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Increasing Prevalence of Toxin A-Negative, Toxin B-Positive Isolates of *Clostridium difficile* in Korea: Impact on Laboratory Diagnosis

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Of 462 Korean *Clostridium difficile* isolates, 77.5% were toxin B positive but 21.4% were toxin A negative (A− B+). The binary toxin gene was detected in nine isolates. A higher fluoroquinolone resistance of A− B+ strains may contribute to the increase of these strains. Toxin A detection alone may underdiagnose *C. difficile*-associated disease.

*Clostridium difficile*-associated disease (CDAD) is due to strains producing toxins A (enterotoxin) and B (cytotoxin), which are encoded by *tcdA* and *tcdB*, respectively (4, 5). Toxin A-negative, toxin B-positive (A− B+) strains of *C. difficile*, described in the early 1990s (3), have been increasingly reported in some parts of the world (6, 15). A− B+ strains fail to produce toxin A due to deletion of the repetitive domain of the *tcdA* gene but can cause CDAD, including fatal pseudomembranous colitis (15). Some *C. difficile* strains also produce binary toxin (actin-specific ADP-riboseyltransferase [CDT]), which contributes to CDAD. Two genes, *cdtA* and *cdtB*, encode the enzymatic and binding components of the toxin (14).

Clindamycin in the 1970s and cephalosporins in the late 1980s and through the 1990s were the antimicrobial agents recommended to improve the diagnosis. The presence of A− B+ strains may profoundly affect the diagnosis of CDAD, depending on the kinds of tests used, but the prevalence of this type of strain in Korea is not well known.

Laboratory diagnosis of CDAD includes detecting cytotoxin and/or toxin A and toxin B proteins (1). Besides direct toxin assay from stool specimens, toxigenic *C. difficile* culture is recommended to improve the diagnosis. The presence of A− B+ strains may profoundly affect the diagnosis of CDAD, depending on the kinds of tests used, but the prevalence of this type of strain in Korea is not well known.

The aim of this study was to determine the prevalence of A− B+ isolates and the presence of CDT+ strains of *C. difficile* in Korea. The susceptibility to fluoroquinolones was also determined.

The *C. difficile* strains were isolated between 1980 and 2006 from stool specimens of suspected CDAD patients at a tertiary care hospital in Korea. Cycloserine-cefoxitin-fructose agar was used for the isolation (1), and the isolates were identified by using conventional tests and the ATB 32A system (bioMerieux, Marcy-l’Etoile, France). The control *C. difficile* strains, VPI 10463 (A+ B+), 3608/03 (A− B+), 1470 (A− B+), and SE844 (CDT+), were obtained from Maja Rupnik in Slovenia. Strain NAPA1/027 was provided by one of the authors of the present report (T. V. Riley). The *C. difficile* toxin genes were detected by PCR as described previously (17). The primer pairs used were NK2-NK3 for *tcdA*, NK9-NK11 for the repetitive domain of *tcdA*, NK104-NK105 for *tcdB*, cdtA pos-cdtA rev for *cdtA*, and cdtB pos-cdtB rev for *cdtB*. PCR ribotyping was performed as described previously (13).

The antimicrobial susceptibilities were determined by the National Committee for Clinical Laboratory Standards-recommended agar dilution method (12), using norfloxacin, ciprofloxacin, ofloxacin, and levofloxacin (Sigma-Aldridge, St. Louis, MO), gatifloxacin (Grunenthal, Aachen, Germany), and moxifloxacin (Bayer, Wuppertal, Germany).

Of the 462 isolates tested, 358 (77.5%) were either A+ B+ (259; 56.1%) or A− B+ (99; 21.4%). A− B+ strains, which were first detected in 1995 in samples from two patients, steadily increased in the following years.

<table>
<thead>
<tr>
<th>Year of isolation (no. of isolates tested)</th>
<th>A+ B+ CDT+</th>
<th>A− B+ CDT+</th>
<th>A− B− CDT−</th>
<th>A− B+ CDT−</th>
</tr>
</thead>
<tbody>
<tr>
<td>1980 (3)</td>
<td>2 (66.7%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>1 (33.3%)</td>
</tr>
<tr>
<td>1990 (12)</td>
<td>9 (75.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>3 (25.0%)</td>
</tr>
<tr>
<td>1995 (48)</td>
<td>34 (70.8%)</td>
<td>1 (2.1%)</td>
<td>2 (4.2%)</td>
<td>11 (22.9%)</td>
</tr>
<tr>
<td>2002 (46)</td>
<td>28 (60.9%)</td>
<td>0 (0.0%)</td>
<td>6 (13.0%)</td>
<td>12 (26.1%)</td>
</tr>
<tr>
<td>2003 (105)</td>
<td>67 (63.8%)</td>
<td>1 (2.5%)</td>
<td>16 (15.2%)</td>
<td>22 (21.0%)</td>
</tr>
<tr>
<td>2004 (53)</td>
<td>22 (41.5%)</td>
<td>1 (1.9%)</td>
<td>21 (39.6%)</td>
<td>9 (17.0%)</td>
</tr>
<tr>
<td>2005 (40)</td>
<td>15 (37.5%)</td>
<td>1 (2.5%)</td>
<td>12 (30.0%)</td>
<td>12 (30.0%)</td>
</tr>
<tr>
<td>2006 (155)</td>
<td>73 (47.1%)</td>
<td>6 (3.9%)</td>
<td>42 (27.1%)</td>
<td>34 (21.9%)</td>
</tr>
<tr>
<td>Total (462)</td>
<td>250 (54.1%)</td>
<td>9 (2.0%)</td>
<td>99 (21.4%)</td>
<td>104 (22.5%)</td>
</tr>
</tbody>
</table>

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The prevalence of CDT decreased of this toxin type for which continuous study is required.

In conclusion, testing for both toxin A and toxin B became very important for the accurate laboratory diagnosis and epidemiologic study of CDAD with the increasing prevalence of A^- B^+ strains in Korea. CDT^+ strains have emerged in Korea, although the ribotype 027 strain was not found.

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REFERENCES


