the study period, the data were finalized and subsequently transferred to the Research Coordinator's office.~~

During the case–control phase of the study, CDI development was treated as the outcome and odds ratios (ORs) were calculated from bivariate analysis comparing the use of different types of antibiotics as outcome causes. For each type of antibiotic, those used for ≥3 days were designated "used" while all others were designated "unused." A dummy variable regression was subsequently performed. Statistical significance in the bivariate analysis was tested by the chi-square test. Logistic regression analysis was performed using the individual patient characteristics and other assumed confounding variables as independent variables. The 95% confidence intervals (CIs) for each variable were used to determine the relationships between the various predictive variables and outcomes.

In the cohort study, gastrointestinal perforations, toxic megacolon, CDI-related surgeries, and the all-cause in-hospital mortality of patients within 30 days of CDI development were recorded. The clinical outcome of mortality within 30 days was set as the dependent variable, and the relationships among the underlying diseases, nutritional status, probiotic use, and types of anti-CDI drugs used were subjected to bivariate and multivariate analyses. Like the case–control phase, bivariate analysis were conducted using the chi-square test, and the multivariate analysis was conducted using logistic regression. The significance level for all analyses was set at $p < 0.05$. We used IBM SPSS Statistics version 20 for statistical analysis.

Ethics Committee Approval and Informed Consent

This study was conducted with the approval of the Central Ethics Committee of the NHO. In principle, individual patients who met the inclusion criteria were not given direct explanations of the study, and no direct consent was sought. Information about the study was made public through postings on facility notice boards and webpages. Patients and their representative agents had the right to refuse study participation.

Results

Participating Facilities

Among the 47 facilities, a total of 1,026 CDI cases were registered at 42 facilities throughout Japan, from Hokkaido in the north to Okinawa in the south. No CDI cases were recorded at the remaining 5 participating facilities, more than 280 patient beds (Table 1). ~~The regional locations of the 47 facilities were as follows: 5 in Hokkaido and Tohoku, 10 in Kanto and Koshinetsu, 2 in Tokai and Hokuriku, 9 in Kinki, 10 in Chugoku and Shikoku, and 11 in Kyushu and Okinawa.~~

Table 1. Number of registered cases of CDI and characteristics of hospitals included in the surveillance of CDI in the NHO (from november 2010 through october 2011)

Region	No. patient beds	No. patient days	No. patients registered		30-day all-cause mortality in CDI group	~~Bacteriological survey~~ Laboratory tests used	
			CDI group	Control group		~~EIA detection: toxins A and B~~ EIA for toxins A and B	Culture
Hokkaido, tohoku	698	208,388	55	55	3 (5%)	+	+
	500	150,603	42	32	1 (2%)	+	+
	310	82,687	28	19	2 (7%)		+
	310	72,144	17	12	2 (12%)	+	+
	220	76,539	1	1	0 (0%)	+	+
Kanto, koshinetsu	780	238,420	124	121	15 (12%)	+	+
	455	151,622	36	36	3 (8%)	+	
	560	158,921	35	30	4 (11%)	+	+
	243	60,155	34	34	6 (18%)	+	+
	350	109,025	22	22	4 (18%)	+	+
	500	159,432	15	14	1 (7%)	+	
	510	166,668	4	4	0 (0%)	+	
	380	109,482	3	2	0 (0%)	+	+
	455	132,483	3	1	0 (0%)	+	
	429	104,802	0	0	– (–)	+	
Tokai, hokuriku	430	195,209	42	26	10 (24%)	+	+
	280	56,475	0	0	– (–)	+	
Kinki	316	103,677	24	22	1 (4%)	+	
	220	47,354	23	23	1 (4%)	+	+
	600	191,041	20	20	3 (15%)	+	
	494	70,455	15	15	6 (40%)	+	+
	520	145,299	13	9	1 (8%)	+	
	500	142,409	6	6	1 (17%)	+	
	180	55,721	3	3	1 (33%)	+	
	346	118,014	2	2	0 (0%)	+	
	370	94,722	0	0	– (–)	+	
Chugoku, shikoku	388	99,728	54	49	5 (9%)	+	+
	700	211,595	49	48	4 (8%)	+	+
	506	119,356	33	8	1 (3%)	+	+
	400	122,846	30	30	5 (17%)	+	
	401	108,303	26	0	2 (8%)	+	+
	250	80,558	21	21	0 (0%)	+	
	424	128,868	12	10	0 (0%)	+	
	365	125,645	10	10	3 (30%)	+	+
	300	87,061	0	0	– (–)		+
	459	66,454	0	0	– (–)	+	
Kyushu, okinawa	424	137,827	46	22	5 (11%)	+	
	702	239,448	38	37	1 (3%)	+	
	190	54,038	33	31	9 (27%)	+	
	550	189,417	27	26	3 (11%)	+	
	285	58,185	25	25	3 (12%)	+	
	500	140,371	24	23	2 (8%)	+	
	300	90,457	14	14	4 (29%)	+	
	320	103,315	6	5	1 (17%)	+	+
	280	79,580	4	4	2 (50%)	+	
	366	112,906	4	4	0 (0%)	+	
	368	89,195	3	2	2 (67%)	+	
Total	19,486	5,592,077	1,026	878	117 (11%)	45	20

Patient Grouping

A total of 1,026 CDI cases that met the study definitions were recorded at the various institutions. We were unable to collect clinical records regarding medical treatments for 1 case; therefore, this case was excluded from the case–control study, and the remaining 1,025 cases were analyzed. A total of 962 patients (93.9%) developed CDI within 48 hours after hospital admittance. The control group comprised 878 patients who were selected from 41 of the 42 facilities. In the cohort study, we analyzed the data from 924 of the 1,025 CDI group patients, excluding 101 patients with no available recent serum albumin level data (i.e., within 30 days prior to CDI development (Figure 1).

Case–Control Study of CDI Development

The mean ages of the CDI and control groups were 75.8 and 75.4 years, respectively. The majority of the subjects were of advanced age: 64.0% and 62.5% of the CDI and control group patients were aged ≥75 years, respectively. No significant differences were identified between the CDI and control groups in the univariate analysis of age distribution, sex differences, or underlying disease (Table 2). Among the medical treatments administered before CDI development, the following were significantly more prevalent in the CDI group than the control group: disruption of feeding (48.6% vs. 30.4%), parenteral nutrition (24.7% vs. 10.3%), and enteral feeding (24.8% vs. 9.1%). Antibiotics were used prior to CDI development in 85.8% of cases. The use of all types of intravenous antibiotics was significantly more prevalent in the CDI group. No significant differences were identified between the 2 groups with respect to oral antibiotic use. Meanwhile, in the univariate analysis, proton pump inhibitor use was significantly more prevalent in the CDI group than the control group (40.3% vs. 31.2%).

We used logistic regression analysis to determine the risk factors for CDI development. The following medical treatments prior to CDI development were identified as significant risk factors in comparison to the control group: disruption of feeding (odds ratio[OR], 1.31; 95% confidence interval[CI], 1.05 to 1.64), parenteral nutrition (OR, 1.63; 95%CI, 1.21 to

2.20) and enteral feeding (OR, 2.16; 95%CI, 1.60 to 2.92).The following intravenous antibiotics were also identified as statistically significant risk factors for CDI development: first- and second-generation cephems (OR, 1.44; 95%CI, 1.10 to 1.87), third- and fourth-generation cephems (OR, 1.86; 95%CI, 1.48 to 2.33), and carbapenems (OR, 1.87; 95%CI, 1.44 to 2.42). However, penicillin (OR, 1.04; 95%CI, 0.82 to 1.33), fluoroquinolones (OR, 1.16; 95%CI, 0.74 to 1.83), clindamycin/lincomycin (OR, 1.35; 95%CI, 0.81 to 2.26), and proton pump inhibitor use (OR, 1.17; 95%CI, 0.95 to 1.44) were not identified as risk factors.

Table 2. Univariate and multivariate analyses of CDI development-related risk factors

Characteristics	CDI group %	Control group %	Univariate analysis P value	Multivariate analysis Odds ratio (95% CI)	Multivariate analysis P value
All	(1,025)	(878)	—	—	—
Age					
≤74 years	36.0 (369)	37.5 (329)	0.67	Ref.	—
75–84 years	37.0 (379)	37.2 (327)		1.02 (0.81 to 1.28)	0.88
≥85 years	27.0 (277)	25.3 (222)		1.09 (0.84 to 1.41)	0.52
Sex					
Women	43.0 (441)	42.6 (374)	0.85	1.11 (0.91 to 1.36)	0.28
Underlying disease					
Respiratory infections	15.8 (162)	17.5 (154)	0.14	—	—
Other infectious conditions	16.9 (173)	14.2 (125)		—	—
Gastrointestinal conditions	8.1 (83)	9.0 (79)		—	—
Malignant tumors	22.6 (232)	24.3 (213)		—	—
Cardiovascular conditions	7.7 (79)	9.8 (86)		—	—
Other conditions	28.9 (296)	25.2 (221)		—	—
Medical treatment prior to CDIdevelopment					
Disruption of feeding	48.6 (498)	30.4 (267)	<0.001	1.31 (1.05 to 1.64)	<0.05
Parenteral nutrition	24.7 (253)	10.3 (90)	<0.001	1.63 (1.21 to 2.20)	<0.01
Enteral feeding	24.8 (254)	9.1 (80)	<0.001	2.16 (1.60 to 2.92)	<0.001
Surgery with general anesthetic	18.2 (187)	15.6 (137)	0.14	0.89 (0.67 to 1.18)	0.41
Cancer drugs	11.3 (116)	14.2 (125)	0.06	0.86 (0.62 to 1.18)	0.35
Antibiotics use	85.8 (879)	66.5 (584)	<0.001	—	—
Intravenous					
Penicillins	27.6 (283)	21.0 (184)	<0.01	1.04 (0.82 to 1.33)	0.75
First/second-generation cephems	22.7 (233)	15.6 (137)	<0.001	1.44 (1.10 to 1.87)	<0.01
Third/fourth-generation cephems	35.2 (361)	19.9 (175)	<0.001	1.86 (1.48 to 2.33)	<0.001
Carbapenems	31.8 (326)	15.0 (132)	<0.001	1.87 (1.44 to 2.42)	<0.001
fluoroquinolones	7.5 (77)	4.0 (35)	<0.01	1.16 (0.74 to 1.83)	0.52
Clindamycin/lincomycin	6.5 (67)	2.8 (25)	<0.001	1.35 (0.81 to 2.26)	0.25
MRSA drugs	10.7 (110)	4.3 (38)	<0.001	1.10 (0.71 to 1.72)	0.66
Anti-fungal drugs	6.9 (71)	3.2 (28)	<0.001	1.01 (0.60 to 1.70)	0.96
Others(aminoglycosides, monobactam,etc.)	8.5 (87)	5.9 (52)	<0.05	1.19 (0.80 to 1.77)	0.39
Oral					
Cephems	5.6 (57)	4.4 (39)	0.29	1.49 (0.95 to 2.32)	0.08
fluoroquinolones	14.5 (149)	11.5 (101)	0.06	1.11 (0.82 to 1.51)	0.49
Others (macrolides, penicillins, etc.)	14.0 (144)	13.9 (122)	0.95	0.84 (0.63 to 1.13)	0.26
Proton pump inhibitors	40.3 (413)	31.2 (274)	<0.001	1.17 (0.95 to 1.44)	0.14

Cohort Study on Mortality among Patients with CDI

The cohort study examined mortality among the 924 patients from the 1,025 CDI group patients in the case–control study for whom serum albumin level data before CDI development were available.

Among the 924 patients, 102 (11.0%) died within 30 days of developing CDI. Among those cases, the cause of death was attributed to CDI in 11 cases (1.2%). Of 11 patients, a patient had gastrointestinal perforation, another patient had CDI-related surgery, and the others were not reported as severe complications. The toxic megacolon was reported in 2 patients however, they were not died within 30 days of CDI development. The mean age of the 102 patients who died during the study was 80.1 ± 8.3 years. Patients ≥75 years old were especially prevalent in this subgroup, accounting for 77.5% (79/102) of the cases.

~~Some patients developed severe complications within 30 days of CDI development, including gastrointestinal perforation in 1 patient (0.1%) and toxic megacolon in 2 patients (0.2%); 1 patient (0.1%) underwent a CDI-related surgery.~~ Among the 714 cases in which CDI was treated directly, recurrence within 30 days was observed in 34 cases (4.8%).

The univariate analysis indicated that comorbidities of heart and respiratory failure were significantly more prevalent among CDI patients. In addition, lower serum albumin levels were significantly associated with mortality. Among CDI treatments, mortality was significantly lower among cases in which probiotics were administered.

A logistic regression analysis of the 102 cases in which the patients died within 30 days of CDI development was performed to identify the factors associated with the risk of mortality. Compared to patients ≤74 years old, the odds ratio of mortality among patients aged 75–84 years was 2.08 (95%CI, 1.19 to 3.62). Among underlying diseases, heart failure (OR, 2.12; 95%CI, 1.26 to 3.55) and respiratory failure (OR, 1.98; 95%CI, 1.19 to 3.32) were identified as risk factors for mortality within 30 days of CDI development. Regarding nutritional status, neither parenteral nutrition nor enteral nutrition was identified as a risk factor for mortality. However, low serum albumin level (i.e., ≤2.6 g/dL) was identified as a significant risk factor for mortality (OR, 3.50; 95%CI, 1.33 to 9.22). Among CDI treatments, probiotic use (OR, 0.66;

95%CI, 0.42 to 1.04) was not identified as a risk factor for mortality. However, compared to cases in which no anti-CDI drugs were administered, vancomycin administration yielded an odds ratio of 0.43 (95%CI, 0.25 to 0.75), indicating a significantly lowered risk of mortality in the CDI group. Meanwhile, no such lowered mortality was observed in cases treated with metronidazole (OR, 0.85; 95%CI, 0.48 to 1.51).

Table 3. Univariate and multivariate analyses of all-cause mortality in CDI patients

Characteristics	All-cause mortality rate %	Univariate analysis P value	Multivariate analysis Odds ratio (95% CI)	Multivariate analysis P value
All	11.0 (102/924)	—	—	—
Age				
≤74 years	7.1 (23/326)		Ref.	
75–84 years	13.3 (47/353)	<0.05	2.08 (1.19 to 3.62)	<0.05
≥85 years	13.1 (32/245)		1.86 (0.98 to 3.55)	0.06
Sex				
Men	12.2 (64/524)		Ref.	
Women	9.5 (38/400)	0.21	0.78 (0.49 to 1.24)	0.29
Underlying disease				
Non-infectious	10.3 (64/619)		Ref.	
Infectious	12.5 (38/305)	0.37	0.99 (0.60 to 1.62)	0.97
Comorbidities				
Malignant tumors				
Not present	10.6 (67/630)		Ref.	
Present	11.9 (35/294)	0.57	1.54 (0.94 to 2.53)	0.09
Diabetes				
Not present	11.6 (89/765)		Ref.	
Present	8.2 (13/159)	0.27	0.71 (0.37 to 1.35)	0.29
Renal failure				
Not present	10.7 (84/784)		Ref.	
Present	12.9 (18/140)	0.46	0.90 (0.49 to 1.65)	0.73
Heart failure				
Not present	9.3 (70/756)		Ref.	
Present	19.0 (32/168)	<0.01	2.12 (1.26 to 3.55)	<0.01
Respiratory failure				
Not present	9.2 (69/754)		Ref.	
Present	19.4 (33/170)	<0.001	1.98 (1.19 to 3.32)	<0.01
Cirrhosis				
Not present	11.2 (100/895)		Ref.	
Present	6.9 (2/29)	0.76	0.61 (0.13 to 2.83)	0.53
Indicators of nutritional status				
Parenteral nutrition or enteral feeding				
Not present	9.4 (53/563)		Ref.	
Present	13.6 (49/361)	0.05	1.16 (0.73 to 1.84)	0.53
Serum albumin (g/dL)				
≥3.5	4.0 (5/124)		Ref.	
2.7–3.4	7.2 (27/376)	<0.001	1.55 (0.57 to 4.21)	0.39
≤2.6	16.5 (70/424)		3.50 (1.33 to 9.22)	<0.05
CDI treatments				
Cessation of antibiotics				
Not present	12.5 (65/519)		Ref.	
Present	9.1 (37/405)	0.11	0.77 (0.48 to 1.22)	0.26
Probiotics (for intestine treatment)				
Not present	13.8 (52/378)		Ref.	
Present	9.2 (50/546)	<0.05	0.66 (0.42 to 1.04)	0.08
Anti-CDI drugs				
Not present	15.2 (32/210)		Ref.	
Vancomycin alone	7.4 (32/433)	<0.05	0.43 (0.25 to 0.75)	<0.01
Metronidazole alone	13.5 (32/237)		0.85 (0.48 to 1.51)	0.59
Vancomycin and metronidazole	13.6 (6/44)		0.75 (0.27 to 2.08)	0.57

Discussion

This is the first large-scale clinical study of CDI in Japan. This study examined 1,026 cases of CDI recorded over 1 year at the nationwide facilities of Japan's largest hospital group. The findings of this investigation are similar to those reported in previous studies conducted in Europe, North America, and Australia with respect to the identification of several risk factors for CDI development, including age, severity of the underlying condition, ~~and~~ artificial feeding and mortality.~~ [2, 4, 5]~~[11, 13, 14] Antibiotic use is a known risk factor for CDI development.~~ [6]~~[15] The present case–control study confirms that intravenous cephems and carbapenems are important risk factors. Some studies report a low risk of CDI development owing to intravenous penicillin administration.~~ [7, 8]~~[16, 17] Concordantly, penicillin use was not identified as a risk factor in the present study. ~~Although~~ The proton pump inhibitor use was discussed as a risk factor for CDI development in the previous studies. ~~[9, 10].~~[18, 19, 20] In the present logistic regression analysis, it was not identified as a risk factor. ~~This finding might be influenced by the relatively high *Helicobacter pylori* infection rate in elderly Japanese people; proton pump inhibitors might produce smaller changes in pH levels in such patients than American and European patients. [11]~~

In this study, 11.0 % of CDI patients died within 30days. In comparison, higher 30-day mortality rates have been reported in previous outbreaks: 24.8% in the ribotype 027 strain outbreak in Canada, and 36.7% in an examination of a single intensive care unit in the USA.~~ [12, 13]~~[21, 22] However, reports of non-outbreak conditions indicate mortality rates of 13%, similar to the findings of the present study.~~ [14]~~[23] Some reports state that the CDI-associated mortality rate has increased 2.5 fold, possibly indicating that CDI cases are more severe and contribute more significantly to mortality than previously thought.~~ [3, 14]~~[12, 23] The mortality rate of CDI patients is reported to increase with age.~~[15]~~[24] Concordantly, the present study also found a significantly elevated risk of death in patients ≥75 years old.

The findings of this study indicate that the mortality risk of CDI patients was not reduced as a result of metronidazole treatment but was reduced with vancomycin treatment, corroborating the existing recommendation.~~[16]~~[25] It is worth noting that metronidazole is less expensive than vancomycin, making it economically advantageous. a patient's condition

must be carefully evaluated when selecting anti-CDI drugs. In particular, for patients in the present study who had conditions associated with a greater mortality risk, including advanced age (i.e., ≥75 years), heart or respiratory failure, or malnutrition as determined by low serum albumin levels, the use of vancomycin rather than metronidazole for treatment appears to have provided better outcomes. The recurrence rate was low (4.8%) in this study compared to the previous studies. [11, 26] We did not investigate the patients neither after 30 days of CDI development nor the patients who discharge even if within 30 days of CDI development. Therefore, the recurrence rate might be underestimated.

Regardless, this study has also several methodological limitations. The most salient limitation is the low number of registered CDI cases from quite a few participants. In the definition of CDI, the times of diarrhea were not investigated. ~~The most salient~~ Another limitation of the case–control study phase is the existence of many confounding factors. In particular, probiotic use, which was recently discussed ~~shown~~ to be correlated with CDI prevention, was not included in the predictive model of this study. ~~[17]~~[10, 11, 27] When interpreting the findings of this study, it is necessary to consider the influence of confounding factors that were not included in the analytical models. Regarding antibiotic use, the present analyses included independent explanatory variables for each antibiotic. However, actual antibiotic use is more complicated. Therefore, it is difficult to clearly determine the roles of individual antibiotics as risk factors for CDI development. Concerning matching process, we tried to adopt 1 to 1 pair sampling matched with sex, age group and main diagnosis. Some hospital could not find appropriate control sample well matched with case sample. So total number of the control group was less than that of the case sample. In addition, although data for the control group were analyzed during the entire study period until hospital discharge, only data from the period prior to CDI development were analyzed in the CDI group. Therefore, the risks might be underestimated, because the control group had a longer period of exposure risk than the CDI group. Confounding factors that were not included in the present analyses also represent a

limitation of the cohort study phase. Furthermore, issues of data quality among the facilities affect all aspects of this study. More than 40 different facilities participated in this study. While some facilities registered nearly all of their CDI patients, other facilities registered smaller proportions of patients. Only C. *difficile* culture but not toxin test was used for the laboratory test in two facilities. Finally, there might have been differences with regard to individual researchers' understanding of the outcome definitions.

~~As the Japanese population continues to age, the number of elderly patients suffering from multiple ailments is increasing as well. As the number of patients requiring intravenous administration of broad-spectrum antibiotics has increased, close and careful monitoring of CDI epidemiology is necessary.~~ In order to ensure appropriate antibiotic use and control the incidence of CDI, it is important to create institutional measures such as infection control teams ~~and to not limit such controls to the efforts of individual doctors~~. The cost-effective treatment of CDI may necessitate the appropriate use of less-expensive metronidazole. However, in cases expected to become severe or life-threatening, the more expensive drug vancomycin should be administered. ~~In countries facing an aging population,~~ CDI is one of many issues concerning medicine and medical treatment costs. Accordingly, further and more proactive research into CDI epidemiology is needed.

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Contributors

MT conceived the idea for the study, designed the study, developed the protocol, was responsible for study management and data collection, interpreted the findings, and drafted the paper. NM contributed to data analysis and interpretation of findings and drafted the paper. SB designed this study, developed the protocol, performed data analysis, and interpreted findings, and drafted the paper. All authors read and approved the final manuscript.

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Competing interests

None.

Ethics approval

The Central Ethics Committee of the NHO.

Provenance and peer review

Not commissioned; externally peer reviewed.

Data sharing statement

No additional data are available.

Contributorship Statement

All authors had full access to all of the data and can take responsibility for the integrity of the data and the accuracy of the data analysis.The lead author affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned have been explained.

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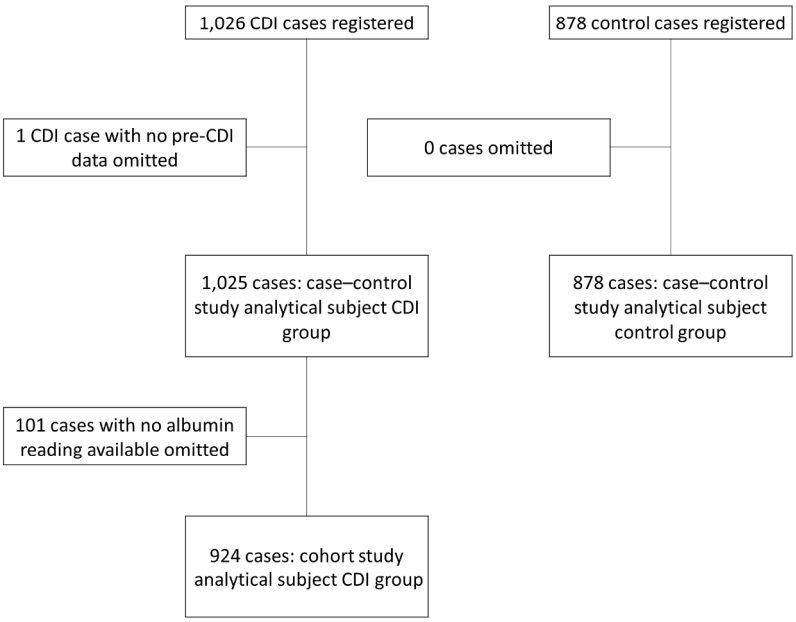

Figure 1. Study populations for the analysis of patients with *Clostridium difficile* infection (CDI) and controls.

595x793mm (96 x 96 DPI)

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STROBE Statement—Checklist of items that should be included in reports of ***case-control studies***

	Item No	Recommendation
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported
Objectives	3	State specific objectives, including any prespecified hypotheses
Methods		
Study design	4	Present key elements of study design early in the paper
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	(a) Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls
		(b) For matched studies, give matching criteria and the number of controls per case
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding
		(b) Describe any methods used to examine subgroups and interactions
		(c) Explain how missing data were addressed
		(d) If applicable, explain how matching of cases and controls was addressed
		(e) Describe any sensitivity analyses
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
Outcome data	15*	Report numbers in each exposure category, or summary measures of exposure
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(b) Report category boundaries when continuous variables were categorized
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

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STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found
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Background/rationale	2	Explain the scientific background and rationale for the investigation being reported
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Study design	4	Present key elements of study design early in the paper
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up
		(b) For matched studies, give matching criteria and number of exposed and unexposed
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding
		(b) Describe any methods used to examine subgroups and interactions
		(c) Explain how missing data were addressed
		(d) If applicable, explain how loss to follow-up was addressed
		(e) Describe any sensitivity analyses
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
		(c) Summarise follow-up time (eg, average and total amount)
Outcome data	15*	Report numbers of outcome events or summary measures over time
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(b) Report category boundaries when continuous variables were categorized
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Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for exposed and unexposed groups.

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Title

Multi-institution Case-control and Cohort Study of Risk Factors for the Development and Mortality of *Clostridium difficile* Infections in Japan

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Abstract

Objective: To examine risk factors for *Clostridium difficile* infection (CDI) morbidity and mortality in Japan.

Design: Multi-method investigation including a case–control study and cohort study.

Setting: Forty-seven participating facilities of the National Hospital Organization (NHO).

Participants: One thousand twenty six CDI patients and 878 patients in control group over the age of 18 years admitted to the subject NHO facilities from November 2010 to October 2011.

Main Outcome Measures: In case-control study, we identify risk factors for CDI development. Next, in cohort study, we identify risk factors for all-cause mortality within 30 days following CDI onset.

Results: A total of 1,026 cases of CDI meeting the definitions of this investigation were identified, encompassing 878 patients at 42 of the 47 subject facilities. In the case–control study, we identified, compared with no antibiotics use, use of first- and second-generation cephem antibiotics (odds ratio[OR], 1.44; 95% confidence interval [CI], 1.10 to 1.87), use of third- and fourth-generation cephem antibiotics(OR, 1.86; 95%CI, 1.48 to 2.33), and use of carbapenem antibiotics (OR, 1.87; 95%CI, 1.44 to 2.42) were risk factors for CDI development. However, use of penicillin was not identified as risk factors. In the cohort study, sufficient data for analysis was available for 924 CDI cases; 102 of them (11.0%) resulted in death within 30 days of CDI onset. Compared with no anti-CDI drug use, use of vancomycin was associated with reduced risk of mortality (OR, 0.43; 95%CI, 0.25 to 0.75) whereas metronidazole was not.

Conclusions: The findings mirror those of previous studies from Europe and North America, identifying the administration of broad-spectrum antibiotics as a risk factor for CDI development. The use of vancomycin is associated with a decreased risk of mortality.

Strengths and limitations of this study

- This study is the first large-scale nationwide multi-center CDI investigation in Japan.
- Most of the epidemiological data of CDI has been limited in the North America and Europe. Our data plays a role of completion of the missing data in Asia.
- Use of β-lactam antibiotics except penicillin was the risk factor for CDI development in the first Japanese large-scale investigation. Appropriate antibiotic use is necessary in order to control the incidence of CDI.
- Vancomycin administration for CDI was associated with decreased risk of mortality. Although the cost-effective treatment of CDI may necessitate the appropriate use of less-expensive metronidazole, vancomycin should be administered in case expected to become severe or life-threatening.
- The limitation of this study is the low number of registered CDI cases from quite a few participants and the existence of many confounding factors.

Introduction

Clostridium difficile is the main causative pathogen of antibiotic-associated colitis. Since 2000, outbreaks of BI/NAP1/027 strain *C. difficile* infections (CDI) have been reported in North American and European hospitals and elder care facilities. The numbers of CDI patients as well as severe and intractable cases have increased simultaneously. Consequently, epidemiological surveillance systems have been set up in several countries. However, very few countries have implemented such national-level measures.

CDI epidemiological studies in Japan to date have been based on scattered data from individual medical facilities. Consequently, the phenomenon of CDI in Japan is not sufficiently understood.[1, 2, 3, 4, 5, 6, 7, 8, 9]

Previous studies report that antibiotic administration is the largest risk factor for CDI development. Other risk factors include advanced age and proton pump inhibitor use. [10, 11] CDI mortality rates differ depending on the presence or absence of an outbreak as well as the relevant definitions of epidemiological surveillance. Furthermore, it is especially difficult to objectively determine precise CDI-related mortality rates because of factors such as underlying patient conditions. [12]

This report documents a case–control study of CDI in Japan based on data from the National Hospital Organization (NHO), which is Japan's largest group of hospitals and includes facilities located nationwide. In addition, a cohort investigation of mortality among CDI cases was conducted.

Materials and Methods

Research Design

This multicenter study is a collaborative effort of the 47 facilities that met our facility standards from among the 143 NHO facilities in Japan. The study was planned as a part of the NHO's "National Hospital Organization Multi-Center Clinical Research for Evidence-Based Medicine" project. This study was conducted with the approval of the Central Ethics

Committee of the NHO. The CDI group in this study included in principal all newly diagnosed CDI cases among patients hospitalized from November 1, 2010 to October 31, 2011; cases were registered continuously.

In the case–control study of CDI development, CDI cases newly diagnosed during the investigation period were registered in the CDI group; meanwhile, age-, sex-, and underlying disease-matched patients in the same facilities were registered to the control group. In addition, a prospective cohort study of CDI group patients who died within 30 days of CDI development was conducted. This investigation is a multi-method study using standard case–control and cohort study designs.

Definition of CDI

CDI was defined as the presence of any gastrointestinal symptoms accompanied by a clinical suspicion of CDI as well as a positive result for *C. difficile* toxins from rapid stool testing or *C. difficile* isolation from stool cultures or both. Final determinations were made by the attending physician or the facility's infection control team.

Enzyme immunoassay testing kits for *C. difficile* toxins A and B were used as the rapid testing method (Immunocard CD toxin A&B, Meridian Bioscience Inc., Cincinnati, OH, USA; C. Diff Quik Chek, Alere Medical Co. Ltd., Tokyo, Japan; Tox A/B Quik Chek, Nissui Pharmaceutical Co., Ltd., Tokyo, Japan; X/pect Toxin A/B, Kanto Chemical Co Ltd., Tokyo, Japan). Cycloserine-cefoxitin mannitol agar (Nissuipure-to CCMA baichi EX, Nissui Pharmaceutical Co. Ltd., Tokyo, Japan), cycloserine-cefoxitin fructose agar (CCFA baichi, Becton, Dickinson and Company Co. Ltd., Tokyo, Japan; Poamedhia® CCFA® kairyoubaichi, Eiken Chemical Co., Ltd., Tokyo, Japan), and brucella HK agar (RS) (brucella HK agar (RS), Kyokuto Pharmaceutical Industrial Co. Ltd., Tokyo, Japan) were used in the *C. difficile* isolation cultures.

Case–Control Study of CDI Development

No additional information besides age, sex, and date of diagnosis was gathered when new patients were registered in the CDI group. After the end of the study registration period, additional patient clinical data were gathered, including clinical

department, underlying diseases, dates of hospital admittance and discharge, and medical treatments administered for ≥3 days between admittance and CDI development. Recorded treatments included disruption of feeding, parenteral nutrition, enteral feeding, surgery with general anesthetic, cancer drugs, antibiotics (excluding external-use antibiotics), proton pump inhibitors (oral or intravenous). We also collected data regarding the use of intravenous antibiotics including penicillins, first- and second-generation cephems, third- and fourth-generation cephems, carbapenems, fluoroquinolones, clindamycin/lincomycin, anti–Methicillin-resistant *Staphylococcus aureus* (MRSA) drugs, and anti-fungal drugs, and others. Finally, we collected data regarding the use of oral antibiotics including cephems, fluoroquinolones, and others.

The control group was divided into three subgroups according to age: ≤74, 75–84, and ≥85 years. The control patients were selected from among patients at the same facilities who did not contract CDI and were matched to the CDI patients with respect to age, sex, underlying disease, and hospital stays of ≥5 days within the same month as a counterpart's CDI diagnosis. The control group cases were selected regardless of gastrointestinal symptoms such as diarrhea. We strove to ensure that the CDI and control groups were as matched as possible. The same data were collected from both groups. The control patients were registered, and relevant patient data were gathered after the end of the CDI group study registration period.

Cohort Study on Mortality among CDI Patients

The prospective cohort study of registered CDI group patients from the case–control study examined all-cause mortality within 30 days as the primary outcome. If the registered patients discharged within 30 days, clinical outcomes were not investigated after discharge in this study. The following data were collected: whether the underlying disease was infectious and whether comorbidities were related to malignant tumors (i.e., gastrointestinal, respiratory, blood/lymph, gynecologic, urological, or other tumors including cancers of the ear, nose, and throat), diabetes, renal failure, heart failure, respiratory failure, or cirrhosis. We also considered patient nutritional status including whether the patient was

subjected to parenteral nutrition or enteral feeding as well as serum albumin levels measured within 30 days prior to CDI development (i.e., ≥3.5, 2.7–3.4, or ≤2.6 g/dL). In addition, we examined CDI treatment factors including whether antibiotic use was halted, probiotic use, and the type of anti-CDI drugs used (i.e., vancomycin and metronidazole). All patient data for the cohort investigation were collected after the end of the registration period.

Data Management and Statistical Analysis

All input data were verified by a designated study data manager. Data from each facility were entered directly into a web-based case report form and subsequently encrypted for security. The data management center was responsible for confirming any missing data and directly inquiring the relevant facilities as necessary.

During the case–control phase of the study, CDI development was treated as the outcome and odds ratios (ORs) were calculated from bivariate analysis comparing the use of different types of antibiotics as outcome causes. For each type of antibiotic, those used for ≥3 days were designated "used" while all others were designated "unused." A dummy variable regression was subsequently performed. Statistical significance in the bivariate analysis was tested by the chi-square test. Logistic regression analysis was performed using the individual patient characteristics and other assumed confounding variables as independent variables. The 95% confidence intervals (CIs) for each variable were used to determine the relationships between the various predictive variables and outcomes.

In cohort study, the definition of severe complications were gastrointestinal perforations, toxic megacolon, CDI-related surgeries. Severe complications and the all-cause in-hospital mortality of patients within 30 days of CDI development were recorded. The clinical outcome of mortality within 30 days was set as the dependent variable, and the relationships among the underlying diseases, nutritional status, probiotic use, and types of anti-CDI drugs used were subjected to bivariate and multivariate analyses. Like the case–control phase, bivariate analysis were conducted using the chi-square test, and the multivariate analysis was conducted using logistic regression. The significance level for all analyses was set at $p < 0.05$. We

used IBM SPSS Statistics version 20 for statistical analysis.

Ethics Committee Approval and Informed Consent

This study was conducted with the approval of the Central Ethics Committee of the NHO. In principle, individual patients who met the inclusion criteria were not given direct explanations of the study, and no direct consent was sought. Information about the study was made public through postings on facility notice boards and webpages. Patients and their representative agents had the right to refuse study participation.

Results

Participating Facilities

Among the 47 facilities, a total of 1,026 CDI cases were registered at 42 facilities throughout Japan, from Hokkaido in the north to Okinawa in the south. No CDI cases were recorded at the remaining 5 participating facilities, more than 280 patient beds (Table 1).

Table 1. Number of registered cases of CDI and characteristics of hospitals included in the surveillance of CDI in the NHO (from november 2010 through october 2011)

Region	No. patient beds	No. patient days	No. patients registered		30-day all-cause mortality in CDI group	Laboratory tests used	
			CDI group	Control group		EIA for toxins A and B	Culture
Hokkaido, tohoku	698	208,388	55	55	3 (5%)	+	+
	500	150,603	42	32	1 (2%)	+	+
	310	82,687	28	19	2 (7%)		+
	310	72,144	17	12	2 (12%)	+	+
	220	76,539	1	1	0 (0%)	+	+
Kanto, koshinetsu	780	238,420	124	121	15 (12%)	+	+
	455	151,622	36	36	3 (8%)	+	
	560	158,921	35	30	4 (11%)	+	+
	243	60,155	34	34	6 (18%)	+	+
	350	109,025	22	22	4 (18%)	+	+
	500	159,432	15	14	1 (7%)	+	
	510	166,668	4	4	0 (0%)	+	
	380	109,482	3	2	0 (0%)	+	+
	455	132,483	3	1	0 (0%)	+	
	429	104,802	0	0	– (–)	+	
Tokai, hokuriku	430	195,209	42	26	10 (24%)	+	+
	280	56,475	0	0	– (–)	+	
Kinki	316	103,677	24	22	1 (4%)	+	
	220	47,354	23	23	1 (4%)	+	+
	600	191,041	20	20	3 (15%)	+	
	494	70,455	15	15	6 (40%)	+	+
	520	145,299	13	9	1 (8%)	+	
	500	142,409	6	6	1 (17%)	+	
	180	55,721	3	3	1 (33%)	+	
	346	118,014	2	2	0 (0%)	+	
	370	94,722	0	0	– (–)	+	
Chugoku, shikoku	388	99,728	54	49	5 (9%)	+	+
	700	211,595	49	48	4 (8%)	+	+
	506	119,356	33	8	1 (3%)	+	+
	400	122,846	30	30	5 (17%)	+	
	401	108,303	26	0	2 (8%)	+	+
	250	80,558	21	21	0 (0%)	+	
	424	128,868	12	10	0 (0%)	+	
	365	125,645	10	10	3 (30%)	+	+
	300	87,061	0	0	– (–)		+
	459	66,454	0	0	– (–)	+	
Kyushu, okinawa	424	137,827	46	22	5 (11%)	+	
	702	239,448	38	37	1 (3%)	+	
	190	54,038	33	31	9 (27%)	+	
	550	189,417	27	26	3 (11%)	+	
	285	58,185	25	25	3 (12%)	+	
	500	140,371	24	23	2 (8%)	+	
	300	90,457	14	14	4 (29%)	+	
	320	103,315	6	5	1 (17%)	+	+
	280	79,580	4	4	2 (50%)	+	
	366	112,906	4	4	0 (0%)	+	
	368	89,195	3	2	2 (67%)	+	
Total	19,486	5,592,077	1,026	878	117 (11%)	45	20

Patient Grouping

A total of 1,026 CDI cases that met the study definitions were recorded at the various institutions. We were unable to collect clinical records regarding medical treatments for 1 case; therefore, this case was excluded from the case–control study, and the remaining 1,025 cases were analyzed. A total of 962 patients (93.9%) developed CDI within 48 hours after hospital admittance. The control group comprised 878 patients who were selected from 41 of the 42 facilities. In the cohort study, we analyzed the data from 924 of the 1,025 CDI group patients, excluding 101 patients with no available recent serum albumin level data (i.e., within 30 days prior to CDI development (Figure 1).

Case–Control Study of CDI Development

The mean ages of the CDI and control groups were 75.8 and 75.4 years, respectively. The majority of the subjects were of advanced age: 64.0% and 62.5% of the CDI and control group patients were aged ≥75 years, respectively. No significant differences were identified between the CDI and control groups in the univariate analysis of age distribution, sex differences, or underlying disease (Table 2). Among the medical treatments administered before CDI development, the following were significantly more prevalent in the CDI group than the control group: disruption of feeding (48.6% vs. 30.4%), parenteral nutrition (24.7% vs. 10.3%), and enteral feeding (24.8% vs. 9.1%). Antibiotics were used prior to CDI development in 85.8% of cases. The use of all types of intravenous antibiotics was significantly more prevalent in the CDI group. No significant differences were identified between the 2 groups with respect to oral antibiotic use. Meanwhile, in the univariate analysis, proton pump inhibitor use was significantly more prevalent in the CDI group than the control group (40.3% vs. 31.2%).

We used logistic regression analysis to determine the risk factors for CDI development. The following medical treatments prior to CDI development were identified as significant risk factors in comparison to the control group: disruption of feeding (odds ratio[OR], 1.31; 95% confidence interval[CI], 1.05 to 1.64), parenteral nutrition (OR, 1.63; 95%CI, 1.21 to

2.20) and enteral feeding (OR, 2.16; 95%CI, 1.60 to 2.92).The following intravenous antibiotics were also identified as statistically significant risk factors for CDI development: first- and second-generation cephems (OR, 1.44; 95%CI, 1.10 to 1.87), third- and fourth-generation cephems (OR, 1.86; 95%CI, 1.48 to 2.33), and carbapenems (OR, 1.87; 95%CI, 1.44 to 2.42). However, penicillin (OR, 1.04; 95%CI, 0.82 to 1.33), fluoroquinolones (OR, 1.16; 95%CI, 0.74 to 1.83), clindamycin/lincomycin (OR, 1.35; 95%CI, 0.81 to 2.26), and proton pump inhibitor use (OR, 1.17; 95%CI, 0.95 to 1.44) were not identified as risk factors.

Table 2. Univariate and multivariate analyses of CDI development-related risk factors

Characteristics	CDI group %	Control group %	Univariate analysis P value	Multivariate analysis Odds ratio (95% CI)	Multivariate analysis P value
All	(1,025)	(878)	—	—	—
Age					
≤74 years	36.0 (369)	37.5 (329)	0.67	Ref.	—
75–84 years	37.0 (379)	37.2 (327)		1.02 (0.81 to 1.28)	0.88
≥85 years	27.0 (277)	25.3 (222)		1.09 (0.84 to 1.41)	0.52
Sex					
Women	43.0 (441)	42.6 (374)	0.85	1.11 (0.91 to 1.36)	0.28
Underlying disease					
Respiratory infections	15.8 (162)	17.5 (154)	0.14	—	—
Other infectious conditions	16.9 (173)	14.2 (125)		—	—
Gastrointestinal conditions	8.1 (83)	9.0 (79)		—	—
Malignant tumors	22.6 (232)	24.3 (213)		—	—
Cardiovascular conditions	7.7 (79)	9.8 (86)		—	—
Other conditions	28.9 (296)	25.2 (221)		—	—
Medical treatment prior to CDIdevelopment					
Disruption of feeding	48.6 (498)	30.4 (267)	<0.001	1.31 (1.05 to 1.64)	<0.05
Parenteral nutrition	24.7 (253)	10.3 (90)	<0.001	1.63 (1.21 to 2.20)	<0.01
Enteral feeding	24.8 (254)	9.1 (80)	<0.001	2.16 (1.60 to 2.92)	<0.001
Surgery with general anesthetic	18.2 (187)	15.6 (137)	0.14	0.89 (0.67 to 1.18)	0.41
Cancer drugs	11.3 (116)	14.2 (125)	0.06	0.86 (0.62 to 1.18)	0.35
Antibiotics use	85.8 (879)	66.5 (584)	<0.001	—	—
Intravenous					
Penicillins	27.6 (283)	21.0 (184)	<0.01	1.04 (0.82 to 1.33)	0.75
First/second-generation cephems	22.7 (233)	15.6 (137)	<0.001	1.44 (1.10 to 1.87)	<0.01
Third/fourth-generation cephems	35.2 (361)	19.9 (175)	<0.001	1.86 (1.48 to 2.33)	<0.001
Carbapenems	31.8 (326)	15.0 (132)	<0.001	1.87 (1.44 to 2.42)	<0.001
fluoroquinolones	7.5 (77)	4.0 (35)	<0.01	1.16 (0.74 to 1.83)	0.52
Clindamycin/lincomycin	6.5 (67)	2.8 (25)	<0.001	1.35 (0.81 to 2.26)	0.25
MRSA drugs	10.7 (110)	4.3 (38)	<0.001	1.10 (0.71 to 1.72)	0.66
Anti-fungal drugs	6.9 (71)	3.2 (28)	<0.001	1.01 (0.60 to 1.70)	0.96
Others(aminoglycosides, monobactam,etc.)	8.5 (87)	5.9 (52)	<0.05	1.19 (0.80 to 1.77)	0.39
Oral					
Cephems	5.6 (57)	4.4 (39)	0.29	1.49 (0.95 to 2.32)	0.08
fluoroquinolones	14.5 (149)	11.5 (101)	0.06	1.11 (0.82 to 1.51)	0.49
Others (macrolides, penicillins, etc.)	14.0 (144)	13.9 (122)	0.95	0.84 (0.63 to 1.13)	0.26
Proton pump inhibitors	40.3 (413)	31.2 (274)	<0.001	1.17 (0.95 to 1.44)	0.14

Cohort Study on Mortality among Patients with CDI

The cohort study examined mortality among the 924 patients from the 1,025 CDI group patients in the case–control study for whom serum albumin level data before CDI development were available.

Among the 924 patients, 102 (11.0%) died within 30 days of developing CDI. Among those cases, the cause of death was attributed to CDI in 11 cases (1.2%). Of 11 patients, a patient had gastrointestinal perforation, another patient had CDI-related surgery, and the others were not reported as severe complications. The toxic megacolon was reported in 2 patients however, they were not died within 30 days of CDI development. The mean age of the 102 patients who died during the study was 80.1 ± 8.3 years. Patients ≥75 years old were especially prevalent in this subgroup, accounting for 77.5% (79/102) of the cases.

Among the 714 cases in which CDI was treated directly, recurrence within 30 days was observed in 34 cases (4.8%).

The univariate analysis indicated that comorbidities of heart and respiratory failure were significantly more prevalent among CDI patients. In addition, lower serum albumin levels were significantly associated with mortality. Among CDI treatments, mortality was significantly lower among cases in which probiotics were administered.

A logistic regression analysis of the 102 cases in which the patients died within 30 days of CDI development was performed to identify the factors associated with the risk of mortality. Compared to patients ≤74 years old, the odds ratio of mortality among patients aged 75–84 years was 2.08 (95%CI, 1.19 to 3.62). Among underlying diseases, heart failure (OR, 2.12; 95%CI, 1.26 to 3.55) and respiratory failure (OR, 1.98; 95%CI, 1.19 to 3.32) were identified as risk factors for mortality within 30 days of CDI development. Regarding nutritional status, neither parenteral nutrition nor enteral nutrition was identified as a risk factor for mortality. However, low serum albumin level (i.e., ≤2.6 g/dL) was identified as a significant risk factor for mortality (OR, 3.50; 95%CI, 1.33 to 9.22). Among CDI treatments, probiotic use (OR, 0.66; 95%CI, 0.42 to 1.04) was not identified as a risk factor for mortality. However, compared to cases in which no anti-CDI drugs were administered, vancomycin administration yielded an odds ratio of 0.43 (95%CI, 0.25 to 0.75), indicating a

significantly lowered risk of mortality in the CDI group. Meanwhile, no such lowered mortality was observed in cases treated with metronidazole (OR, 0.85; 95%CI, 0.48 to 1.51).

Table 3. Univariate and multivariate analyses of all-cause mortality in CDI patients

Characteristics	All-cause mortality rate %	Univariate analysis P value	Multivariate analysis Odds ratio (95% CI)	Multivariate analysis P value
All	11.0 (102/924)	—	—	—
Age				
≤74 years	7.1 (23/326)		Ref.	
75–84 years	13.3 (47/353)	<0.05	2.08 (1.19 to 3.62)	<0.05
≥85 years	13.1 (32/245)		1.86 (0.98 to 3.55)	0.06
Sex				
Men	12.2 (64/524)		Ref.	
Women	9.5 (38/400)	0.21	0.78 (0.49 to 1.24)	0.29
Underlying disease				
Non-infectious	10.3 (64/619)		Ref.	
Infectious	12.5 (38/305)	0.37	0.99 (0.60 to 1.62)	0.97
Comorbidities				
Malignant tumors				
Not present	10.6 (67/630)		Ref.	
Present	11.9 (35/294)	0.57	1.54 (0.94 to 2.53)	0.09
Diabetes				
Not present	11.6 (89/765)		Ref.	
Present	8.2 (13/159)	0.27	0.71 (0.37 to 1.35)	0.29
Renal failure				
Not present	10.7 (84/784)		Ref.	
Present	12.9 (18/140)	0.46	0.90 (0.49 to 1.65)	0.73
Heart failure				
Not present	9.3 (70/756)		Ref.	
Present	19.0 (32/168)	<0.01	2.12 (1.26 to 3.55)	<0.01
Respiratory failure				
Not present	9.2 (69/754)		Ref.	
Present	19.4 (33/170)	<0.001	1.98 (1.19 to 3.32)	<0.01
Cirrhosis				
Not present	11.2 (100/895)		Ref.	
Present	6.9 (2/29)	0.76	0.61 (0.13 to 2.83)	0.53
Indicators of nutritional status				
Parenteral nutrition or enteral feeding				
Not present	9.4 (53/563)		Ref.	
Present	13.6 (49/361)	0.05	1.16 (0.73 to 1.84)	0.53
Serum albumin (g/dL)				
≥3.5	4.0 (5/124)		Ref.	
2.7–3.4	7.2 (27/376)	<0.001	1.55 (0.57 to 4.21)	0.39
≤2.6	16.5 (70/424)		3.50 (1.33 to 9.22)	<0.05
CDI treatments				
Cessation of antibiotics				
Not present	12.5 (65/519)		Ref.	
Present	9.1 (37/405)	0.11	0.77 (0.48 to 1.22)	0.26
Probiotics (for intestine treatment)				
Not present	13.8 (52/378)		Ref.	
Present	9.2 (50/546)	<0.05	0.66 (0.42 to 1.04)	0.08
Anti-CDI drugs				
Not present	15.2 (32/210)		Ref.	
Vancomycin alone	7.4 (32/433)	<0.05	0.43 (0.25 to 0.75)	<0.01
Metronidazole alone	13.5 (32/237)		0.85 (0.48 to 1.51)	0.59
Vancomycin and metronidazole	13.6 (6/44)		0.75 (0.27 to 2.08)	0.57

Discussion

This is the first large-scale clinical study of CDI in Japan. This study examined 1,026 cases of CDI recorded over 1 year at the nationwide facilities of Japan's largest hospital group. The findings of this investigation are similar to those reported in previous studies conducted in Europe, North America, and Australia with respect to the identification of several risk factors for CDI development, including age, severity of the underlying condition, artificial feeding and mortality. Antibiotic use is a known risk factor for CDI development. [15] The present case–control study confirms that intravenous cephems and carbapenems are important risk factors. Some studies report a low risk of CDI development owing to intravenous penicillin administration. [16, 17] Concordantly, penicillin use was not identified as a risk factor in the present study. The proton pump inhibitor use was discussed as a risk factor for CDI development in the previous studies..[18, 19, 20] In the present logistic regression analysis, it was not identified as a risk factor.

In this study, 11.0 % of CDI patients died within 30days. In comparison, higher 30-day mortality rates have been reported in previous outbreaks: 24.8% in the ribotype 027 strain outbreak in Canada, and 36.7% in an examination of a single intensive care unit in the USA. [21, 22] However, reports of non-outbreak conditions indicate mortality rates of 13%, similar to the findings of the present study. [23] Some reports state that the CDI-associated mortality rate has increased 2.5 fold, possibly indicating that CDI cases are more severe and contribute more significantly to mortality than previously thought. [12, 23] The mortality rate of CDI patients is reported to increase with age. [24] Concordantly, the present study also found a significantly elevated risk of death in patients ≥75 years old.

The findings of this study indicate that the mortality risk of CDI patients was not reduced as a result of metronidazole treatment but was reduced with vancomycin treatment, corroborating the existing recommendation. [25] It is worth noting that metronidazole is less expensive than vancomycin, making it economically advantageous. a patient's condition must be carefully evaluated when selecting anti-CDI drugs. In particular, for patients in the present study who had conditions associated with a greater mortality risk, including advanced age (i.e., ≥75 years), heart or respiratory failure, or malnutrition

as determined by low serum albumin levels, the use of vancomycin was expected to reduce the mortality. The recurrence rate was low (4.8%) in this study compared to the previous studies. [11, 26] We did not investigate the patients neither after 30 days of CDI development nor the patients who discharge even if within 30 days of CDI development. Therefore, the recurrence rate might be underestimated.

Regardless, this study has also several methodological limitations. The most salient limitation is the low number of registered CDI cases from quite a few participants. In the definition of CDI, the times of diarrhea were not investigated. Another limitation of the case–control study phase is the existence of many confounding factors. In particular, probiotic use, which was recently discussed to be correlated with CDI prevention, was not included in the predictive model of this study. [10, 11, 27] When interpreting the findings of this study, it is necessary to consider the influence of confounding factors that were not included in the analytical models. Regarding antibiotic use, the present analyses included independent explanatory variables for each antibiotic. However, actual antibiotic use is more complicated. Therefore, it is difficult to clearly determine the roles of individual antibiotics as risk factors for CDI development. Concerning matching process, we tried to adopt 1 to 1 pair sampling matched with sex, age group and main diagnosis. Some hospital could not find appropriate control sample well matched with case sample. So total number of the control group was less than that of the case sample. In addition, although data for the control group were analyzed during the entire study period until hospital discharge, only data from the period prior to CDI development were analyzed in the CDI group. Therefore, the risks might be underestimated, because the control group had a longer period of exposure risk than the CDI group. Confounding factors that were not included in the present analyses also represent a limitation of the cohort study phase. Furthermore, issues of data quality among the facilities affect all aspects of this study. More than 40 different facilities participated in this study. While some facilities registered nearly all of their CDI patients, other facilities registered smaller proportions of patients. Only C. *difficile* culture but not toxin test was used for the laboratory test in two facilities.

Finally, there might have been differences with regard to individual researchers' understanding of the outcome definitions.

In order to ensure appropriate antibiotic use and control the incidence of CDI, it is important to create institutional measures such as infection control teams The cost-effective treatment of CDI may necessitate the appropriate use of less-expensive metronidazole. However, in cases expected to become severe or life-threatening, the more expensive drug vancomycin should be administered. CDI is one of many issues concerning medicine and medical treatment costs. Accordingly, further and more proactive research into CDI epidemiology is needed.

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Contributors

MT conceived the idea for the study, designed the study, developed the protocol, was responsible for study management and data collection, interpreted the findings, and drafted the paper. NM contributed to data analysis and interpretation of findings and drafted the paper. SB designed this study, developed the protocol, performed data analysis, and interpreted findings. and drafted the paper. All authors read and approved the final manuscript.

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None.

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All authors had full access to all of the data and can take responsibility for the integrity of the data and the accuracy of the data analysis.The lead author affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned have been explained.

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Title

Multi-institution Case-control and Cohort Study of Risk Factors for the Development and Mortality of *Clostridium difficile* Infections in Japan

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Abstract

Objective: To examine risk factors for *Clostridium difficile* infection (CDI) morbidity and mortality in Japan.

Design: Multi-method investigation including a case–control study and cohort study.

Setting: Forty-seven participating facilities of the National Hospital Organization (NHO).

Participants: One thousand twenty six CDI patients and 878 patients in control group over the age of 18 years admitted to the subject NHO facilities from November 2010 to October 2011.

Main Outcome Measures: In case-control study, we identify risk factors for CDI development. Next, in cohort study, we identify risk factors for all-cause mortality within 30 days following CDI onset.

Results: A total of 1,026 cases of CDI meeting the definitions of this investigation were identified, encompassing 878 patients at 42 of the 47 subject facilities. In the case–control study, we identified, compared with no antibiotics use, use of first- and second-generation cephem antibiotics (odds ratio[OR], 1.44; 95% confidence interval [CI], 1.10 to 1.87), use of third- and fourth-generation cephem antibiotics(OR, 1.86; 95%CI, 1.48 to 2.33), and use of carbapenem antibiotics (OR, 1.87; 95%CI, 1.44 to 2.42) were risk factors for CDI development. However, use of penicillin was not identified as risk factors. In the cohort study, sufficient data for analysis was available for 924 CDI cases; 102 of them (11.0%) resulted in death within 30 days of CDI onset. Compared with no anti-CDI drug use, use of vancomycin was associated with reduced risk of mortality (OR, 0.43; 95%CI, 0.25 to 0.75) whereas metronidazole was not.

Conclusions: The findings mirror those of previous studies from Europe and North America, identifying the administration of broad-spectrum antibiotics as a risk factor for CDI development. The use of vancomycin is associated with a decreased risk of mortality.

Strengths and limitations of this study

- This study is the first large-scale nationwide multi-center CDI investigation in Japan.
- Most of the epidemiological data of CDI has been limited in the North America and Europe. Our data plays a role of completion of the missing data in Asia.
- Use of β-lactam antibiotics except penicillin was the risk factor for CDI development in the first Japanese large-scale investigation. Appropriate antibiotic use is necessary in order to control the incidence of CDI.
- Vancomycin administration for CDI was associated with decreased risk of mortality. Although the cost-effective treatment of CDI may necessitate the appropriate use of less-expensive metronidazole, vancomycin should be administered in case expected to become severe or life-threatening.
- The limitation of this study is the low number of registered CDI cases from quite a few participants and the existence of many confounding factors.

Introduction

Clostridium difficile is the main causative pathogen of antibiotic-associated colitis. Since 2000, outbreaks of BI/NAP1/027 strain *C. difficile* infections (CDI) have been reported in North American and European hospitals and elder care facilities. The numbers of CDI patients as well as severe and intractable cases have increased simultaneously. Consequently, epidemiological surveillance systems have been set up in several countries. However, very few countries have implemented such national-level measures.

CDI epidemiological studies in Japan to date have been based on scattered data from individual medical facilities. Consequently, the phenomenon of CDI in Japan is not sufficiently understood~~, including *C. difficile* typing~~.[1, 2, 3, 4, 5, 6, 7, 8, 9]

Previous studies report that antibiotic administration is the largest risk factor for CDI development. Other risk factors include advanced age and proton pump inhibitor use. [10, 11] CDI mortality rates differ depending on the presence or absence of an outbreak as well as the relevant definitions of epidemiological surveillance. Furthermore, it is especially difficult to objectively determine precise CDI-related mortality rates because of factors such as underlying patient conditions. [12]

This report documents a case–control study of CDI in Japan based on data from the National Hospital Organization (NHO), which is Japan's largest group of hospitals and includes facilities located nationwide. In addition, a cohort investigation of mortality among CDI cases was conducted.

Materials and Methods

Research Design

This multicenter study is a collaborative effort of the 47 facilities that met our facility standards from among the 143 NHO facilities in Japan. The study was planned as a part of the NHO's "National Hospital Organization Multi-Center

Clinical Research for Evidence-Based Medicine" project. This study was conducted with the approval of the Central Ethics Committee of the NHO. The CDI group in this study included in principal all newly diagnosed CDI cases among patients hospitalized from November 1, 2010 to October 31, 2011; cases were registered continuously.

In the case–control study of CDI development, CDI cases newly diagnosed during the investigation period were registered in the CDI group; meanwhile, age-, sex-, and underlying disease-matched patients in the same facilities were registered to the control group. In addition, a prospective cohort study of CDI group patients who died within 30 days of CDI development was conducted. This investigation is a multi-method study using standard case–control and cohort study designs.

Definition of CDI

CDI was defined as the presence of any gastrointestinal symptoms accompanied by a clinical suspicion of CDI as well as a positive result for *C. difficile* toxins from rapid stool testing or *C. difficile* isolation from stool cultures or both. Final determinations were made by the attending physician or the facility's infection control team.

Enzyme immunoassay testing kits for *C. difficile* toxins A and B were used as the rapid testing method (Immunocard CD toxin A&B, Meridian Bioscience Inc., Cincinnati, OH, USA; C. Diff Quik Chek, Alere Medical Co. Ltd., Tokyo, Japan; Tox A/B Quik Chek, Nissui Pharmaceutical Co., Ltd., Tokyo, Japan; X/pect Toxin A/B, Kanto Chemical Co Ltd., Tokyo, Japan). Cycloserine-cefoxitin mannitol agar (Nissuipure-to CCMA baichi EX, Nissui Pharmaceutical Co. Ltd., Tokyo, Japan), cycloserine-cefoxitin fructose agar (CCFA baichi, Becton, Dickinson and Company Co. Ltd., Tokyo, Japan; Poamedhia® CCFA® kairyoubaichi, Eiken Chemical Co., Ltd., Tokyo, Japan), and brucella HK agar (RS) (brucella HK agar (RS), Kyokuto Pharmaceutical Industrial Co. Ltd., Tokyo, Japan) were used in the *C. difficile* isolation cultures.

Case–Control Study of CDI Development

No additional information besides age, sex, and date of diagnosis was gathered when new patients were registered in the

CDI group. After the end of the study registration period, additional patient clinical data were gathered, including clinical department, underlying diseases, dates of hospital admittance and discharge, and medical treatments administered for ≥3 days between admittance and CDI development. Recorded treatments included disruption of feeding, parenteral nutrition, enteral feeding, surgery with general anesthetic, cancer drugs, antibiotics (excluding external-use antibiotics), proton pump inhibitors (oral or intravenous). We also collected data regarding the use of intravenous antibiotics including penicillins, first- and second-generation cephems, third- and fourth-generation cephems, carbapenems, fluoroquinolones, clindamycin/lincomycin, anti–Methicillin-resistant *Staphylococcus aureus* (MRSA) drugs, and anti-fungal drugs, and others. Finally, we collected data regarding the use of oral antibiotics including cephems, fluoroquinolones, and others.

The control group was divided into three subgroups according to age: ≤74, 75–84, and ≥85 years. The control patients were selected from among patients at the same facilities who did not contract CDI and were matched to the CDI patients with respect to age, sex, underlying disease, and hospital stays of ≥5 days within the same month as a counterpart's CDI diagnosis. The control group cases were selected regardless of gastrointestinal symptoms such as diarrhea. We strove to ensure that the CDI and control groups were as matched as possible. The same data were collected from both groups. The control patients were registered, and relevant patient data were gathered after the end of the CDI group study registration period.

Cohort Study on Mortality among CDI Patients

The prospective cohort study of registered CDI group patients from the case–control study examined all-cause mortality within 30 days as the primary outcome. ~~Clinical outcomes of patients who discharged within 30 days of CDI development were not investigated in this study.~~ If the registered patients discharged within 30 days, clinical outcomes were not investigated after discharge in this study. The following data were collected: whether the underlying disease was infectious and whether comorbidities were related to malignant tumors (i.e., gastrointestinal,

respiratory, blood/lymph, gynecologic, urological, or other tumors including cancers of the ear, nose, and throat), diabetes, renal failure, heart failure, respiratory failure, or cirrhosis. We also considered patient nutritional status including whether the patient was subjected to parenteral nutrition or enteral feeding as well as serum albumin levels measured within 30 days prior to CDI development (i.e., ≥3.5, 2.7–3.4, or ≤2.6 g/dL). In addition, we examined CDI treatment factors including whether antibiotic use was halted, probiotic use, and the type of anti-CDI drugs used (i.e., vancomycin and metronidazole). All patient data for the cohort investigation were collected after the end of the registration period.

Data Management and Statistical Analysis

All input data were verified by a designated study data manager. Data from each facility were entered directly into a web-based case report form and subsequently encrypted for security. The data management center was responsible for confirming any missing data and directly inquiring the relevant facilities as necessary.

During the case–control phase of the study, CDI development was treated as the outcome and odds ratios (ORs) were calculated from bivariate analysis comparing the use of different types of antibiotics as outcome causes. For each type of antibiotic, those used for ≥3 days were designated "used" while all others were designated "unused." A dummy variable regression was subsequently performed. Statistical significance in the bivariate analysis was tested by the chi-square test. Logistic regression analysis was performed using the individual patient characteristics and other assumed confounding variables as independent variables. The 95% confidence intervals (CIs) for each variable were used to determine the relationships between the various predictive variables and outcomes.

~~In the cohort study, gastrointestinal perforations, toxic megacolon, CDI-related surgeries, and the all-cause in-hospital mortality of patients within 30 days of CDI development were recorded.~~ In cohort study, the definition of severe complications were gastrointestinal perforations, toxic megacolon, CDI-related surgeries. Severe complications and the all-cause in-hospital mortality of patients within 30 days of CDI development were recorded. The clinical outcome of

mortality within 30 days was set as the dependent variable, and the relationships among the underlying diseases, nutritional status, probiotic use, and types of anti-CDI drugs used were subjected to bivariate and multivariate analyses. Like the case–control phase, bivariate analysis were conducted using the chi-square test, and the multivariate analysis was conducted using logistic regression. The significance level for all analyses was set at $p < 0.05$. We used IBM SPSS Statistics version 20 for statistical analysis.

Ethics Committee Approval and Informed Consent

This study was conducted with the approval of the Central Ethics Committee of the NHO. In principle, individual patients who met the inclusion criteria were not given direct explanations of the study, and no direct consent was sought. Information about the study was made public through postings on facility notice boards and webpages. Patients and their representative agents had the right to refuse study participation.

Results

Participating Facilities

Among the 47 facilities, a total of 1,026 CDI cases were registered at 42 facilities throughout Japan, from Hokkaido in the north to Okinawa in the south. No CDI cases were recorded at the remaining 5 participating facilities, more than 280 patient beds (Table 1).

Table 1. Number of registered cases of CDI and characteristics of hospitals included in the surveillance of CDI in the NHO (from november 2010 through october 2011)

Region	No. patient beds	No. patient days	No. patients registered		30-day all-cause mortality in CDI group	Laboratory tests used	
			CDI group	Control group		EIA for toxins A and B	Culture
Hokkaido, tohoku	698	208,388	55	55	3 (5%)	+	+
	500	150,603	42	32	1 (2%)	+	+
	310	82,687	28	19	2 (7%)		+
	310	72,144	17	12	2 (12%)	+	+
	220	76,539	1	1	0 (0%)	+	+
Kanto, koshinetsu	780	238,420	124	121	15 (12%)	+	+
	455	151,622	36	36	3 (8%)	+	
	560	158,921	35	30	4 (11%)	+	+
	243	60,155	34	34	6 (18%)	+	+
	350	109,025	22	22	4 (18%)	+	+
	500	159,432	15	14	1 (7%)	+	
	510	166,668	4	4	0 (0%)	+	
	380	109,482	3	2	0 (0%)	+	+
	455	132,483	3	1	0 (0%)	+	
	429	104,802	0	0	– (–)	+	
Tokai, hokuriku	430	195,209	42	26	10 (24%)	+	+
	280	56,475	0	0	– (–)	+	
Kinki	316	103,677	24	22	1 (4%)	+	
	220	47,354	23	23	1 (4%)	+	+
	600	191,041	20	20	3 (15%)	+	
	494	70,455	15	15	6 (40%)	+	+
	520	145,299	13	9	1 (8%)	+	
	500	142,409	6	6	1 (17%)	+	
	180	55,721	3	3	1 (33%)	+	
	346	118,014	2	2	0 (0%)	+	
	370	94,722	0	0	– (–)	+	
Chugoku, shikoku	388	99,728	54	49	5 (9%)	+	+
	700	211,595	49	48	4 (8%)	+	+
	506	119,356	33	8	1 (3%)	+	+
	400	122,846	30	30	5 (17%)	+	
	401	108,303	26	0	2 (8%)	+	+
	250	80,558	21	21	0 (0%)	+	
	424	128,868	12	10	0 (0%)	+	
	365	125,645	10	10	3 (30%)	+	+
	300	87,061	0	0	– (–)		+
	459	66,454	0	0	– (–)	+	
Kyushu, okinawa	424	137,827	46	22	5 (11%)	+	
	702	239,448	38	37	1 (3%)	+	
	190	54,038	33	31	9 (27%)	+	
	550	189,417	27	26	3 (11%)	+	
	285	58,185	25	25	3 (12%)	+	
	500	140,371	24	23	2 (8%)	+	
	300	90,457	14	14	4 (29%)	+	
	320	103,315	6	5	1 (17%)	+	+
	280	79,580	4	4	2 (50%)	+	
	366	112,906	4	4	0 (0%)	+	
	368	89,195	3	2	2 (67%)	+	
Total	19,486	5,592,077	1,026	878	117 (11%)	45	20

Patient Grouping

A total of 1,026 CDI cases that met the study definitions were recorded at the various institutions. We were unable to collect clinical records regarding medical treatments for 1 case; therefore, this case was excluded from the case–control study, and the remaining 1,025 cases were analyzed. A total of 962 patients (93.9%) developed CDI within 48 hours after hospital admittance. The control group comprised 878 patients who were selected from 41 of the 42 facilities. In the cohort study, we analyzed the data from 924 of the 1,025 CDI group patients, excluding 101 patients with no available recent serum albumin level data (i.e., within 30 days prior to CDI development (Figure 1).

Case–Control Study of CDI Development

The mean ages of the CDI and control groups were 75.8 and 75.4 years, respectively. The majority of the subjects were of advanced age: 64.0% and 62.5% of the CDI and control group patients were aged ≥75 years, respectively. No significant differences were identified between the CDI and control groups in the univariate analysis of age distribution, sex differences, or underlying disease (Table 2). Among the medical treatments administered before CDI development, the following were significantly more prevalent in the CDI group than the control group: disruption of feeding (48.6% vs. 30.4%), parenteral nutrition (24.7% vs. 10.3%), and enteral feeding (24.8% vs. 9.1%). Antibiotics were used prior to CDI development in 85.8% of cases. The use of all types of intravenous antibiotics was significantly more prevalent in the CDI group. No significant differences were identified between the 2 groups with respect to oral antibiotic use. Meanwhile, in the univariate analysis, proton pump inhibitor use was significantly more prevalent in the CDI group than the control group (40.3% vs. 31.2%).

We used logistic regression analysis to determine the risk factors for CDI development. The following medical treatments prior to CDI development were identified as significant risk factors in comparison to the control group: disruption of feeding (odds ratio[OR], 1.31; 95% confidence interval[CI], 1.05 to 1.64), parenteral nutrition (OR, 1.63; 95%CI, 1.21 to

2.20) and enteral feeding (OR, 2.16; 95%CI, 1.60 to 2.92).The following intravenous antibiotics were also identified as statistically significant risk factors for CDI development: first- and second-generation cephems (OR, 1.44; 95%CI, 1.10 to 1.87), third- and fourth-generation cephems (OR, 1.86; 95%CI, 1.48 to 2.33), and carbapenems (OR, 1.87; 95%CI, 1.44 to 2.42). However, penicillin (OR, 1.04; 95%CI, 0.82 to 1.33), fluoroquinolones (OR, 1.16; 95%CI, 0.74 to 1.83), clindamycin/lincomycin (OR, 1.35; 95%CI, 0.81 to 2.26), and proton pump inhibitor use (OR, 1.17; 95%CI, 0.95 to 1.44) were not identified as risk factors.

Table 2. Univariate and multivariate analyses of CDI development-related risk factors

Characteristics	CDI group %	Control group %	Univariate analysis P value	Multivariate analysis Odds ratio (95% CI)	Multivariate analysis P value
All	(1,025)	(878)	—	—	—
Age					
≤74 years	36.0 (369)	37.5 (329)	0.67	Ref.	—
75–84 years	37.0 (379)	37.2 (327)		1.02 (0.81 to 1.28)	0.88
≥85 years	27.0 (277)	25.3 (222)		1.09 (0.84 to 1.41)	0.52
Sex					
Women	43.0 (441)	42.6 (374)	0.85	1.11 (0.91 to 1.36)	0.28
Underlying disease					
Respiratory infections	15.8 (162)	17.5 (154)	0.14	—	—
Other infectious conditions	16.9 (173)	14.2 (125)		—	—
Gastrointestinal conditions	8.1 (83)	9.0 (79)		—	—
Malignant tumors	22.6 (232)	24.3 (213)		—	—
Cardiovascular conditions	7.7 (79)	9.8 (86)		—	—
Other conditions	28.9 (296)	25.2 (221)		—	—
Medical treatment prior to CDIdevelopment					
Disruption of feeding	48.6 (498)	30.4 (267)	<0.001	1.31 (1.05 to 1.64)	<0.05
Parenteral nutrition	24.7 (253)	10.3 (90)	<0.001	1.63 (1.21 to 2.20)	<0.01
Enteral feeding	24.8 (254)	9.1 (80)	<0.001	2.16 (1.60 to 2.92)	<0.001
Surgery with general anesthetic	18.2 (187)	15.6 (137)	0.14	0.89 (0.67 to 1.18)	0.41
Cancer drugs	11.3 (116)	14.2 (125)	0.06	0.86 (0.62 to 1.18)	0.35
Antibiotics use	85.8 (879)	66.5 (584)	<0.001	—	—
Intravenous					
Penicillins	27.6 (283)	21.0 (184)	<0.01	1.04 (0.82 to 1.33)	0.75
First/second-generation cephems	22.7 (233)	15.6 (137)	<0.001	1.44 (1.10 to 1.87)	<0.01
Third/fourth-generation cephems	35.2 (361)	19.9 (175)	<0.001	1.86 (1.48 to 2.33)	<0.001
Carbapenems	31.8 (326)	15.0 (132)	<0.001	1.87 (1.44 to 2.42)	<0.001
fluoroquinolones	7.5 (77)	4.0 (35)	<0.01	1.16 (0.74 to 1.83)	0.52
Clindamycin/lincomycin	6.5 (67)	2.8 (25)	<0.001	1.35 (0.81 to 2.26)	0.25
MRSA drugs	10.7 (110)	4.3 (38)	<0.001	1.10 (0.71 to 1.72)	0.66
Anti-fungal drugs	6.9 (71)	3.2 (28)	<0.001	1.01 (0.60 to 1.70)	0.96
Others(aminoglycosides, monobactam,etc.)	8.5 (87)	5.9 (52)	<0.05	1.19 (0.80 to 1.77)	0.39
Oral					
Cephems	5.6 (57)	4.4 (39)	0.29	1.49 (0.95 to 2.32)	0.08
fluoroquinolones	14.5 (149)	11.5 (101)	0.06	1.11 (0.82 to 1.51)	0.49
Others (macrolides, penicillins, etc.)	14.0 (144)	13.9 (122)	0.95	0.84 (0.63 to 1.13)	0.26
Proton pump inhibitors	40.3 (413)	31.2 (274)	<0.001	1.17 (0.95 to 1.44)	0.14

Cohort Study on Mortality among Patients with CDI

The cohort study examined mortality among the 924 patients from the 1,025 CDI group patients in the case–control study for whom serum albumin level data before CDI development were available.

Among the 924 patients, 102 (11.0%) died within 30 days of developing CDI. Among those cases, the cause of death was attributed to CDI in 11 cases (1.2%). Of 11 patients, a patient had gastrointestinal perforation, another patient had CDI-related surgery, and the others were not reported as severe complications. The toxic megacolon was reported in 2 patients however, they were not died within 30 days of CDI development. The mean age of the 102 patients who died during the study was 80.1 ± 8.3 years. Patients ≥75 years old were especially prevalent in this subgroup, accounting for 77.5% (79/102) of the cases.

Among the 714 cases in which CDI was treated directly, recurrence within 30 days was observed in 34 cases (4.8%).

The univariate analysis indicated that comorbidities of heart and respiratory failure were significantly more prevalent among CDI patients. In addition, lower serum albumin levels were significantly associated with mortality. Among CDI treatments, mortality was significantly lower among cases in which probiotics were administered.

A logistic regression analysis of the 102 cases in which the patients died within 30 days of CDI development was performed to identify the factors associated with the risk of mortality. Compared to patients ≤74 years old, the odds ratio of mortality among patients aged 75–84 years was 2.08 (95%CI, 1.19 to 3.62). Among underlying diseases, heart failure (OR, 2.12; 95%CI, 1.26 to 3.55) and respiratory failure (OR, 1.98; 95%CI, 1.19 to 3.32) were identified as risk factors for mortality within 30 days of CDI development. Regarding nutritional status, neither parenteral nutrition nor enteral nutrition was identified as a risk factor for mortality. However, low serum albumin level (i.e., ≤2.6 g/dL) was identified as a significant risk factor for mortality (OR, 3.50; 95%CI, 1.33 to 9.22). Among CDI treatments, probiotic use (OR, 0.66; 95%CI, 0.42 to 1.04) was not identified as a risk factor for mortality. However, compared to cases in which no anti-CDI drugs were administered, vancomycin administration yielded an odds ratio of 0.43 (95%CI, 0.25 to 0.75), indicating a

significantly lowered risk of mortality in the CDI group. Meanwhile, no such lowered mortality was observed in cases treated with metronidazole (OR, 0.85; 95%CI, 0.48 to 1.51).

Table 3. Univariate and multivariate analyses of all-cause mortality in CDI patients

Characteristics	All-cause mortality rate %	Univariate analysis P value	Multivariate analysis Odds ratio (95% CI)	Multivariate analysis P value
All	11.0 (102/924)	—	—	—
Age				
≤74 years	7.1 (23/326)		Ref.	
75–84 years	13.3 (47/353)	<0.05	2.08 (1.19 to 3.62)	<0.05
≥85 years	13.1 (32/245)		1.86 (0.98 to 3.55)	0.06
Sex				
Men	12.2 (64/524)		Ref.	
Women	9.5 (38/400)	0.21	0.78 (0.49 to 1.24)	0.29
Underlying disease				
Non-infectious	10.3 (64/619)		Ref.	
Infectious	12.5 (38/305)	0.37	0.99 (0.60 to 1.62)	0.97
Comorbidities				
Malignant tumors				
Not present	10.6 (67/630)		Ref.	
Present	11.9 (35/294)	0.57	1.54 (0.94 to 2.53)	0.09
Diabetes				
Not present	11.6 (89/765)		Ref.	
Present	8.2 (13/159)	0.27	0.71 (0.37 to 1.35)	0.29
Renal failure				
Not present	10.7 (84/784)		Ref.	
Present	12.9 (18/140)	0.46	0.90 (0.49 to 1.65)	0.73
Heart failure				
Not present	9.3 (70/756)		Ref.	
Present	19.0 (32/168)	<0.01	2.12 (1.26 to 3.55)	<0.01
Respiratory failure				
Not present	9.2 (69/754)		Ref.	
Present	19.4 (33/170)	<0.001	1.98 (1.19 to 3.32)	<0.01
Cirrhosis				
Not present	11.2 (100/895)		Ref.	
Present	6.9 (2/29)	0.76	0.61 (0.13 to 2.83)	0.53
Indicators of nutritional status				
Parenteral nutrition or enteral feeding				
Not present	9.4 (53/563)		Ref.	
Present	13.6 (49/361)	0.05	1.16 (0.73 to 1.84)	0.53
Serum albumin (g/dL)				
≥3.5	4.0 (5/124)		Ref.	
2.7–3.4	7.2 (27/376)	<0.001	1.55 (0.57 to 4.21)	0.39
≤2.6	16.5 (70/424)		3.50 (1.33 to 9.22)	<0.05
CDI treatments				
Cessation of antibiotics				
Not present	12.5 (65/519)		Ref.	
Present	9.1 (37/405)	0.11	0.77 (0.48 to 1.22)	0.26
Probiotics (for intestine treatment)				
Not present	13.8 (52/378)		Ref.	
Present	9.2 (50/546)	<0.05	0.66 (0.42 to 1.04)	0.08
Anti-CDI drugs				
Not present	15.2 (32/210)		Ref.	
Vancomycin alone	7.4 (32/433)	<0.05	0.43 (0.25 to 0.75)	<0.01
Metronidazole alone	13.5 (32/237)		0.85 (0.48 to 1.51)	0.59
Vancomycin and metronidazole	13.6 (6/44)		0.75 (0.27 to 2.08)	0.57

Discussion

This is the first large-scale clinical study of CDI in Japan. This study examined 1,026 cases of CDI recorded over 1 year at the nationwide facilities of Japan's largest hospital group. The findings of this investigation are similar to those reported in previous studies conducted in Europe, North America, and Australia with respect to the identification of several risk factors for CDI development, including age, severity of the underlying condition, artificial feeding and mortality. Antibiotic use is a known risk factor for CDI development. [15] The present case–control study confirms that intravenous cephems and carbapenems are important risk factors. Some studies report a low risk of CDI development owing to intravenous penicillin administration. [16, 17] Concordantly, penicillin use was not identified as a risk factor in the present study. The proton pump inhibitor use was discussed as a risk factor for CDI development in the previous studies..[18, 19, 20] In the present logistic regression analysis, it was not identified as a risk factor.

In this study, 11.0 % of CDI patients died within 30days. In comparison, higher 30-day mortality rates have been reported in previous outbreaks: 24.8% in the ribotype 027 strain outbreak in Canada, and 36.7% in an examination of a single intensive care unit in the USA. [21, 22] However, reports of non-outbreak conditions indicate mortality rates of 13%, similar to the findings of the present study. [23] Some reports state that the CDI-associated mortality rate has increased 2.5 fold, possibly indicating that CDI cases are more severe and contribute more significantly to mortality than previously thought. [12, 23] The mortality rate of CDI patients is reported to increase with age. [24] Concordantly, the present study also found a significantly elevated risk of death in patients ≥75 years old.

The findings of this study indicate that the mortality risk of CDI patients was not reduced as a result of metronidazole treatment but was reduced with vancomycin treatment, corroborating the existing recommendation. [25] It is worth noting that metronidazole is less expensive than vancomycin, making it economically advantageous. a patient's condition must be carefully evaluated when selecting anti-CDI drugs. In particular, for patients in the present study who had conditions associated with a greater mortality risk, including advanced age (i.e., ≥75 years), heart or respiratory failure, or malnutrition

as determined by low serum albumin levels, ~~the use of vancomycin rather than metronidazole for treatment appears to have provided better outcomes.~~ the use of vancomycin was expected to reduce the mortality. The recurrence rate was low (4.8%) in this study compared to the previous studies. [11, 26] We did not investigate the patients neither after 30 days of CDI development nor the patients who discharge even if within 30 days of CDI development. Therefore, the recurrence rate might be underestimated.

Regardless, this study has also several methodological limitations. The most salient limitation is the low number of registered CDI cases from quite a few participants. In the definition of CDI, the times of diarrhea were not investigated. Another limitation of the case–control study phase is the existence of many confounding factors. In particular, probiotic use, which was recently discussed to be correlated with CDI prevention, was not included in the predictive model of this study. [10, 11, 27] When interpreting the findings of this study, it is necessary to consider the influence of confounding factors that were not included in the analytical models. Regarding antibiotic use, the present analyses included independent explanatory variables for each antibiotic. However, actual antibiotic use is more complicated. Therefore, it is difficult to clearly determine the roles of individual antibiotics as risk factors for CDI development. Concerning matching process, we tried to adopt 1 to 1 pair sampling matched with sex, age group and main diagnosis. Some hospital could not find appropriate control sample well matched with case sample. So total number of the control group was less than that of the case sample. In addition, although data for the control group were analyzed during the entire study period until hospital discharge, only data from the period prior to CDI development were analyzed in the CDI group. Therefore, the risks might be underestimated, because the control group had a longer period of exposure risk than the CDI group. Confounding factors that were not included in the present analyses also represent a limitation of the cohort study phase. Furthermore, issues of data quality among the facilities affect all aspects of this study. More than 40 different facilities participated in this study. While some facilities registered nearly all of their CDI patients, other facilities registered

smaller proportions of patients. Only C. *difficile* culture but not toxin test was used for the laboratory test in two facilities. Finally, there might have been differences with regard to individual researchers' understanding of the outcome definitions.

In order to ensure appropriate antibiotic use and control the incidence of CDI, it is important to create institutional measures such as infection control teams The cost-effective treatment of CDI may necessitate the appropriate use of less-expensive metronidazole. However, in cases expected to become severe or life-threatening, the more expensive drug vancomycin should be administered. CDI is one of many issues concerning medicine and medical treatment costs. Accordingly, further and more proactive research into CDI epidemiology is needed.

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Contributors

MT conceived the idea for the study, designed the study, developed the protocol, was responsible for study management and data collection, interpreted the findings, and drafted the paper. NM contributed to data analysis and interpretation of findings and drafted the paper. SB designed this study, developed the protocol, performed data analysis, and interpreted findings. and drafted the paper. All authors read and approved the final manuscript.

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Competing interests

None.

Ethics approval

The Central Ethics Committee of the NHO.

Provenance and peer review

Not commissioned; externally peer reviewed.

Data sharing statement

No additional data are available.

Contributorship Statement

All authors had full access to all of the data and can take responsibility for the integrity of the data and the accuracy of the data analysis.The lead author affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned have been explained.

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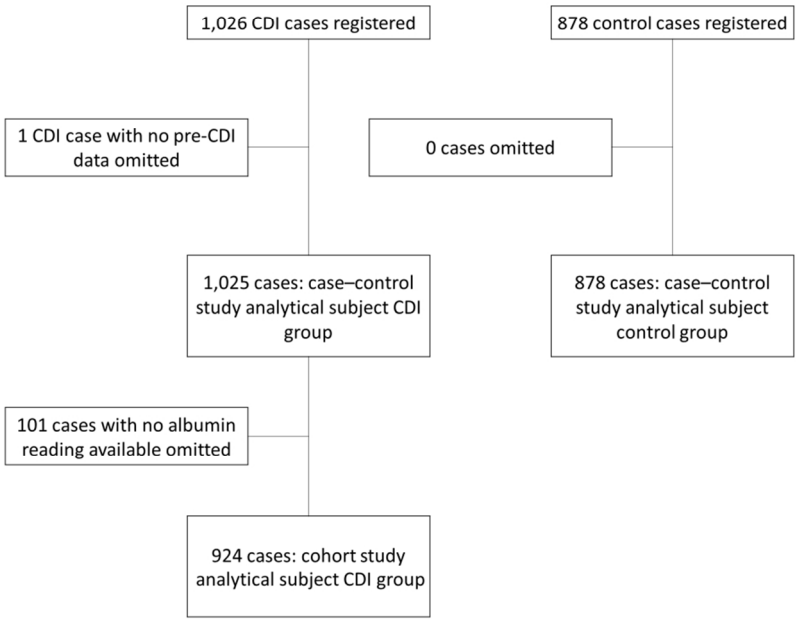

Figure 1. Study populations for the analysis of patients with *Clostridium difficile* infection (CDI) and controls.

90x119mm (300 x 300 DPI)

TAKAHASHI et. al.

STROBE Statement—Checklist of items that should be included in reports of ***case-control studies***

	Item No	Recommendation
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported
Objectives	3	State specific objectives, including any prespecified hypotheses
Methods		
Study design	4	Present key elements of study design early in the paper
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	(a) Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls
		(b) For matched studies, give matching criteria and the number of controls per case
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding
		(b) Describe any methods used to examine subgroups and interactions
		(c) Explain how missing data were addressed
		(d) If applicable, explain how matching of cases and controls was addressed
		(e) Describe any sensitivity analyses
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
Outcome data	15*	Report numbers in each exposure category, or summary measures of exposure
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(b) Report category boundaries when continuous variables were categorized
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

TAKAHASHI et. al.

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported
Objectives	3	State specific objectives, including any prespecified hypotheses
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Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up
		(b) For matched studies, give matching criteria and number of exposed and unexposed
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding
		(b) Describe any methods used to examine subgroups and interactions
		(c) Explain how missing data were addressed
		(d) If applicable, explain how loss to follow-up was addressed
		(e) Describe any sensitivity analyses
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
		(c) Summarise follow-up time (eg, average and total amount)
Outcome data	15*	Report numbers of outcome events or summary measures over time
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(b) Report category boundaries when continuous variables were categorized
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.