**Case–Control Study of Clostridium innocuum Infection, Taiwan**

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Vancomycin-resistant *Clostridium innocuum* was recently identified as an etiologic agent for antibiotic-associated diarrhea in humans. We conducted a case–control study involving 152 *C. innocuum*-infected patients during 2014–2019 in Taiwan, using 304 cases of *Clostridioides difficile* infection (CDI) matched by diagnosis year, age (+2 years), and sex as controls. The baseline characteristics were similar between the 2 groups. *C. innocuum*-infected patients experienced more extraintestinal clostridial infection and gastrointestinal tract–related complications than did patients with CDI. The 30-day mortality rate among *C. innocuum*-infected patients was 14.5%, and the overall rate was 23.0%. Chronic kidney disease, solid tumor, intensive care unit admission, and shock status were 4 independent risk factors for death. *C. innocuum* identified from clinical specimens should be recognized as a pathogen requiring treatment, and because of its intrinsic vancomycin resistance, precise identification is necessary to guide appropriate and timely antimicrobial therapy.

*Clostridium* species are obligate anaerobic, endospore-forming bacilli that usually colonize in the gastrointestinal tracts of humans. Of the >200 species of *Clostridium*, >30 are potential pathogens in humans, such as *C. perfringens* and *Clostridioides difficile*. However, *C. innocuum* has rarely been described as associated with human disease.

*C. innocuum* was first identified in the 1960s among 8 patients in the United States; the name, innocuum, described its lack of virulence (1,2). It was challenging to distinguish *C. innocuum* from other *Clostridium* species (especially *C. ramosum* and *C. clostridioforme*, together called the RIC group) because of their similar phenotypes of atypical clostridial colonial morphology, rare spore-forming features, and fatty acid pattern (3–5). Identifying *C. innocuum* has become faster and more accurate after the introduction of molecular techniques such as 16S RNA sequencing and matrix-associated laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (6).

In 1995, Cutrona et al. reported the first case of endocarditis caused by *C. innocuum* (7). Although the bacterium was considered less pathogenic and seldom caused infections previously, more and more clinical evidence has emerged since 2000s, suggesting *C. innocuum* might be a potential cause of antibiotic-associated diarrhea and of extraintestinal clostridial infection (EICI), such as bacteremia, intra-abdominal infection, and endocarditis (8–10). However, we are not aware of a study of *C. innocuum* infection with a large enough cohort of patients to describe its clinical characteristics.

Precise diagnosis of *C. innocuum* is necessary because of its unique intrinsic resistance to vancomycin, presumably caused by the presence of 2 chromosomal genes that enable the synthesis of a peptidoglycan precursor terminating in serine with low vancomycin affinity (9,11). Although vancomycin is one of the recommended antimicrobial drugs to treat infections caused by *Clostridium* species, especially *C. difficile*, intrinsic resistance to vancomycin in *C. innocuum* poses the risk for inappropriate treatment for patients who acquire *C. innocuum* infection (12). *C. difficile* is one of the most representative clostridial species to cause human disease and has been well investigated. In the United States, ≈500,000 infections were identified annually, and 15,000–30,000 deaths were associated with *C. difficile* infection (CDI) (12–14)

In previous studies, we demonstrated *C. innocuum* as a potential invasive pathogen causing severe colitis and EICI in a small case series and proved its cellular toxicity in vitro (8,9). Herein, we conducted a retrospective case–control study to describe and

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evaluate the clinical characteristics and outcomes of infections caused by *C. innocuum*. To this end, we selected case-patients with CDI as the control group.

Institute Review Boards in Chang Gung Memorial Hospital (CGMH; Taoyuan, Taiwan) approved the study, allowing review of the medical data of the patients (IRB#201900906B). A waiver of consent was granted given the retrospective nature of the project and anonymous analysis of the clinical information of patients.

**Methods**

**Study Design, Clinical Setting, and Case Enrollment**

We conducted a retrospective case–control study at CGMH during 2014–2019. CGMH is a tertiary medical center accommodating 3,700 patient beds. We selected *C. difficile* as the control to better illustrate the clinical features of *C. innocuum* infection. The case and control groups were assigned in a 1:2 ratio and matched in the diagnosed year, age, and sex.

We identified cases with *C. innocuum* and *C. difficile* infections using the rapid ID 32A system (bioMérieux, https://www.biomerieux.com) and MALDI-TOF mass spectrometry Biotaiper (Bruker Daltonik GmbH, https://www.bruker.com) (15–17). MALDI-TOF mass spectrometry was introduced in 2009 in the clinical microbiology laboratory of CGMH, but *C. innocuum* was not reported routinely because it was considered a clinically insignificant microorganism. To trace the cases infected with *C. innocuum*, we reviewed the original reporting database from the MALDI-TOF mass spectrometry system directly and identified the samples reporting *C. innocuum*. Our definition of a microbiologically confirmed *C. innocuum* infection was that the original report from the MALDI-TOF mass spectrometry database revealed *C. innocuum* in the strongest 2 signals and had signal scores >2.00. We defined *C. difficile* infections by the same rationale.

We reviewed baseline information of each patient and enlisted all patients with *C. innocuum* infection in the study. We defined *C. difficile* infection as a positive PCR-based toxin assay with presence of clinical symptoms compatible with the infection, or a positive culture of *C. difficile* with compatible clinical symptoms (e.g., documented diarrhea or radiologic features of toxic megacolon). We excluded cases with concomitant *C. innocuum* and *C. difficile* isolated from the same clinical sample from the study. For the case-control matching, 3 authors (Y.-C. Chen, Y.-C. Kuo, and M.-C. Chen) reviewed baseline information of all cases with *C. innocuum* and *C. difficile* infection. We randomly selected 2 controls for each case, matched by diagnostic year, age (±2 years), and sex of the index case. If no controls were eligible from these 3 matching variables, then we dropped the sex criterium, followed by the age criterium if necessary. After the matching process, we further reviewed the clinical information of these patients.

**Clinical Data Resources, Variables, and Definition**

We collected demographic data, laboratory testing results, images, and microbiology reports through an electronic medical record system (EMR). Demographic data were age, sex, race, underlying systemic diseases, and acquisition modality (community vs. hospital). We defined hospital-acquired infection as the symptoms that occurred >48 hours after admission, or <4 weeks after discharge from a healthcare facility; otherwise, it was classified as a community-acquired infection (18). We calculated the Charlson Comorbidity Index score for each patient to represent the baseline physiologic condition affected by underlying disease. The index is composed of 19 underlying conditions in 4 categories. Each category had a weighted score based on the risk for 1- and 10-year mortality rate (19).

We recorded clinical symptoms such as diarrhea, fever, bloody stool, abdominal pain, vomiting, and abdominal distension. We also reviewed disease-related complications, including toxic megacolon, ileus, bowel perforation, and shock. Recurrent infection was defined if the patient had a repeated microbiological culture from the same specimen source within 8 weeks of initial documented symptoms resolution (20,21). Outcome assessment included 30-day, 90-day, and overall deaths after the infection. We reviewed previous antibiotic exposures according to each class: penicillins, cephalosporins, carbapenems, carbapenems, carbapenems, fluoroquinolones, aminoglycosides, macrolides, tetracyclines, glycopeptides, oxazolidins, polymyxins, lincosamides, and metronidazole. We defined antibiotic exposure rates as the percentage of patients who received any drugs <30 days before *C. innocuum* or *C. difficile* infection and duration of antibiotic exposure as total days of any antimicrobial drug use in a patient <30 days before the event of *C. innocuum* or *C. difficile* infection.

**Bacterial Isolation and Identification**

We performed anaerobic bacterial cultures in the clinical microbiology laboratory, as described
Table 1. Baseline characteristics and clinical diagnoses in 2 groups of patients by the infecting Clostridium species in case–control study of C. innocuum infection, Taiwan*

| Variable | Total, N = 456 | C. innocuum, n = 152 | Clostridiodes difficile, n = 304 | OR (95% CI) | p value |
|----------|----------------|-----------------------|-------------------------------|-------------|---------|
| Age, mean (SD)† | 66.7 (18.2) | 66.6 (18.3) | 66.7 (18.1) | NA | 0.978 |
| Sex | | | | | |
| M | 266 (58.3) | 97 (63.8) | 169 (55.6) | 1.41 (0.94–2.10) | 0.094 |
| F | 190 (41.7) | 55 (36.2) | 135 (44.4) | 0.71 (0.48–1.06) | 0.094 |
| Hospitalization | | | | | |
| No. days, median (IQR, range)‡ | 22 (36, 0–492) | 14 (33, 0–492) | 26 (36, 0–409) | NA | <0.001 |
| Charlson Comorbidity Index, mean (SD)† | 6.1 (3.2) | 5.7 (3.2) | 6.2 (3.3) | NA | 0.100 |
| Diabetes mellitus | 135 (29.6) | 52 (34.2) | 83 (27.3) | 1.39 (0.91–2.11) | 0.128 |
| Chronic kidney disease | 122 (26.8) | 28 (18.4) | 94 (30.9) | 0.50 (0.31–0.81) | 0.005 |
| Congestive heart failure | 45 (9.9) | 12 (7.9) | 33 (10.9) | 0.70 (0.35–1.40) | 0.315 |
| AIDS | 4 (0.9) | 1 (0.7) | 3 (1.0) | 0.66 (0.07–6.44) | 0.724 |
| Solid tumor | 138 (30.3) | 42 (27.6) | 96 (31.6) | 0.83 (0.54–1.27) | 0.387 |
| Initial ICU admission | 65 (14.3) | 36 (23.7) | 29 (9.5) | 2.94 (1.72–5.03) | <0.001 |
| Acquisition of infection | | | | | |
| Hospital acquired | 354 (77.6) | 101 (66.4) | 253 (83.2) | 0.40 (0.25–0.63) | <0.001 |
| Community acquired | 102 (22.4) | 51 (33.6) | 51 (16.8) | 2.50 (1.60–3.93) | <0.001 |

Clinical diagnosis

| Variable | Total, N = 456 | C. innocuum, n = 152 | Clostridiodes difficile, n = 304 | OR (95% CI) | p value |
|----------|----------------|-----------------------|-------------------------------|-------------|---------|
| Clostridium-associated diarrhea | 375 (82.2) | 96 (63.2) | 279 (91.8) | 0.15 (0.09–0.26) | <0.001 |
| Extraintestinal clostridial infection | 81 (17.8) | 56 (36.8) | 25 (8.2) | 6.51 (3.85–11.01) | <0.001 |
| Bacteremia | 8 (1.8) | 7 (4.6) | 1 (0.3) | 14.63 (1.78–120.00) | 0.012 |
| Intra-abdominal infection | 31 (6.8) | 21 (13.8) | 10 (3.2) | 4.71 (2.16–10.23) | <0.001 |
| Biliary tract infection | 4 (0.9) | 3 (2.0) | 1 (0.3) | 6.10 (0.63–59.15) | 0.119 |
| Recurrent infection | 15 (3.3) | 0 (0) | 15 (4.9) | NA | NA |
| Skin and soft tissue infection | 36 (7.9) | 23 (15.1) | 13 (4.3) | 3.99 (1.96–8.13) | <0.001 |
| Genital tract infection§ | 2 (0.4) | 1 (2.3) | 0 (0) | NA | NA |
| Complication | | | | | |
| Ileus | 34 (7.5) | 17 (11.2) | 17 (5.6) | 2.12 (1.05–4.29) | 0.035 |
| Bowel perforation | 14 (3.0) | 11 (7.2) | 3 (1.0) | 7.83 (2.15–28.50) | 0.002 |
| Hypovolemic or septic shock | 43 (9.4) | 22 (14.5) | 21 (6.9) | 2.28 (1.21–4.30) | 0.011 |
| Mortality | | | | | |
| 30-day mortality | 81 (17.7) | 22 (14.5) | 59 (19.4) | 0.70 (0.41–1.20) | 0.195 |
| 90-day mortality | 97 (21.3) | 24 (15.8) | 73 (24.0) | 0.59 (0.36–0.99) | 0.045 |
| Overall mortality | 122 (26.7) | 35 (23.0) | 87 (28.6) | 0.77 (0.49–1.21) | 0.264 |

*Values are presented as no (%) unless otherwise indicated. NA, not applicable for continuous variables or too few events (<5) to calculate a stable OR; OR, odds ratio.
†By independent t test.
‡By Mann-Whitney test.
§Two cases were diagnosed as pyospermia and bacterial vaginitis.

Previously (9), we streaked all the anaerobic samples onto the selective agar plate, including CDC-ANA-BAP (anaerobic blood agar plate), CDC-ANA-PEA (anaerobic phenylethyl alcohol blood agar plate), and BBE/KVLB (Bacteroides bile esculin and laked kanamycin) bi-plate. We incubated agar plates in anaerobic conditions (90% N₂/10% CO₂) at 37°C for 5 days. We grossly reviewed the growing colonies on

Table 2. Antibiotic exposure before Clostridium infection in case–control study of C. innocuum infection*

| Antibiotic exposure | C. innocuum, n = 152 | Clostridiodes difficile, n = 304 | Odds ratio (95% CI) | p value |
|---------------------|-----------------------|-------------------------------|-------------------|---------|
| Any antibiotic exposure | 121 (79.6) | 289 (95.1) | 0.20 (0.11–0.39) | <0.001 |
| Mean duration of antibiotic exposure, d (SD)† | 13.7 (8.6) | 15.6 (8.3) | NA | 0.039 |
| Antibiotic exposure rate by drug class | | | | |
| Penicillins | 36 (23.7) | 107 (35.2) | 0.57 (0.37–0.89) | 0.013 |
| Cephalosporins | 81 (53.3) | 206 (67.8) | 0.54 (0.36–0.81) | 0.003 |
| Carbapenems | 36 (23.7) | 102 (33.6) | 0.62 (0.40–0.96) | 0.031 |
| Fluoroquinolones | 31 (20.4) | 108 (35.5) | 0.47 (0.29–0.74) | 0.001 |
| Aminoglycosides | 13 (8.6) | 20 (6.6) | 1.33 (0.64–2.75) | 0.444 |
| Macrolides | 3 (2.0) | 8 (2.6) | 0.75 (0.20–2.85) | 0.667 |
| Tetracyclines | 6 (3.9) | 5 (1.6) | 2.46 (0.74–8.19) | 0.143 |
| Glycopeptides | 52 (34.2) | 92 (30.3) | 1.20 (0.79–1.81) | 0.393 |
| Oxazolids | 0 (0) | 2 (0.7) | NA | NA |
| Polymyxins | 6 (3.9) | 11 (3.6) | 1.10 (0.40–3.02) | 0.861 |
| Lincosamides | 6 (3.9) | 21 (6.9) | 0.55 (0.22–1.40) | 0.213 |
| Metronidazole | 16 (10.5) | 33 (10.9) | 0.97 (0.51–1.81) | 0.915 |

*Data are presented as no (%) unless otherwise indicated. NA, not applicable for continuous variables or too few events (<5) to calculate a stable odds ratio.
†By independent student t test. The p value of odds ratio was analyzed by univariate logistic regression.
plate and analyzed 1 representative colony for each agar plate by the rapid ID 32A system (bioMérieux) for identification of the microorganisms.

Antimicrobial Susceptibility Testing
We tested antimicrobial susceptibilities to clindamycin, metronidazole, penicillin, piperacillin, and ampicillin/sulbactam by the break-point agar dilution method according to Clinical and Laboratory Standards Institute criteria (document M11-A8) for anaerobic bacteria (22). We used interpretive criteria in document M100S to determine susceptibility (22).

Statistical Analysis
We performed statistical analysis by SPSS Statistics 24.0 (SPSS Inc., https://www.ibm.com/products/spss-statistics). For continuous variables, we determined significance by using the independent t test or Mann-Whitney U test as appropriate. If the continuous variable had outliers and did not fit the normal distribution, variables were shown as median (interquartile range, range). We analyzed the categorical variables by \( \chi^2 \) test and considered \( p<0.05 \) statistically significant. We obtained odds ratios (ORs) from cross-tabulation and analyzed the \( p \) value of ORs by univariate logistic regression. We estimated mortality rate at 30 days and 90 days after the positive culture and analyzed by Kaplan-Meier survival analysis using methods described previously (23). In addition, we examined risk factors associated with 30- and 90-day mortality in both groups by logistic regression.

Results

Participants and Demographic Information
By the MALDI-TOF mass spectrometry system, 180 samples yielded the growth of \( C. \) innocuum. We excluded 22 of those from further analysis because of lack of access to clinical information and 6 because of concomitant isolation of \( C. \) innocuum and \( C. \) difficile from the same sample (CI group). We matched the control group with \( C. \) innocuum samples in accordance with the study criteria. From 1,134 \( C. \) difficile cases during the study period, we enrolled 304 cases as controls (CD group). All control cases were

Table 3. Clinical and laboratory characteristics by the infecting \( Clostridium \) species in case–control study of \( C. \) innocuum infection, Taiwan*

| Characteristic                          | \( C. \) innocuum, \( n=152 \) | \( Clostridioides \) difficile, \( n=304 \) | \( p \) value |
|----------------------------------------|-------------------------------|---------------------------------|-------------|
| Clinical symptoms                       |                               |                                 |             |
| Diarrhea                                | 56 (36.8)                     | 217 (71.4)                      | <0.001      |
| Fever                                   | 29 (19.1)                     | 92 (30.3)                       | 0.011       |
| Abdominal pain                          | 37 (24.3)                     | 54 (17.8)                       | 0.998       |
| Vomiting                                | 13 (8.6)                      | 29 (9.5)                        | 0.731       |
| Abdominal distension                    | 25 (16.4)                     | 41 (13.5)                       | 0.391       |
| Blood testing                           |                               |                                 |             |
| Leukocytes, cells/\( \mu L \)†          | 10,454 (6,773)                | 11,005 (6,788)                  | 0.783       |
| Hemoglobin, g/dL†                       | 10.7 (2.4)                    | 9.8 (2.0)                       | <0.001      |
| Platelet count \( \times 1,000/\mu L \)†| 243 (110.4)                   | 231 (135.0)                     | 0.134       |
| CRP, mg/L, median (IQR)‡                | 55.7 (104.7)                  | 55.7 (97.2)                     | 0.108       |
| Stool routine, no. positive/total (%)   |                               |                                 |             |
| Occult blood                            | 53/73 (72.6)                  | 175/216 (81.0)                  | 0.128       |
| Mucus                                   | 9/70 (12.8)                   | 39/205 (19.0)                   | 0.241       |
| Pus cells                               | 8/70 (11.4)                   | 30/205 (14.6)                   | 0.502       |
| Sample site                             |                               |                                 |             |
| Stool                                   | 96 (63.2)                     | 279 (91.8)                      | <0.001      |
| Blood                                   | 7 (4.6)                       | 1 (0.3)                         | 0.001       |
| Ascites                                 | 13 (8.5)                      | 8 (2.7)                         | 0.002       |
| Bile§                                   | 2 (1.3)                       | 1 (0.3)                         | 0.219       |
| Pus/abscess§                            | 16 (10.5)                     | 3 (1.0)                         | <0.001      |
| Wound/deep tissue§                      | 16 (10.5)                     | 12 (3.6)                        | 0.006       |
| Endocervix§                             | 1 (0.7)                       | 0                               | 0.592       |
| Semen§                                  | 1 (0.7)                       | 0                               | 0.592       |

Antimicrobial susceptibility#

|                     | \( C. \) innocuum              | \( Clostridioides \) difficile | \( p \) value |
|---------------------|--------------------------------|--------------------------------|-------------|
| Metronidazole       | 20/20 (100)                    | 53/53 (100)                    | 1.000       |
| Clindamycin§        | 30/44 (68.2)                   | 17/20 (85.0)                   | 0.158       |
| Penicillin§         | 35/44 (79.5)                   | 12/20 (60.0)                   | 0.101       |
| Ampicillin/sulbactam| 21/21 (100)                    | 44/44 (100)                    | 1.000       |

*Values are no. (%) patients except as indicated. Among patients with \( C. \) difficile (CD)–associated diarrhea, the CD toxin assay positive rate was 65%. \( \chi^2 \) tests were used to compare all the categorical variables listed in the table except as noted. CRP, C-reactive protein; IQR, interquartile range.
†By independent t test.
‡By Mann-Whitney test.
§By Fisher exact test.
#Data are expressed as susceptible isolate number/total isolate number (%).
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matched precisely on diagnostic year and age (+2 years); 25 controls were not matched on sex. The mean patient age for the 456 cases was 66.7 years, and 58.3% of patients were male (Table 1). Both groups were similar regarding age, sex, and Charlson Comorbidity Index score (5.7 ± 3.2 for CI and 6.2 ± 3.3 for CD). Subgroup analysis of each age group (<50, 50–60, 60–70, 70–80, and >80 years) also revealed no statistical difference. Overall, 8 pediatric patients were recruited, 3 in the CI group and 5 in the CD group. Regarding underlying systemic diseases, the CD group showed more patients with chronic kidney disease (18.4% vs. 30.9%; p = 0.005) (Table 1). Of note, more patients acquired the infection in the community in the CI group (33.6% vs. 16.8%; odds ratio [OR] 2.5, 95% CI 1.6–3.9; p<0.001) (Table 1).

Disease Characteristics and Severity

We observed notable differences in disease characteristics between the 2 groups. Those in the CI group had a 6.5 times higher risk of developing EICI, including bacteremia, intra-abdominal infection, biliary tract infection, skin and soft tissue infection, pyospermia, and bacterial vaginitis (36.8% for CI vs. 8.2% for CD; OR 6.5, 95% CI 3.9–11.0; p<0.001) (Table 1). On the contrary, most disease manifestation in the CD group was confined to the intestine and colon, mainly C. difficile–associated diarrhea. Most patients had antibiotic exposure 30 days before the CI or CD infection event. CD group showed higher 30-day antibiotic exposure rate (95.1%) than CI group (79.6%; p<0.001) (Table 2) and longer duration (mean 15.6 days, SD 8.3) than CI group (mean 13.7 days, SD 8.6; p<0.001). Patients in CD group received more penicillins, cephalosporins, carbapenems, and fluoroquinolones (Table 2).

Regarding disease severity, most of the patients in both groups required hospitalization (93.4% in the CI group and 97.7% in the CD group; p = 0.03) (Table 1). Although most patients in CD group had intestinal infections, gastrointestinal tract–related complications of ileus, bowel perforation, clinical sepsis, and shock occurred more frequently in the CI group (26.3%) than CD group (11.2%; OR 2.8, 95% CI 1.7–4.7; p<0.001). CI group also showed a higher rate of intensive care unit (ICU) admission (23.6% vs. 9.5%; OR 2.9, 95% CI 1.7–5.0; p<0.001) (Table 1). All the data indicated that the disease severity at the acute stage was more severe and invasive in the C. innocuum–infected patients. Furthermore, we saw no recurrence of infection in CI group but recurrence of infection in 4.9% of CD group (p = 0.005). We observed no statistically significant differences in clinical presentations, but patients with C. innocuum infection had fewer diarrheal symptoms
and less fever. In the laboratory testing results, patients experienced anemia more commonly in the CD group than CI group; hemoglobin counts were 9.8 (2.0) g/dL in CD and 10.7 (2.4) g/dL in CI (p<0.001) (Table 3). We observed no difference in other systemic inflammatory markers. A limited number of patients received colonoscopy examination, and we found no pseudomembranous colitis in the CI group.

Outcome and Risk Factor for Mortality Rate
The 30-day mortality rate in the CI group was 14.5%; the 90-day rate, 15.8%, and the overall rate, 23.0%. Although the 90-day mortality rate was slightly higher in the CD group with a significant difference (p value of log rank test = 0.05) in Kaplan-Meier survival analysis, the overall mortality rate did not show a statistically significant difference between the 2 groups (Figure). Using logistic regression, we identified chronic kidney disease (OR 8.6, 95% CI 2.6–28.4; p<0.001), solid tumor (OR 3.5, 95% CI 1.0–12.0; p = 0.051), ICU admission (OR 7.3, 95% CI 2.4–21.9; p<0.001), and shock status (odds ratio 8.0, 95% CI 2.4–27.2; p<0.001) as 4 independent risk factors for both 30-day and overall mortality rates in the patients with C. innocuum infection. We identified 7 bacteremias caused by C. innocuum in this study. Two of those patients experienced septic shock, and 1 needed ICU hospitalization. The 30-day mortality rate for the 7 patients was 42.9% (3/7) and 90-day rate was 57.1% (4/7).

Microbiologic Result and Antimicrobial Susceptibility
Among the 152 C. innocuum isolates, we recovered 96 (63.2%) isolates from stool specimens; the rest were from the blood (7), ascites (13), pus/abscess (16), wound/deep tissue (16), bile juice (2), endocervix (1), and semen (1). We detected 18 polymicrobial infections in the CI group, most of which were from ascites and pus/abscess samples. More C. innocuum isolates (36.8%) than C. difficile isolates (8.2%) were from extraintestinal specimens (p<0.001) (Table 3), which is compatible with our clinical observation. We performed antimicrobial susceptibility testing on limited isolates. In the C. innocuum isolates, we observed the highest susceptibility rate for metronidazole (20/20, 100%) and ampicillin/sulbactam (21/21, 100%), followed by penicillin (35/44, 79.5%) and clindamycin 30/44 (68.2%).

Discussion
Genus Clostridium is large and heterogeneous; it includes ≤200 species. Accurate species identification has been difficult. In recent years, several new species have been recognized and others reclassified using newer molecular diagnostic methods, such as 16S rRNA gene sequencing (24). Among the medically important Clostridium spp., C. perfringens is the predominant species isolated from cases of bacteremia. The severity of EICI varies; for bacteremia, the mortality rate was found to be 48%–52% by different studies (25–27). The risk factors for disease acquisition and death were related to an underlying immunocompromised condition such as hemodialysis, malignancy, immunosuppressant use, and Crohn’s disease (25). The main portal of entry is the hepatobiliary and gastrointestinal tract. We believe this is also the case in C. innocuum because stool was a common source for the C. innocuum isolates and gastrointestinal tract–related complications were not uncommon in C. innocuum–infected patients.
A recent study by Ha et al. (28) also found that \textit{C. innocuum} is one of the most common bacteria that could translocate from intestine to mesenteric tissue in patients with Crohn’s disease and further induce adipogenesis and local fibrosis, known together as creeping fat.

We found that among anaerobic clostridial species, \textit{C. innocuum} has long been overlooked as a human pathogen. Our study is to date the most comprehensive observational study to depict the clinical manifestations and outcome of \textit{C. innocuum} infection; not only it is more invasive than most \textit{Clostridium} species, but it can cause more gastrointestinal tract complications following intestinal infection. Case reports of EICI related to \textit{C. innocuum} infection have been published from the United States, Spain, Japan, and Taiwan (10,29–32) (Table 4). Bacteremia and intraabdominal infection were the most common manifestations, which is compatible with our observations. All the infections occurred in patients with underlying conditions; prolonged antimicrobial therapy was required to treat these patients, whose mortality rate (20%) was similar to that observed in our study (23%). Compared to \textit{C. difficile}, which is known to be a nosocomial pathogen, nearly one third of the \textit{C. innocuum} infections occurred in the community. This observation indicates that \textit{C. innocuum} could be more virulent and competitive than \textit{C. difficile}.

Among the EICI, bacteremia is the most severe form of infection. In a recent study by Morel et al. (33), non-\textit{C. difficile} \textit{Clostridium} bacteremia requiring ICU hospitalization showed an aggressive clinical course and was usually life-threatening. The 28-day mortality rate was 55% and the 90-day mortality rate was 71% (33). This report is compatible with our findings of 30-day (42.9%) and 90-day (57.1%) mortality rates in the CI bacteremic patients.

Identifying \textit{C. innocuum} infection is important because the microorganism expresses intrinsic resistance to vancomycin, because of the synthesis of peptidoglycan precursors with low affinity for vancomycin (MIC 4–16 mg/L) (8,26). Moreover, highly vancomycin-resistant strains (MIC >16 mg/L) could develop if the bacteria were previously exposed to vancomycin (34). Because oral vancomycin has been recommended as the first-line therapy for \textit{C. difficile} infection, distinguishing \textit{C. innocuum} from other clostridial species becomes essential to avoid treatment failure caused by inappropriate antimicrobial use. Metronidazole and clindamycin appear to be appropriate choices for treating \textit{C. innocuum} infection, according to our antimicrobial susceptibility testing results.

The main limitation of our study is the retrospective study design and the inevitable missing data. The lack of standardized medical record format prevented us from precisely defining every case-patient’s diagnosis, especially antibiotic-associated diarrhea and acute colitis, which have similar clinical descriptions in the medical records. Some objective data were not available, which may potentially compromise the accuracy of the estimated rates of presentations and diagnoses among the patients. However, the proportion of missing data appeared small and should not significantly affect the results of the study. Second, not all the \textit{C. innocuum} isolates from the enrolled patients were tested for antimicrobial susceptibility, and that testing did not include vancomycin. Third, the study does not advance our understanding on virulence mechanism of \textit{C. innocuum}. It is possible that \textit{C. innocuum} possesses a unique virulence mechanism to cause gastrointestinal as well as extraintestinal infections, such as the lipopolysaccharide-like structure we described in our previous study (9). \textit{C. difficile} also contains surface lipocarbohydrate, which has a similar biologic activity to the lipopolysaccharide in gram-negative bacteria (35); this hypothesis needs further experimental verification.

In conclusion, \textit{C. innocuum} should be considered an important \textit{Clostridium} species causing EICI and gastrointestinal infection that has a risk for severe complications and a high mortality rate in immunocompromised patients; physicians should recognize it as a pathogen to treat clinically. More studies are needed to understand the virulence mechanism of \textit{C. innocuum}. Precise identification of \textit{C. innocuum} will guide appropriate and timely antimicrobial therapy for patients because of its intrinsic vancomycin resistance.

The study was financially supported by grants (CIRPG3H0031-2 and CIRPG3H0041-2) from Chang Gung Memorial Hospital, Taiwan, and the Maintenance Project of the Center for Big Data Analytics and Statistics at Chang Gung Memorial Hospital (grant CLRPG3D0048) for statistical consultation and data analysis.

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References

1. Smith LD, King E. Clostridium innocuum, sp. n., a sporeforming anaerobe isolated from human infections. J Bacteriol. 1962;83:938–9. https://doi.org/10.1128/jb.83.4.938-939.1962

2. Alexander CJ, Citron DM, Brazier JS, Goldstein EJ. Identification and antimicrobial resistance patterns of clinical isolates of Clostridium clindamicos, Clostridium innocuum, and Clostridium ramosum compared with those of clinical isolates of Clostridium perfringens. J Clin Microbiol. 1995;33:3209–15. https://doi.org/10.1128/jcm.33.12.3209-3215.1995

3. Stokes NA, Hylemon PB. Characterization of delta 4-3-ketosteroid-5 beta-reductase and 3 beta-hydroxysteroid dehydrogenase in cell extracts of Clostridium innocuum. Biochim Biophys Acta. 1985;836:255–61. https://doi.org/10.1016/0006-8073(85)90073-6

4. Carlier JP, Sellier N. Identification by gas chromatography-mass spectrometry of short-chain hydroxy acids produced by Fusobacterium species and Clostridium innocuum. J Chromatogr A. 1987;420:121–8. https://doi.org/10.1016/0021-9681(87)80161-5

5. Johnston NC, Goldfine H, Fischer W. Novel polar lipid composition of Clostridium innocuum as the basis for an assessment of its taxonomic status. Microbiology (Reading). 1994;140:105–11. https://doi.org/10.1099/13500872-140-1-105

6. Li Y, Shan M, Zhu Z, Mao X, Yan M, Chen Y, et al. Application of MALDI-TOF MS to rapid identification of anaerobic bacteria. BMC Infect Dis. 2019;19:941. https://doi.org/10.1186/s12879-019-4584-0

7. Cutrona AF, Watanakunakorn C, Schaub CR, Jagetia A. Clostridium innocuum endocarditis. Clin Infect Dis. 1995;21:1306–7. https://doi.org/10.1093/clinids/21.5.1306

8. Chia JH, Wu TS, Wu TL, Chen CL, Chang CH, Su LH, et al. Clostridium innocuum is a vancomycin-resistant pathogen that may cause antibiotic-associated diarrhoea. Clin Microbiol Infect. 2018;24:1195–9. https://doi.org/10.1016/j.cmi.2018.02.015

9. Chia JH, Peng Y, Su LH, Wu TL, Chen CL, Liang YH, et al. Clostridium innocuum is a significant vancomycin-resistant pathogen for extraintestinal clostridial infection. Clin Microbiol Infect. 2017;23:560–6. https://doi.org/10.1016/j.cmi.2017.02.025

10. Castiglioni B, Gautam A, Citron DM, Pasculle W, Goldstein EJC, Strollo D, et al. Clostridium innocuum bacteremia secondary to infected hematoma with gas formation in a kidney transplant recipient. Transpl Infect Dis. 2003;5:199–202. https://doi.org/10.1111/j.1399-3062.2003.00037.x

11. David V, Bozdogan B, Mainardi JL, Legrand R, Gutmann L, Leclercq R. Mechanism of intrinsic resistance to vancomycin in Clostridium innocuum NCIB 10674. J Bacteriol. 2004;186:3415–22. https://doi.org/10.1128/JB.186.11.3415-3422.2004

12. Peng Z, Ling L, Stratton CW, Li C, Polage CR, Wu B, et al. Advances in the diagnosis and treatment of Clostridium difficile infections. Emerg Microbes Infect. 2018;7:15. https://doi.org/10.1038/s41426-017-0019-4

13. Jlewkes J, Larson HE, Price AB, Sanderson PJ, Davies HA. Aetiology of acute diarrhoea in adults. Gut. 1981;22:388–92. https://doi.org/10.1136/gut.22.5.388

14. McDonald LC, Gerding DN, Johnson S, Bakken JS, Carroll KC, Coffin SE, et al. Clinical Practice guidelines for Clostridium difficile infection in adults and children: 2017 update by the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA). Clin Infect Dis. 2018;66:987–94. https://doi.org/10.1093/cid/ciy149

15. Shannon S, Kronemann D, Patel R, Schuetz AN. Routine use of MALDI-TOF MS for anaerobic bacterial identification in clinical microbiology. Anaerobe. 2018;54:191–6. https://doi.org/10.1016/j.anaerobe.2018.07.001

16. Veloo AC, de Vries ED, Jean-Pierre H, Justesen US, Morris T, Urban E, et al.; ENTRA workgroup. The optimization and validation of the Biotyper MALDI-TOF MS database for the identification of Gram-positive anaerobic cocci. Clin Microbiol Infect. 2016;22:793–8. https://doi.org/10.1016/j.cmi.2016.06.016

17. Li Y, Shan M, Zhu Z, Mao X, Yan M, Chen Y, et al. Application of MALDI-TOF MS to rapid identification of anaerobic bacteria. BMC Infect Dis. 2019;19:941. https://doi.org/10.1186/s12879-019-4584-0

18. Khanna S, Pardi DS, Aronson SL, Kammerstein R, St Sauver JL, et al. The epidemiology of community-acquired Clostridium difficile infection: a population-based study. Am J Gastroenterol. 2012;107:89–95. https://doi.org/10.1038/ajg.2011.398

19. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J Chronic Dis. 1987;40:373–83. https://doi.org/10.1016/0021-9681(87)90171-8

20. Pepin J, Alary ME, Valiquette L, Raiche E, Ruel J, Fulop K, et al. Increasing risk of relapse after treatment of Clostridium difficile colitis in Quebec, Canada. Clin Infect Dis. 2005;40:1591–7. https://doi.org/10.1086/430315

21. Lee HY, Hsiao HL, Chia CY, Cheng CW, Tsai TC, Deng ST, et al. Risk factors and outcomes of Clostridium difficile infection in hospitalized patients. Biomed J. 2019;42:99–106. https://doi.org/10.1016/j.bi j.2018.12.002

22. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing: 27th edition (M100-S27). Wayne (PA): The Institute; 2017.

23. Van Dalee E, Van de Putte D, Ceelen W, Van Nieuwenhove Y, Pattyn P. Risk factors and consequences of anastomotic leakage after Ivor Lewis oesophagectomy. Interact Cardiovasc Thorac Surg. 2016;22:32–7. https://doi.org/10.1093/icvts/ivy276

24. Finegold SM, Song Y, Liu C, Hecht DW, Summanen P, Könnönen E, et al. Clostridium clindamicos: a mixture of three clinically important species. Eur J Clin Microbiol Infect Dis. 2005;24:319–24. https://doi.org/10.1007/s10096-005-1334-6

25. Leal J, Gregson DB, Ross T, Church DL, Laupland KB. Epidemiology of Clostridium species bacteremia in Calgary, Canada, 2000–2006. J Infect. 2008;57:198–203. https://doi.org/10.1016/j.jinf.2008.06.018

26. Bodey GP, Rodriguez S, Fainstein V, Elting LS. Clostridial bacteremia in cancer patients. A 12-year experience. Cancer. 1991;67:1928–42. https://doi.org/10.1002/1097-0142(19910401)67:7<1928:AID-CANCR2820670718>3.0.CO;2-9

27. Shah M, Bishburg E, Baran DA, Chan T. Epidemiology and outcomes of clostridial bacteremia at a tertiary-care institution. ScientificWorldJournal. 2009;9:144–8. https://doi.org/10.11021/swj.2009.08.018

28. Ha CWY, Martin A, Sepich-Poore GD, Shi B, Wang Y, Gouin K, et al. Translocation of viable gut microbiota to mesenteric adipose drives formation of creeping fat in humans. Cell. 2020;183:666–83. https://doi.org/10.1016/j.cell.2020.09.009

29. Crum-Cianflone N. Clostridium innocuum bacteremia in a patient with acquired immunodeficiency syndrome. Am J Med Sci. 2009;337:480–2. https://doi.org/10.1097/MAJ.0b013e31819f1e95

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30. Hung YP, Lin HJ, Wu CJ, Chen PL, Lee JC, Liu HC, et al. Vancomycin-resistant *Clostridium innocuum* bacteremia following oral vancomycin for *Clostridium difficile* infection. Anaerobe. 2014;30:24–6. https://doi.org/10.1016/j.anaerobe.2014.07.009

31. Mutoh Y, Hirai R, Tanimura A, Matono T, Morino E, Kutsuna S, et al. Osteomyelitis due to *Clostridium innocuum* in a patient with acute lymphoblastic leukemia: case report and literature review. Springerplus. 2015;4:385. https://doi.org/10.1186/s40064-015-1176-3

32. Aroca-Ferri M, Suárez-Hormiga L, Bosch-Benitez-Parodi E, Bolaños-Rivero M. Peritonitis by *Clostridium innocuum* associated to peritoneal dialysis [in Spanish]. Rev Esp Quimioter. 2019;32:192–3.

33. Morel G, Mulier G, Ghrenassia E, Abdel Nabey M, Tandjaoui Y, Kouatchet A, et al. Non–*C. difficile* *Clostridioides* bacteremia in intensive care patients, France. Emerg Infect Dis. 2021;27:1840–9. https://doi.org/10.3201/eid2707.203471

34. Cherny KE, Ozer EA, Kochan TJ, Johnson S, Kociolek LK. Complete genome sequence of *Clostridium innocuum* strain LC–LUMC-CI-001, isolated from a patient with recurrent antibiotic-associated diarrhea. Microbiol Resour Announc. 2020;9:e00365–20. https://doi.org/10.1128/MRA.00365-20

35. Sánchez-Hurtado K, Poxton IR. Enhancement of the cytotoxic activity of *Clostridium difficile* toxin A by surface-associated antigens. J Med Microbiol. 2008;57:739–44. https://doi.org/10.1099/jmm.0.47678-0

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