The increase in the incidence, severity, and mortality rates of *Clostridium difficile* infection (CDI) is a matter of serious concern (1,2). The reported prevalence of CDI differs across hospitals and depends on detection methods (3). In Thailand, the reported prevalence of *Clostridium difficile*-associated diarrhea (CDAD) has varied from 5% to 25% (4–7). The prevalence of CDAD has probably been underestimated because the widely used enzyme immunoassay (EIA) for detecting stool *C. difficile* (8) has low sensitivity. This study aims to investigate the prevalence of CDAD in patients with nosocomial diarrhea using different methods, including EIA and direct polymerase chain reaction (PCR) of stool samples for the *tcdB* gene, and to determine factors associated with and the treatment outcome in CDAD.

This prospective cohort study was conducted from May 2010 through January 2011 at Ramathibodi Hospital, a 1,000-bed tertiary-care university hospital. The patients included in this study were adult patients (age, ≥15 years) who were admitted to the medicine ward and developed diarrhea during their hospitalization (8). Patients whose stool samples were not available for EIA or direct PCR were excluded from the study. In this study, CDAD was defined as diarrhea with the presence of *C. difficile* toxins in stools, as detected by EIA, or positive PCR results for *tcdB*. This study was approved by the institutional review board.

The clinical records of patients were reviewed for demographics, clinical characteristics, laboratory investigations, the treatment received, and its outcomes.

EIA was performed using the *C. difficile* Tox A&B kit (VIDAS®; bioMérieux, Marcy l’Etoile, France). Equivocal values were interpreted as negative results in our study.

PCR was undertaken for detecting the *tcdB* gene. Genomic bacterial DNA was isolated directly from stool samples and was used as a template for PCR; amplification was achieved using in-house primers that targeted a highly conserved region of the *tcdB* gene, which is present in most toxigenic *C. difficile* strains (9). The sequence of the forward primer was 5‘-GAAGATTT AGGAAATGAAGAAGGTGA-3‘ and that of the reverse primer was 5‘-AACCACTATAATTCACCTGC TTGTC-3‘. The PCR conditions were as follows: initial denaturation at 92°C for 5 min, followed by 30 cycles of 92°C for 30 s, 55°C for 30 s, and 68°C for 60 s. After the final cycle, the samples were heated at 68°C for 5 min and cooled to 4°C. The PCR products were then analyzed by performing agarose gel electrophoresis.

Data were analyzed using the SPSS version 16.0 for Windows (SPSS Inc., Chicago, Ill., USA). Patients were categorized into two groups on the basis of their CDAD status. The 95% confidence interval (CI) for CDAD prevalence was calculated using a Microsoft Excel statistical function. Chi-square or Fisher’s exact tests were used for comparing categorical variables. Student’s *t* test and Mann-Whitney *U* tests were used to compare the means and medians of continuous varia-
Fig. 1. Positive test for *Clostridium difficile*-associated diarrhea based on detection method (*n* = 175).

Table 1. Univariate and multivariate analyses of factors associated with CDAD cases

<table>
<thead>
<tr>
<th>Factor</th>
<th>Crude OR (95% CI)</th>
<th><em>P</em></th>
<th>Adjusted OR (95% CI)</th>
<th><em>P</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Onset after admission ≥ 48 h</td>
<td>4.37 (1.46–13.05)</td>
<td>0.008</td>
<td>2.95 (0.74–11.75)</td>
<td>0.126</td>
</tr>
<tr>
<td>Heart diseases</td>
<td>1.94 (0.90–4.19)</td>
<td>0.093</td>
<td>1.81 (0.79–4.15)</td>
<td>0.164</td>
</tr>
<tr>
<td>Diarrhea onset after ≥ 10 days of antibiotic administration</td>
<td>2.39 (1.10–5.17)</td>
<td>0.028</td>
<td>2.71 (1.14–6.44)</td>
<td>0.024</td>
</tr>
<tr>
<td>Proton pump inhibitors</td>
<td>2.03 (0.95–4.36)</td>
<td>0.069</td>
<td>1.07 (0.42–2.73)</td>
<td>0.891</td>
</tr>
<tr>
<td>Steroid</td>
<td>1.96 (0.97–3.95)</td>
<td>0.062</td>
<td>1.63 (0.67–3.93)</td>
<td>0.278</td>
</tr>
<tr>
<td>Leukocyte count &gt; 15,000 cells/mm³</td>
<td>2.20 (1.04–4.64)</td>
<td>0.039</td>
<td>3.12 (1.24–7.88)</td>
<td>0.016</td>
</tr>
</tbody>
</table>

CDAD, *Clostridium difficile* associated-diarrhea; OR, odds ratio; CI, confidence interval.
difficile has been reported to be 7.1–8.7 on the basis of EIAs and direct PCR of stool samples. The all-cause mortality rate was 31.9% within 10 days after treatment. The median (interquartile range [IQR]) time from treatment to cessation of diarrhea in 27 patients who received oral metronidazole and in 9 patients who received oral vancomycin was 5 (2–8) days and 2 (1–10) days, respectively. Furthermore, 75.9% of the diarrheal episodes improved within 10 days after treatment. The all-cause mortality rate was 31.9% in the CDAD group and 35.9% in the non-CDAD group (P = 0.721). C. difficile-related mortality rate was 6.4%.

In Thailand, the prevalence of CDAD as determined on the basis of EIAs and direct PCR of stool samples has been reported to be 7.1–8.7% (5,7) and 8.4% (7), respectively. The prevalence of CDAD as determined by either of the 2 tests in our study was higher than that reported previously. This might imply that the incidence of CDAD in Thailand is on a rise over time, as indicated by studies from other countries (1,10).

The total agreement between EIA and direct PCR of stool samples was 83.2%; however, the kappa coefficient showed moderate agreement (κ = 0.46). We found that the EIA and PCR tests yielded concordant negative results but discordant positive test results. Direct PCR for stool samples gave approximately 2 times higher positive cases than EIA of stool toxins. The limitation of the EIA test was its low sensitivity (75–83%) (11). C. difficile toxins are unstable and may be degraded within 2 h after collection (12). This may explain the low sensitivity of EIA. Direct PCR of stool has been shown to exhibit higher sensitivity (>90%) (13). Our results support a previous study which indicated that the performance of PCR is superior, thus offering clinical benefits in CDAD detection (14). However, molecular detection of C. difficile toxin genes may yield false-positive results for the DNA in the absence of toxin production; thus, positive PCR results must be carefully interpreted. One of the known risks of CDAD, among others, is antibiotic therapy. We also found that the duration of antibiotic administration was significantly longer in the CDAD group (14 days versus 8 days; P = 0.003), which is in accordance with previously reported findings (15), and thus unnecessarily prolonged treatment with antibiotics should be discouraged.

The significant laboratory finding in our study was that the CDAD group had a higher number of patients with a leukocyte count of >15,000 cells/mm³ on the day of diarrhea onset. In an observational study, leukocytosis was found in 50% of patients with CDI (16). In another study on patients with unexplained leukocytosis, 58% of patients had CDI (17).

Previous randomized-treatment trials showed that the symptoms of patients improved within 1 or 2 days after therapy, with a mean time of 3–6 days for diarrhea resolution (18). In our study, treatment with oral vancomycin resulted in shorter median time (IQR) to improvement of diarrhea than treatment with oral metronidazole (2 days versus 5 days). However, 4 of 5 patients for whom the treatment was changed from oral metronidazole to oral vancomycin showed improvement of diarrhea on the first day of switching therapy. It is difficult to conclude whether this was the late effect of oral metronidazole or the early effect of oral vancomycin. A relatively poor response to metronidazole was observed, with patients continuing to have symptoms of colitis for 10 days or more despite treatment (19). The overall non-response rate in the case of metronidazole and vancomycin in our study was 24.1%, which was comparable to the 22–29% in other studies (19,20).

The limitations of this study include the lack of another diagnostic test such as cell cytotoxicity or toxinogenic C. difficile culture, because of which we could not identify the false positives or negatives in the cases of discordant test results. However, the analytical sensitivity and specificity of our PCR assay with tcdA + tcdB+ C. difficile, tcdA−/tcdB+ C. difficile isolates, other Clostridium spp., and enteric bacterial organisms showed no false-positive or false-negative results. Furthermore, as previously described, the relatively small number of CDAD cases makes it difficult to identify the risk factors for CDAD.

In conclusion, the prevalence of CDAD infection has shown an increasing trend over time. Our direct PCR of stool for tcdB showed a higher number of positive results than the EIA results for stool toxins A and B. Diarrhea in patients who receive antibiotics for 10 days or more or those who have a high leukocyte count of >15,000 cells/mm³ should alert clinicians to examine the patient for CDAD. The performance of the PCR test was superior, and PCR can serve as an effective diagnostic test for CDAD detection.

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Conflict of interest None to declare.

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