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Epidemiology and clinical features of toxigenic culture-confirmed hospital-onset *Clostridium difficile* infection: a multicentre prospective study in tertiary hospitals of South Korea

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Hypervirulent *Clostridium difficile* strains, most notably BI/NAP1/027, have been increasingly emerging in Western countries as local epidemics. We performed a prospective multicentre observational study from December 2011 to May 2012 to identify recent incidences of toxigenic culture-confirmed hospital-onset *C. difficile* infections (CDI) and their associated clinical characteristics in South Korea. Patients suspected of having been suffering from CDI more than 48 h after admission and aged ⩾20 years were prospectively enrolled and provided loose stool specimens. Toxigenic *C. difficile* culture (anaerobic culture + toxin A/B/binary gene PCR) and PCR ribotyping were performed in one central laboratory. We enrolled 98 toxigenic culture-confirmed CDI-infected patients and 250 toxigenic culture-negative participants from three hospitals. The incidence of toxigenic culture-confirmed hospital-onset CDI cases was 2.7 per 10 000 patient-days. The percentage of severe CDI cases was relatively low at only 3.1 %. UK ribotype 018 was the predominant type (48.1 %). There were no hypervirulent BI/NAP1/027 isolates identified. The independent risk factors for toxigenic culture-confirmed hospital-onset CDI were invasive procedure (odds ratio (OR) 7.3, P=0.003) and past CDI history within 3 months (OR 28.5, P=0.003). In conclusion, the incidence and severity of CDI in our study were not higher than reported in Western countries.

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INTRODUCTION

Clostridium difficile is a Gram-positive, spore-forming anaerobic bacterium that is emerging as an important ubiquitous pathogen that causes classical healthcare-associated infections that range from mild self-limiting diarrhoea to life-threatening severe pseudomembranous colitis (PMC), toxic megacolon, bowel perforation, severe sepsis, and death (Bartlett *et al.*, 1978; Lo Vecchio & Zacur, 2012; Rubin *et al.*, 1995; Safdar, 2012). *C. difficile* is estimated to be responsible for 10–25 % of all cases of antibiotic-associated diarrhoea and for almost all cases of PMC (Anand *et al.*, 1994; Bartlett, 1994). Another clinical significance of *C. difficile* infection (CDI) is its relatively high recurrence rate (5–47 % of cases) (Barbut *et al.*, 2000; Bauer *et al.*, 2011). CDI is a growing concern worldwide, as it not only increases the duration of time a patient is required to stay hospitalized, which increases the associated cost to the hospital, but also causes morbidity and mortality (Kyne *et al.*, 2002).

C. difficile toxin A (308 kDa) is commonly referred to as the enterotoxin, while toxin B (270 kDa) is referred to as the cytotoxin. These toxins damage intestinal epithelial cells and are the cause of the associated clinical illness (Kuehne *et al.*, 2010). Recently, hypervirulent strains, most notably BI/NAP1/027, which is detectable by PCR ribotype 027, as it produces binary toxin as well as PCR ribotype 078, have been increasingly emerging in Western countries as local

Abbreviations: CDI, *Clostridium difficile* infection; CI, confidence interval; EIA, enzyme immunoassay; IBD, inflammatory bowel disease; OR, odds ratio; PMC, pseudomembranous colitis.

epidemics (Bauer *et al.*, 2011; Clements *et al.*, 2010; Goorhuis *et al.*, 2008; Kim *et al.*, 2011). These strains have resulted in a change in CDI epidemiology and have also increased the incidence and severity of CDI (Barbut *et al.*, 2005; Freeman *et al.*, 2010; Gilca *et al.*, 2010; Ricciardi *et al.*, 2007).

The well-known risk factors for CDI that lead to clinically apparent disease in adults include prolonged antimicrobial therapy, hospitalization, malnutrition, previous gastrointestinal surgery, older age ($\geqslant$65 years of age), and increased duration of hospital stay (Freeman *et al.*, 2010; Lo Vecchio & Zacur, 2012). However, in recent years, the epidemiology of CDI has shifted from almost exclusively infecting elderly patients in whom the gut microbiota has been disturbed by antibiotics to now infecting individuals of all age groups with no recent antibiotic exposure, and includes community-onset or -acquired CDI (Lessa *et al.*, 2012). In addition, inflammatory bowel disease (IBD), cystic fibrosis and solid organ transplantation have emerged as new risk factors for CDI (Lo Vecchio & Zacur, 2012; Patriarchi *et al.*, 2011).

It has been reported that the increased usage of broad-spectrum antibiotics and the rate of antimicrobial resistance in major hospital-acquired pathogens are high in South Korea (Kim, 2005; Lee *et al.*, 2006, 2011). However, a local epidemic caused by hypervirulent strains of *C. difficile* has not occurred until recently in South Korea, in spite of the uncommon reports of PCR ribotype 027 infection (Kim *et al.*, 2011, 2013a; Shin *et al.*, 2012; Tae *et al.*, 2009). There are only a few reports of prospective multicentre epidemiological studies on CDI in South Korea. The objective of this study was to estimate the incidence and to determine the risk factors of toxigenic culture-confirmed hospital-onset CDI cases by studying hospitalized adult patients in South Korea through a multicentre prospective cohort.

METHODS

Study design. This prospective, multicentre observational, epidemiological study was conducted in three university-affiliated tertiary care centre hospitals in Seoul, South Korea, between December 2011 and May 2012. An individual who met both of the following criteria was eligible for enrolment: (1) hospitalized adult patient $\geqslant$20 years of age and (2) clinically suspected of having CDI any time after the first 48 h of hospitalization, as evidenced by the passage of three or more unformed or loose stools (diarrhoea) per day for more than 2 days (Kyne *et al.*, 2000; Zar *et al.*, 2007). We excluded patients that were currently taking laxatives or had loose stools that were definitely associated with an enema. The final protocol was approved by each of the applicable independent ethics committees prior to the start of the trial. All participants were required to provide a written informed consent form prior to being included in the study. This study was registered at Clinicaltrials.gov (NCT01560832).

Loose stool samples of the enrolled patients were submitted to the clinical microbiology laboratory of the associated hospital. These samples were transferred in an airtight container (to allow for an anaerobic environment) to a central laboratory (Severance Hospital, Yonsei University College of Medicine), and the anaerobic culture and enzyme immunoassay (EIA) for toxins A and B were performed within 24 h after sample collection (Crobach *et al.*, 2009). If *C. difficile* was cultured from a stool sample, then PCR assays for toxins A and B and the binary toxin genes, as well as PCR ribotyping assay, were conducted.

Data collection. Patients were enrolled prospectively at each site. Relevant clinical and laboratory data were obtained from the electronic medical records and/or by prospectively interviewing the patient or his/her legally responsible representative in order to complete a standardized questionnaire. Information was obtained regarding the symptoms experienced and the frequency of diarrhoea. The electronic medical records were used to identify the demographic variables, laboratory findings, comorbid diseases, and history of admission and antibiotic use. We categorized the invasive procedures into the following seven types, with possible multiple selection in one patient: urinary tract procedures including urinary catheter insertion, invasive blood stream procedures including central catheter insertion and haemodialysis and/or continuous renal replacement therapy, respiratory tract or pleural cavity procedures including endotracheal tube insertion and mechanical ventilation, gastrointestinal surgery, non-gastrointestinal surgery, non-surgical gastrointestinal tract procedures including naso-gastric (Levin) tube insertion, and central nervous system procedures including cerebrospinal fluid tapping. The total number of patients admitted and the number of days each patient was hospitalized during the entire study period were obtained by using each hospital's computerized database to assess the number of patient-days, which was used for the calculation of the incidence of CDI.

Definitions. The CDI severity score was calculated for all of the participants, with severe CDI being defined as when a patient has a score greater than 2 (Zar *et al.*, 2007). The criteria for a severity score of 1 are: (1) >60 years of age, (2) temperature >38.3 °C, (3) peripheral white blood cell count >15 000 μl^{-1}, or (4) serum albumin level <2.5 mg dl^{-1} (Zar *et al.*, 2007). The criteria for a severity score of 2 are: (1) endoscopic evidence of PMC or (2) treatment in the intensive care unit (Zar *et al.*, 2007). Toxigenic culture-confirmed CDI was defined as when a case has a positive result for the A/B and/or binary toxin gene PCR test (i.e. $A^+B^+CDT^+$, $A^+B^+CDT^-$, $A^-B^+CDT^-$) of an anaerobically cultured colony of the *C. difficile* strain.

A hospital-onset case was defined as a patient with diarrhoea onset more than 48 h after the time of admission to a hospital (Kyne *et al.*, 2000; Zar *et al.*, 2007). The incidence rate of toxigenic culture-confirmed CDI cases was expressed as the number of cases per 10 000 patient-days.

***C. difficile* anaerobic culture.** A total of 348 fresh, non-duplicated stool specimens were collected from patients with suspected CDI at the three hospitals throughout the study period. Alcohol-shocked stool specimens were cultured anaerobically on *C. difficile* selective agar (CDSA; Becton Dickinson) for 48 h at 37 °C. Suspected *C. difficile* colonies were used to make Gram-stained smears to observe the typical morphology. Species were identified using the ATB 32A system (bioMérieux).

Toxin analysis by EIA and PCR. The commercial *C. difficile* toxin A & B EIA (VIDAS CDAB assay; bioMérieux) was performed according to the manufacturer's instructions. The EIA test results were interpreted as positive, negative or equivocal for both toxins A and B. Specimens with equivocal results were retested.

C. difficile toxin genes were detected by PCR as described previously (Kato *et al.*, 1998; Stubbs *et al.*, 2000). The primer pairs used were NK9/NK11 for the repetitive domain of the toxin A gene (*tcdA* rep), NK104/NK105 for the toxin B gene (*tcdB*), *cdtA* pos/*cdtA* rev for the *C. difficile* binary toxin A gene (*cdtA*), and *cdtB* pos/*cdtB* rev for the *C. difficile* binary toxin B gene (*cdtB*) (Kato *et al.*, 1998; Stubbs *et al.*, 2000; Terhes *et al.*, 2004). The reference strains VPI 10463

($A^+B^+CDT^-$), 3608/03 ($A^-B^-CDT^-$), SE844 ($A^+B^+CDT^+$), 48489 (CDT^+), 1470 ($A^-B^+CDT^-$) and UK078 were used as controls (Alonso *et al.*, 2005; Kim *et al.*, 2010).

PCR ribotyping. PCR ribotyping was performed as previously described with primers 5′-CTGGGGTGAAGTCGTAACAAGG-3′ (position 1445 to 1466 of the 16S rRNA gene) and 5′-GCGCCCTT-TGTAGCTTGACC-3′ (position 20 to 1 of the 23S rRNA gene) (Stubbs *et al.*, 1999). The PCR ribotyping patterns were compared visually. Ribotype patterns that differed by at least one band were assigned to different ribotype groups, which were designated by upper- and lower-case letters combined with a number.

Statistical analyses. The data are expressed as number (percentage), mean ± standard deviation (SD) or median (interquartile range). Either the two-tailed unpaired Student's *t*-test or the Mann–Whitney *U*-test was used for comparisons of continuous variables with or without a normal distribution, respectively, between the *C. difficile* toxigenic culture-positive (confirmed) and -negative groups. We used the analysis of variance (ANOVA) test and the Kruskal–Wallis test as the parametric and non-parametric method, respectively, for the comparisons of continuous variables between the three hospitals. For the nominal variables, either Pearson's χ^2-test or Fisher's exact test was used in the univariate analyses. We performed a multivariate logistic regression analysis using the variables with a *P*-value less than 0.05 in the univariate analyses to identify the risk factors of toxigenic culture-confirmed hospital-onset CDI. The final regression model was expressed using the odds ratio (OR) and 95 % confidence interval (CI). A two-tailed $P<0.05$ was considered to be statistically significant. All of the statistical analyses in this study were performed using SPSS 20.0 software (SPSS).

RESULTS

Incidence of toxigenic culture-confirmed hospital-onset CDI

The total incidence of toxigenic culture-confirmed hospital-onset CDI in hospitalized adult patients was 2.70 cases per 10 000 patient-days. The incidence by hospital was 2.90, 3.14 and 1.03 cases per 10 000 patient-days in hospitals A (2500 beds), B (2000 beds) and C (1000 beds), respectively. The percentage of severe toxigenic culture-confirmed hospital-onset CDI cases was 3.1 % (3 of 98 cases). The crude all-cause in-hospital mortality during the enrolment admission and the attributed mortality of CDI in 98 patients with toxigenic culture-confirmed hospital-onset CDI were 3.1 and 0 %, respectively.

Toxigenic *C. difficile* culture and PCR ribotype

Among the 348 stool specimens collected, 115 (33.0 %) were identified as growing *C. difficile*. Among the 115 anaerobically cultured isolates, toxigenic culture-confirmed CDI was 98 (85.2 %) cases. Among the 98 toxigenic-culture positive specimens, there were 82 (83.7 %) isolates of the $A^+B^+CDT^-$ strain, 11 (11.2 %) isolates of the $A^-B^+CDT^-$ strain and 5 (5.1 %) isolates of the $A^+B^+CDT^+$ strain.

Table 1. Results of PCR ribotyping analyses of the toxigenic *C. difficile* culture-positive stool samples

Kim's ribotype	UK ribotype*	No. (%)			
		Total (*N*=98)	Hospital A† (*N*=24)	Hospital B† (*N*=62)	Hospital C† (*N*=12)
$A^+B^+CDT^-$ strains					
AB1	001	3 (3.1)	0 (0)	3 (4.8)	0 (0)
AB2	014	8 (8.3)	1 (4.2)	7 (11.3)	0 (0)
AB3	NM	9 (9.3)	1 (4.2)	5 (8.1)	3 (25.0)
AB4	161	1 (1.0)	0 (0)	1 (1.6)	0 (0)
AB5	002	1 (1.0)	0 (0)	1 (1.6)	0 (0)
AB6	012	2 (2.0)	1 (4.2)	1 (1.6)	0 (0)
AB8	163	1 (1.0)	0 (0)	0 (0)	1 (8.3)
AB9	NM	1 (1.0)	0 (0)	1 (1.6)	0 (0)
AB17	018	47 (48.1)	15 (62.5)	25 (40.3)	7 (58.4)
AB19	NM	1 (1.0)	0 (0)	1 (1.6)	0 (0)
AB22	NM	2 (2.0)	0 (0)	2 (3.3)	0 (0)
AB36	NM	1 (1.0)	0 (0)	1 (1.6)	0 (0)
AB45	NM	2 (2.0)	1 (4.2)	1 (1.6)	0 (0)
AB49	NM	2 (2.0)	0 (0)	2 (3.3)	0 (0)
AB50	NM	1 (1.0)	1 (4.2)	0 (0)	0 (0)
$A^-B^+CDT^-$ strains					
aB	017	11 (11.2)	3 (12.5)	8 (12.9)	0 (0)
$A^+B^+CDT^+$ strains					
C2	078	2 (2.0)	0 (0)	1 (1.6)	1 (8.3)
C3	122	2 (2.0)	1 (4.2)	1 (1.6)	0 (0)
C17	267	1 (1.0)	0 (0)	1 (1.6)	0 (0)

*NM, Not matched.

†Hospitals A, B and C have a total of 2500, 2000 and 1000 beds, respectively.

PCR ribotyping analysis revealed 19 different patterns in the 98 toxigenic strains. There were 15 patterns among the $A^+B^+CDT^-$ strains, one among the $A^-B^+CDT^-$ strains, and three among the $A^+B^+CDT^+$ strains. All of the $A^-B^+CDT^-$ strains showed the same ribotype pattern, which was identical to the pattern of the *C. difficile* 1470 strain (UK ribotype 017). UK ribotype 018 was the predominant type (47 of 98 isolates, 48.1 %). The most common type in each of the three centres was UK ribotype 018. The percentage of UK ribotype 017 in hospitals A and B was 12.5 % and 12.9 %, respectively. However, UK ribotype 017 was not found in hospital C, which has a relatively small number of beds. There were no hypervirulent BI/NAP1/027 isolates (UK ribotype 027) identified in this study (Table 1).

Comparison of clinical features between the toxigenic culture-confirmed CDI group and the culture-negative group

The mean age of the toxigenic culture-confirmed CDI group was older than the toxigenic culture-negative group with borderline statistical significance (64.6 ± 14.4 vs 61.1 ± 16.4 years, $P=0.057$), and there was no difference between the two groups with regard to gender. The time between the admission of the patient and the occurrence of diarrhoea was significantly longer in the toxigenic culture-confirmed CDI group than in the culture-negative group (34.1 ± 20.1 vs 14.3 ± 10.0 days, $P=0.013$). The distributions of diarrhoea-associated symptoms, including nausea, abdominal pain and median maximal frequency of diarrhoea, were similar between the two groups. Also, the blood pressure and the clinical or laboratory findings of ileus, megacolon, body temperature, white blood cell count, and serum creatinine level at the time of loose stool sampling were not different between the two groups (Table 2).

The toxigenic culture-confirmed CDI group had significantly higher readmission within 3 months after hospital discharge, and also had a higher rate of having had an invasive procedure during the current admission than the toxigenic culture-negative group (46.9 vs 30.0 %, $P=0.003$, and 90.8 vs 75.2 %, $P<0.001$, respectively). The most common types of invasive procedure were blood stream procedures, including central catheter insertion and haemodialysis and/or continuous renal replacement therapy, in both the toxigenic culture-confirmed CDI group and the culture-negative group. There was no significant difference in the frequency of invasive procedure type between the two groups. The duration from first admission in the previous 3 months to occurrence of diarrhoea was not different between the two groups (47.5 ± 28.5 vs 49.5 ± 24.0 days, $P=0.713$). However, the total admission duration before the first occurrence of diarrhoea within the previous 3 months was significantly longer in the toxigenic culture-confirmed CDI group than in the culture-negative group (32.3 ± 26.9 vs 19.8 ± 17.0 days, $P<0.001$) (Table 2).

Among the comorbid conditions, only the frequency of cerebrovascular disease was significantly higher in the toxigenic culture-confirmed CDI group than in the culture-negative group (21.4 vs 11.2 %, $P<0.001$). Having a positive toxin EIA or toxin gene PCR with a history of CDI within the previous 3 months was significantly higher in the toxigenic culture-confirmed CDI group than in the culture-negative group (12.2 vs 0.4 %, $P<0.001$). However, the enteral nutrition history during the previous 3 months and the mortality rate at the time of the current admission were not significantly different between the two groups (Table 2).

We have summarized the comprehensive epidemiological data of the three centres and marked the variables with statistically significant difference in Table 2. Each centre had different characteristics with regard to patient population, number of beds, etc.

Characteristics of antibiotic use in the toxigenic culture-confirmed CDI group and culture-negative group

We compared the antibiotic exposure history within 3 months of the occurrence of diarrhoea between the two groups. The frequency of previous total antibiotic exposure history was significantly higher in the toxigenic culture-confirmed CDI group than in the culture-negative group (39.8 vs 27.6 %, $P<0.001$). Also, the toxigenic culture-confirmed CDI group had a significantly longer cumulative total antibiotic exposure than the culture-negative group (12.4 ± 10.3 vs 4.9 ± 2.7 days, $P=0.002$). However, in the analyses for each antibiotic class, only the carbepenems showed significant differences between the two groups in both the frequency of exposure history and the duration of cumulative exposure (43.9 vs 34.0 %, $P=0.017$, and 10.6 ± 7.6 vs 6.6 ± 2.6 days, $P=0.020$, respectively). There were no significant differences between the two groups with regard to broad-spectrum penicillin with an antipseudomonal effect, third-generation cephalosporins with or without the antipseudomonal effect, quinolones, intravenous glycopeptides, or clindamycin (Table 3).

Risk factors for toxigenic culture-confirmed CDI

In the multivariate logistic regression analysis, a history of invasive procedure during the current admission (OR 7.3, 95 % CI 2.0–27.1, $P=0.003$) and laboratory (toxin EIA or toxin gene PCR)-confirmed CDI history within the previous 3 months (OR 28.5, 95 % CI 3.0–266.8, $P=0.003$) were independent risk factors for toxigenic culture-confirmed CDI (Table 4).

DISCUSSION

The worldwide incidence of CDI has increased steadily over the past decades, but recent data suggest that its incidence and severity are now markedly increasing in Western countries (O'Connor *et al.*, 2009; Pépin *et al.*,

Table 2. Comparison of demographic and clinical characteristics between the toxigenic culture-confirmed CDI group and the culture-negative group

Variable	Total toxigenic culture-confirmed CDI group (*N*=98)	Total toxigenic culture-negative group (*N*=250)	*P**	Hospital A (*N*=106)†	Hospital B (*N*=191)†	Hospital C (*N*=51)†
Age (years)	64.6±14.4	61.1±16.4	0.057[a]	57.8±14.7	63.4±16.3	66.2±15.0‡
Gender, male	52 (53.1)	141 (56.4)	0.573[b]	67 (63.2)	96 (50.3)	30 (58.8)
Duration from admission to occurrence of diarrhoea (days)	34.1±20.1	14.3±10.0	0.013[a]	10.3±12.6	16.4±18.1	15.1±19.1‡
Maximal frequency of diarrhoea (day^{-1})	5 (4–7)	4 (4–7)	0.260[c]	5 (3–8)	4 (4–6)	5 (3–6)
Associated symptoms, yes						
Abdominal pain	38 (38.8)	80 (32.0)	0.230[b]	26 (24.5)	15 (7.9)	12 (23.5)‡
Fever (⩾38 °C)	16 (16.3)	23 (9.2)	0.058[b]	12 (11.3)	12 (6.3)	15 (29.4)‡
Nausea	7 (7.1)	18 (7.2)	0.985[d]	18 (17.0)	4 (2.1)	5 (5.9)‡
Clinical and laboratory findings at the time of loose stool sampling						
Systolic BP (mmHg)	117.5±18.2	116.7±17.5	0.702[a]	109.3±13.9	123.4±18.1	108.9±13.4‡
Diastolic BP (mmHg)	72.5±12.5	73.2±40.8	0.858[a]	68.9±10.2	77.4±46.3	65.2±7.2‡
Ileus, yes	11 (11.2)	19 (7.6)	0.279[b]	3 (2.8)	13 (6.8)	14 (27.5)‡
Megacolon, yes	0 (0)	0 (0)	–	0 (0)	0 (0)	0 (0)
WBC (mm^{-3})	9787±7801	8799±6728	0.249[a]	8576±8332	9232±6681	9443±5628
Creatinine (mg dl^{-1})	1.14±1.14	1.25±1.42	0.495[a]	0.96±0.66	1.24±1.44	1.62±1.78‡
Body temperature (°C)	37.0±0.8	37.1±0.9	0.231[a]	36.9±0.7	37.1±0.9	37.2±1.0
Admission history within 3 months			0.003[b]			
Initial hospitalization	52 (53.1)	175 (70.0)		65 (61.3)	127 (66.5)	35 (68.6)
Readmission	46 (46.9)	75 (30.0)		41 (38.7)	64 (33.5)	16 (31.4)
Duration from first admission in the previous 3 months to occurrence of diarrhoea (days)	47.5±28.5	49.5±24.0	0.713[a]	64.0±22.1	43.1±24.9	44.1±23.8‡
Total admission duration until occurrence of diarrhoea within the previous 3 months (days)	32.3±26.9	19.8±17.0	<0.001[a]	20.6±21.0	25.3±24.3	21.6±24.6
Invasive procedure during current admission, yes§	89 (90.8)	188 (75.2)	<0.001[b]	77 (72.6)	164 (85.9)	36 (70.6)‡
Urinary tract[e]	46/226 (20.5)	90/454 (19.8)		1/103 (1.0)	117/462 (25.3)	18/112 (16.1)
Blood stream[f]	60/226 (26.5)	136/454 (30.0)		52/103 (50.5)	121/462 (26.2)	23/112 (20.5)
Respiratory or pleural cavity[g]	31/226 (13.7)	50/454 (11.0)		5/103 (4.9)	58/462 (12.6)	18/112 (16.1)
Surgery						
GI tract	6/226 (2.7)	28/454 (6.2)		20/103 (19.4)	11/462 (2.4)	3/112 (2.7)
Non-GI tract	36/226 (15.9)	67/454 (14.8)		18/103 (17.5)	58/462 (12.5)	27/112 (24.0)
Non-surgical GI tract[h]	47/226 (20.7)	80/454 (17.6)		7/103 (6.7)	97/462 (21.0)	20/112 (17.9)
Central nervous system[i]	0/226 (0)	3/454 (0.6)		0/103 (0)	0/462 (0)	3/112 (2.7)
Comorbid diseases, yes						
Any cancer in the last six years	24 (24.5)	67 (26.8)	0.645[b]	6 (5.7)	72 (37.7)	13 (25.5)‡
Type 2 DM	27 (27.6)	67 (26.8)	0.903[b]	21 (19.8)	54 (28.3)	19 (37.3)
Heart disease	15 (15.3)	34 (13.6)	0.691[b]	3 (2.8)	36 (18.8)	10 (19.6)‡
Hypertension	50 (51.0)	110 (44.0)	0.250[b]	39 (36.8)	90 (47.1)	31 (60.8)‡
Renal insufficiency	13 (13.3)	20 (8.0)	0.135[b]	3 (2.8)	16 (8.4)	14 (27.5)‡
Hepatic insufficiency	9 (9.2)	32 (12.8)	0.341[b]	14 (13.2)	18 (9.4)	9 (17.6)

Table 2. cont.

Variable	Total toxigenic culture-confirmed CDI group (N=98)	Total toxigenic culture-negative group (N=250)	P*	Hospital A (N=106)†	Hospital B (N=191)†	Hospital C (N=51)†
Pulmonary disease	14 (14.3)	29 (11.6)	0.502[b]	8 (7.5)	20 (10.5)	15 (29.4)‡
Cerebrovascular disease	21 (21.4)	28 (11.2)	0.014[b]	1 (0.9)	35 (18.3)	13 (25.5)‡
Enteral nutrition history during previous 3 months, yes	11 (11.2)	13 (5.2)	0.088[b]	6 (5.7)	19 (9.9)	5 (9.8)
Laboratory (toxin EIA or toxin gene PCR)-positive CDI history within previous 3 months, yes	12 (12.2)	1 (0.4)	<0.001[d]	3 (2.8)	9 (4.7)	1 (2.0)
Discharge status at current admission, death	3 (3.1)	17 (6.8)	0.200[d]	5 (4.7)	13 (6.8)	2 (3.9)

Data are expressed as mean ± SD, median (interquartile range) or number (percentage). Abbreviations: BP, blood pressure; WBC, white blood cell; GI, gastrointestinal; DM, diabetes mellitus.
*Univariate comparison between the total toxigenic culture-confirmed CDI group and the culture-negative group.
[a]Independent sample Student's t-test, [b]Pearson's χ^2-test, [c]Mann–Whitney U-test, [d]Fisher's exact test.
†Hospitals A, B and C have 2500, 2000 and 1000 beds, respectively. N, Total number of prospectively enrolled patients irrespective of the toxigenic *C. difficile* culture result in each hospital.
‡P<0.05 in comparisons between hospitals A, B and C using one-way ANOVA and χ^2-test in continuous and categorical variables, respectively.
§Possible multiple selection in one patient. Including: [e]urinary catheter insertion; [f]central catheter insertion and haemodialysis and/or continuous renal replacement therapy; [g]endotracheal tube insertion and mechanical ventilation; [h]naso-gastric (Levin) tube insertion; [i]cerebrospinal fluid tapping.

2004; Ricciardi *et al.*, 2007). This multicentre prospective observational study revealed that the incidence of toxigenic culture-confirmed hospital-onset CDI in South Korea was 2.7 cases per 10 000 patient-days. The percentage of these cases having severe CDI was relatively low (3.1 %). To our knowledge, this is the first multicentre prospective epidemiological study to include PCR ribotyping analysis and to assess the risk factors for hospital-onset CDI in South Korea. In another Korean study, performed in a single hospital, the incidence of healthcare-associated CDI, defined as the number of cases for which *C. difficile* cultured from stool specimens contained toxin genes confirmed by multiplex PCR or by a positive toxin A & B EIA among patients who developed diarrhoea with onset at least 3 days after admission or within 2 months of the most recent hospital discharge, was 7.16 cases per 10 000 patient-days (Kim *et al.*, 2013a). There have also been several recent studies that have reported that hospital-acquired CDI has significantly increased in South Korea (Byun *et al.*, 2009; Lee *et al.*, 2010). The most recent multinational data from a Europe-wide, hospital-based survey reported a mean incidence of healthcare-associated CDI of 4.1 cases per 10 000 patient-days (Bauer *et al.*, 2011). However, this incidence was significantly variable between countries, ranging between 0.0 and 36.3 (Bauer *et al.*, 2011). A recent prospective surveillance study in Canada showed that the incidence of healthcare-associated CDI was 6.5 cases per 10 000 patient-days between 2002 and 2007 (Gravel *et al.*, 2009). In the USA, the healthcare facility-onset and -associated CDI incidence was 8.5 cases per 10 000 patient-days in 2006 (Dubberke *et al.*, 2010). The relatively low incidence in our study compared with those in other studies in Korea and Western countries may be due to our more accurate definition of laboratory-confirmed CDI and narrowed restriction to only toxigenic culture-positive cases. In addition, the different rates of CDI between centres could be because of the difference in characteristics of patients among centres.

Shin *et al.* (2008a, b) have reported that toxin A-negative and toxin B-positive ($tcdA^-tcdB^+$) strains were prevalent in Korea from 2003, and that this strain constituted 27 % of toxigenic strains in 2005, which is much higher than reported in other countries, and that this may have been responsible for the high rate of PMC in both a multicentre and a single-centre retrospective study. However, the $A^-B^+CDT^-$ variant strain was only present in 11.2 % of the 98 toxigenic isolates in our multicentre prospective study. Recently, Kim *et al.* (2010) have reported that binary toxin CDT-producing strains are not rare (7.1 %) in Korea. In the present study, there were five CDT-producing strains (5.1 %) among the toxigenic isolates, similar to the results of the previous study (Kim *et al.*, 2010). The most prevalent PCR ribotype was UK 018, and all of the $A^-B^+CDT^-$ strains showed only one pattern (UK 017). In contrast to previous Korean studies, this study had a lower prevalence of the $A^-B^+CDT^-$ variant strain. We did not identify the hypervirulent UK ribotype 027 strains in our study, although we did identify another binary

Table 3. Comparisons of antibiotic exposure history and cumulative duration within 3 months from occurrence of diarrhoea between toxigenic culture-confirmed CDI group and culture-negative group

Variable	Toxigenic culture-confirmed CDI group (N=98)	Toxigenic culture-negative group (N=250)	P
Any antibiotics			
Yes	39 (39.8)	69 (27.6)	<0.001[a]
Cumulative duration (days)	12.4±10.3	4.9±2.7	0.002[b]
Broad-spectrum penicillins with antipseudomonal effect			
Yes	45 (45.9)	118 (47.2)	0.586[a]
Cumulative duration (days)	6.6±5.6	6.0±5.5	0.623[b]
Third-generation cephalosporins without antipseudomonal effect			
Yes	39 (39.8)	97 (38.8)	0.407[a]
Cumulative duration (days)	3.2±2.4	3.9±2.7	0.401[b]
Third-generation cephalosporins with antipseudomonal effect			
Yes	18 (18.4)	30 (12.0)	0.056[a]
Cumulative duration (days)	1.8±0.9	1.2±0.1	0.221[b]
Carbapenems			
Yes	43 (43.9)	85 (34.0)	0.017[a]
Cumulative duration (days)	10.6±7.6	6.6±2.6	0.020[b]
Quinolones			
Yes	49 (50.0)	134 (53.6)	0.813[a]
Cumulative duration (days)	6.9±4.7	6.5±5.9	0.720[b]
Intravenous glycopeptides			
Yes	39 (39.8)	88 (35.2)	0.149[a]
Cumulative duration (days)	8.0±3.3	5.4±3.0	0.102[b]
Clindamycin			
Yes	2 (2.0)	12 (4.8)	0.299[c]
Cumulative duration (days)	0.3±0.1	0.8±0.3	0.336[b]

Data are expressed as either mean±SD or number (percentage). [a]Pearson's χ^2-test, [b]independent sample Student's t-test, [c]Fisher's exact test.

toxin-producing strain, UK ribotype 078. A European multinational hospital-based survey (Bauer *et al.*, 2011) has also identified UK ribotype 018 as the predominant type associated with complicated disease outcome in an area where UK ribotype 027 was absent, similar to our results.

Our study has the unique strength of having prospective data collection from multiple centres and the most accurate method of CDI diagnosis of toxigenic culture-confirmed cases, instead of using an EIA with a low sensitivity of 63–73 %, in contrast with other Korean CDI epidemiological and surveillance studies (Delmée *et al.*, 2005; Peterson *et al.*, 2007). We applied identical definitions and used the same data collection method to measure many of the clinical variables. In addition, the microbiological diagnoses for CDI were uniformly performed using a standardized protocol in one central laboratory. We also used alcohol-shocked enrichment stool specimens in anaerobic *C. difficile* culture to improve the sensitivity of its growth (Riley *et al.*, 1987).

The independent risk factors for the development of hospital-onset CDI in our study were (1) experience of an invasive procedure during the current admission period, and (2) a laboratory (toxin EIA or toxin gene PCR)-confirmed

Table 4. Multivariate logistic regression analysis to identify risk factors of toxigenic culture-confirmed hospital-onset CDI

Independent variables, yes	OR	95 % CI	P
Duration from admission to occurrence of diarrhoea >2 weeks	1.1	0.6–2.2	0.737
Readmission within 3 months	1.6	0.9–3.0	0.139
Total admission duration during previous 3 months to occurrence of diarrhoea >3 weeks	1.4	0.6–2.9	0.417
Invasive procedure during current admission	7.3	2.0–27.1	0.003
Cerebrovascular disease	1.0	0.5–2.2	0.973
Laboratory (toxin EIA or toxin gene PCR)-positive CDI history within previous 3 months	28.5	3.0–266.8	0.003
Exposure history of carbapenems within 3 months from occurrence of diarrhoea	1.1	0.6–1.9	0.847

CDI history. Previously, non-surgical gastrointestinal procedures had been identified as risk factors of *C. difficile*-associated diarrhoea (Bignardi, 1998). However, we did not find an independent relationship between any specific type of invasive procedure and the development of hospital-onset CDI. Unlike the studies from Western countries that had reported IBD as an emerging risk factor for CDI, there were no CDI patients with comorbid IBD in our study. Another nationwide study in South Korea had reported the rate of CDI patients with IBD to be as low as 2.2 % (30 of 1367) (Kim *et al.*, 2013b). In contrast with the previous reports, we did not find the independent relation between the recent exposure history or cumulative duration of any specific antibiotic, including the well-known CDI-triggering antibiotics such as clindamycin, and the development of hospital-onset CDI (Freeman *et al.*, 2010; Lo Vecchio & Zacur, 2012). Although the recent exposure history and cumulative duration of any antibiotics and carbepenems were significantly higher and longer in the toxigenic culture-confirmed CDI group than in the culture-negative group in the univariate analyses, the final multivariate regression model revealed the stronger effect of invasive procedure and past history of a laboratory-confirmed CDI. The frequency of CDI in patients who had not recently received antibiotic treatment was higher in our study than in other studies (Kim *et al.*, 2013a). Hospital-onset toxigenic culture-confirmed CDI developed in as many as 60 % of patients who had not received any antibiotics within the previous 3 months. Our results might reflect the recent epidemiological change in CDI and the importance of *C. difficile* spore acquisition through the hospital environment rather than the disruption of colonic normal flora by antibiotics in the development of hospital-onset CDI (Lessa *et al.*, 2012). Therefore, active and continuous implementation of environmental control polices may have to be emphasized to decrease the incidence of hospital-onset CDI.

This study has several limitations. First, we were unable to perform endoscopic evaluation in all of our patients to assess the exact severity of each CDI case. Therefore, it is possible that we may have underestimated the number of severe CDI cases. Second, we did not collect the history of gastric acid suppressant use, including exposure to proton pump inhibitors, which is proposed as one of the risk factors for CDI (Freeman *et al.*, 2010). However, a recent study found no association between gastric acid suppressants and a more severe course of CDI (Henrich *et al.*, 2009). It is still a matter of ongoing debate as to whether there is a causal association between gastric acid suppressants and CDI (Freeman *et al.*, 2010; Lo Vecchio & Zacur, 2012). Third, our study only used tertiary hospitals with at least 1000 beds; the study subjects from these hospitals tend to be more severely ill than patients that are admitted to smaller hospitals. Therefore, this incidence of 2.7 cases per 10 000 patient-days might be overestimated compared with the general nationwide surveillance data. Finally, the ratio of toxigenic culture-confirmed CDI (N=98) to antibiotic-associated diarrhoea, that is, total collected loose stool specimens (N=348), was relatively high at 28.2 %. Because two centres should send the stool samples to one central laboratory, they possibly would drop the suspected CDI cases, and with this, CDI incidence could be underestimated, especially in hospital C with the relative low incidence. However, despite these limitations, this well-designed and prospectively performed study provides important information concerning the clinical impact of CDI in South Korea without epidemics of hypervilurent UK ribotype 027 strains.

In conclusion, the incidence of toxigenic culture-confirmed hospital-onset CDI in South Korea was 2.7 cases per 10 000 patient-days. Although the incidence and clinical features of hospital-onset CDI are, overall, respectively low and milder than those in Western countries, the considerable burden of CDI suggests that South Korea is no longer a safe area with regard to CDI. Therefore, preventive strategies, including active immunization, should be considered to decrease the mortality and morbidity caused by CDI and to lower healthcare costs associated with hospital-acquired CDI, especially in patients with the identified risk factors.

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REFERENCES

Alonso, R., Martín, A., Peláez, T., Marín, M., Rodríguez-Creixéms, M. & Bouza, E. (2005). Toxigenic status of *Clostridium difficile* in a large Spanish teaching hospital. *J Med Microbiol* **54**, 159–162.

Anand, A., Bashey, B., Mir, T. & Glatt, A. E. (1994). Epidemiology, clinical manifestations, and outcome of *Clostridium difficile*-associated diarrhea. *Am J Gastroenterol* **89**, 519–523.

Barbut, F., Richard, A., Hamadi, K., Chomette, V., Burghoffer, B. & Petit, J. C. (2000). Epidemiology of recurrences or reinfections of *Clostridium difficile*-associated diarrhea. *J Clin Microbiol* **38**, 2386–2388.

Barbut, F., Decré, D., Lalande, V., Burghoffer, B., Noussair, L., Gigandon, A., Espinasse, F., Raskine, L., Robert, J. & other authors (2005). Clinical features of *Clostridium difficile*-associated diarrhoea due to binary toxin (actin-specific ADP-ribosyltransferase)-producing strains. *J Med Microbiol* **54**, 181–185.

Bartlett, J. G. (1994). *Clostridium difficile*: history of its role as an enteric pathogen and the current state of knowledge about the organism. *Clin Infect Dis* **18** (Suppl. 4), S265–S272.

Bartlett, J. G., Chang, T. W., Gurwith, M., Gorbach, S. L. & Onderdonk, A. B. (1978). Antibiotic-associated pseudomembranous colitis due to toxin-producing clostridia. *N Engl J Med* **298**, 531–534.

Bauer, M. P., Notermans, D. W., van Benthem, B. H., Brazier, J. S., Wilcox, M. H., Rupnik, M., Monnet, D. L., van Dissel, J. T., Kuijper, E. J. & ECDIS Study Group (2011). *Clostridium difficile* infection in Europe: a hospital-based survey. *Lancet* **377**, 63–73.

Bignardi, G. E. (1998). Risk factors for *Clostridium difficile* infection. *J Hosp Infect* **40**, 1–15.

Byun, T. J., Han, D. S., Ahn, S. B., Cho, H. S., Kim, T. Y., Eun, C. S., Jeon, Y. C., Sohn, J. H. & Kang, J. O. (2009). [Clinical characteristics and changing epidemiology of *Clostridium difficile*-associated disease (CDAD)]. *Korean J Gastroenterol* **54**, 13–19 (in Korean).

Clements, A. C., Magalhães, R. J., Tatem, A. J., Paterson, D. L. & Riley, T. V. (2010). *Clostridium difficile* PCR ribotype 027: assessing the risks of further worldwide spread. *Lancet Infect Dis* **10**, 395–404.

Crobach, M. J., Dekkers, O. M., Wilcox, M. H. & Kuijper, E. J. (2009). European Society of Clinical Microbiology and Infectious Diseases (ESCMID): data review and recommendations for diagnosing *Clostridium difficile*-infection (CDI). *Clin Microbiol Infect* **15**, 1053–1066.

Delmée, M., Van Broeck, J., Simon, A., Janssens, M. & Avesani, V. (2005). Laboratory diagnosis of *Clostridium difficile*-associated diarrhoea: a plea for culture. *J Med Microbiol* **54**, 187–191.

Dubberke, E. R., Butler, A. M., Yokoe, D. S., Mayer, J., Hota, B., Mangino, J. E., Khan, Y. M., Popovich, K. J. & Fraser, V. J. (2010). Multicenter study of *Clostridium difficile* infection rates from 2000 to 2006. *Infect Control Hosp Epidemiol* **31**, 1030–1037.

Freeman, J., Bauer, M. P., Baines, S. D., Corver, J., Fawley, W. N., Goorhuis, B., Kuijper, E. J. & Wilcox, M. H. (2010). The changing epidemiology of *Clostridium difficile* infections. *Clin Microbiol Rev* **23**, 529–549.

Gilca, R., Hubert, B., Fortin, E., Gaulin, C. & Dionne, M. (2010). Epidemiological patterns and hospital characteristics associated with increased incidence of *Clostridium difficile* infection in Quebec, Canada, 1998-2006. *Infect Control Hosp Epidemiol* **31**, 939–947.

Goorhuis, A., Bakker, D., Corver, J., Debast, S. B., Harmanus, C., Notermans, D. W., Bergwerff, A. A., Dekker, F. W. & Kuijper, E. J. (2008). Emergence of *Clostridium difficile* infection due to a new hypervirulent strain, polymerase chain reaction ribotype 078. *Clin Infect Dis* **47**, 1162–1170.

Gravel, D., Miller, M., Simor, A., Taylor, G., Gardam, M., McGeer, A., Hutchinson, J., Moore, D., Kelly, S. & other authors (2009). Health care-associated *Clostridium difficile* infection in adults admitted to acute care hospitals in Canada: a Canadian Nosocomial Infection Surveillance Program Study. *Clin Infect Dis* **48**, 568–576.

Henrich, T. J., Krakower, D., Bitton, A. & Yokoe, D. S. (2009). Clinical risk factors for severe *Clostridium difficile*-associated disease. *Emerg Infect Dis* **15**, 415–422.

Kato, H., Kato, N., Watanabe, K., Iwai, N., Nakamura, H., Yamamoto, T., Suzuki, K., Kim, S. M., Chong, Y. & Wasito, E. B. (1998). Identification of toxin A-negative, toxin B-positive *Clostridium difficile* by PCR. *J Clin Microbiol* **36**, 2178–2182.

Kim, B. N. (2005). Compliance with an infectious disease specialist's advisory consultations on targeted antibiotic usage. *J Infect Chemother* **11**, 84–88.

Kim, H., Jeong, S. H., Roh, K. H., Hong, S. G., Kim, J. W., Shin, M. G., Kim, M. N., Shin, H. B., Uh, Y. & other authors (2010). Investigation of toxin gene diversity, molecular epidemiology, and antimicrobial resistance of *Clostridium difficile* isolated from 12 hospitals in South Korea. *Korean J Lab Med* **30**, 491–497.

Kim, H., Lee, Y., Moon, H. W., Lim, C. S., Lee, K. & Chong, Y. (2011). Emergence of *Clostridium difficile* ribotype 027 in Korea. *Korean J Lab Med* **31**, 191–196.

Kim, J., Kang, J. O., Kim, H., Seo, M. R., Choi, T. Y., Pai, H., Kuijper, E. J., Sanders, I. & Fawley, W. (2013a). Epidemiology of *Clostridium difficile* infections in a tertiary-care hospital in Korea. *Clin Microbiol Infect* **19**, 521–527.

Kim, Y. S., Han, D. S., Kim, Y. H., Kim, W. H., Kim, J. S., Kim, H. S., Kim, H. S., Park, Y. S., Song, H. J. & other authors (2013b). Incidence and clinical features of *Clostridium difficile* infection in Korea: a nationwide study. *Epidemiol Infect* **141**, 189–194.

Kuehne, S. A., Cartman, S. T., Heap, J. T., Kelly, M. L., Cockayne, A. & Minton, N. P. (2010). The role of toxin A and toxin B in *Clostridium difficile* infection. *Nature* **467**, 711–713.

Kyne, L., Warny, M., Qamar, A. & Kelly, C. P. (2000). Asymptomatic carriage of *Clostridium difficile* and serum levels of IgG antibody against toxin A. *N Engl J Med* **342**, 390–397.

Kyne, L., Hamel, M. B., Polavaram, R. & Kelly, C. P. (2002). Health care costs and mortality associated with nosocomial diarrhea due to *Clostridium difficile*. *Clin Infect Dis* **34**, 346–353.

Lee, K., Park, K. H., Jeong, S. H., Lim, H. S., Shin, J. H., Yong, D., Ha, G.-Y., Chong, Y. & KONSAR (2006). Further increase of vancomycin-resistant *Enterococcus faecium*, amikacin- and fluoroquinolone-resistant *Klebsiella pneumoniae*, and imipenem-resistant *Acinetobacter* spp. in Korea: 2003 KONSAR surveillance. *Yonsei Med J* **47**, 43–54.

Lee, Y. J., Choi, M. G., Lim, C. H., Jung, W. R., Cho, H. S., Sung, H. Y., Nam, K. W., Chang, J. H., Cho, Y. K. & other authors (2010). [Change of *Clostridium difficile* colitis during recent 10 years in Korea]. *Korean J Gastroenterol* **55**, 169–174 (in Korean).

Lee, K., Kim, M. N., Kim, J. S., Hong, H. L., Kang, J. O., Shin, J. H., Park, Y. J., Yong, D., Jeong, S. H., Chong, Y. & KONSAR Group (2011). Further increases in carbapenem-, amikacin-, and fluoroquinolone-resistant isolates of *Acinetobacter* spp. and *P. aeruginosa* in Korea: KONSAR study 2009. *Yonsei Med J* **52**, 793–802.

Lessa, F. C., Gould, C. V. & McDonald, L. C. (2012). Current status of *Clostridium difficile* infection epidemiology. *Clin Infect Dis* **55** (Suppl 2), S65–S70.

Lo Vecchio, A. & Zacur, G. M. (2012). *Clostridium difficile* infection: an update on epidemiology, risk factors, and therapeutic options. *Curr Opin Gastroenterol* **28**, 1–9.

O'Connor, J. R., Johnson, S. & Gerding, D. N. (2009). *Clostridium difficile* infection caused by the epidemic BI/NAP1/027 strain. *Gastroenterology* **136**, 1913–1924.

Patriarchi, F., Rolla, M., Maccioni, F., Menichella, A., Scacchi, C., Ambrosini, A., Costantino, A. & Quattrucci, S. (2011). *Clostridium difficile*-related pancolitis in lung-transplanted patients with cystic fibrosis. *Clin Transplant* **25**, E46–E51.

Pépin, J., Valiquette, L., Alary, M. E., Villemure, P., Pelletier, A., Forget, K., Pépin, K. & Chouinard, D. (2004). *Clostridium difficile*-associated diarrhea in a region of Quebec from 1991 to 2003: a changing pattern of disease severity. *CMAJ* **171**, 466–472.

Peterson, L. R., Manson, R. U., Paule, S. M., Hacek, D. M., Robicsek, A., Thomson, R. B., Jr & Kaul, K. L. (2007). Detection of toxigenic *Clostridium difficile* in stool samples by real-time polymerase chain reaction for the diagnosis of *C. difficile*-associated diarrhea. *Clin Infect Dis* **45**, 1152–1160.

Ricciardi, R., Rothenberger, D. A., Madoff, R. D. & Baxter, N. N. (2007). Increasing prevalence and severity of *Clostridium difficile* colitis in hospitalized patients in the United States. *Arch Surg* **142**, 624–631, discussion 631.

Riley, T. V., Brazier, J. S., Hassan, H., Williams, K. & Phillips, K. D. (1987). Comparison of alcohol shock enrichment and selective enrichment for the isolation of *Clostridium difficile*. *Epidemiol Infect* **99**, 355–359.

Rubin, M. S., Bodenstein, L. E. & Kent, K. C. (1995). Severe *Clostridium difficile* colitis. *Dis Colon Rectum* **38**, 350–354.

Safdar, N. (2012). *Clostridium difficile*: the emerging epidemic. *Mayo Clin Proc* **87**, 1037–1039.

Shin, B. M., Kuak, E. Y., Yoo, H. M., Kim, E. C., Lee, K., Kang, J. O., Whang, D. H. & Shin, J. H. (2008a). Multicentre study of the prevalence of toxigenic *Clostridium difficile* in Korea: results of a retrospective study 2000–2005. *J Med Microbiol* **57**, 697–701.

Shin, B. M., Kuak, E. Y., Yoo, S. J., Shin, W. C. & Yoo, H. M. (2008b). Emerging toxin $A^{-}B^{+}$ variant strain of *Clostridium difficile* responsible for pseudomembranous colitis at a tertiary care hospital in Korea. *Diagn Microbiol Infect Dis* **60**, 333–337.

Shin, S., Kim, M., Kim, M., Lim, H., Kim, H., Lee, K. & Chong, Y. (2012). Evaluation of the Xpert *Clostridium difficile* assay for the diagnosis of *Clostridium difficile* infection. *Annals Lab Med* **32**, 355–358.

Stubbs, S. L., Brazier, J. S., O'Neill, G. L. & Duerden, B. I. (1999). PCR targeted to the 16S-23S rRNA gene intergenic spacer region of *Clostridium difficile* and construction of a library consisting of 116 different PCR ribotypes. *J Clin Microbiol* **37**, 461–463.

Stubbs, S., Rupnik, M., Gibert, M., Brazier, J., Duerden, B. & Popoff, M. (2000). Production of actin-specific ADP-ribosyltransferase (binary toxin) by strains of *Clostridium difficile*. *FEMS Microbiol Lett* **186**, 307–312.

Tae, C. H., Jung, S. A., Song, H. J., Kim, S. E., Choi, H. J., Lee, M., Hwang, Y., Kim, H. & Lee, K. (2009). The first case of antibiotic-associated colitis by *Clostridium difficile* PCR ribotype 027 in Korea. *J Korean Med Sci* **24**, 520–524.

Terhes, G., Urbán, E., Sóki, J., Hamid, K. A. & Nagy, E. (2004). Community-acquired *Clostridium difficile* diarrhea caused by binary toxin, toxin A, and toxin B gene-positive isolates in Hungary. *J Clin Microbiol* **42**, 4316–4318.

Zar, F. A., Bakkanagari, S. R., Moorthi, K. M. & Davis, M. B. (2007). A comparison of vancomycin and metronidazole for the treatment of *Clostridium difficile*-associated diarrhea, stratified by disease severity. *Clin Infect Dis* **45**, 302–307.