Glutamate Dehydrogenase Is Highly Conserved among Clostridium difficile Ribotypes

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Glutamate dehydrogenase (GDH), encoded by the gluD gene, is a metabolic enzyme produced by Clostridium difficile. There are now numerous studies demonstrating the utility of GDH as a marker for the presence of C. difficile in fecal specimens. Because GDH is produced by both toxigenic and nontoxigenic strains, its diagnostic utility is based on GDH as a screening marker, followed by confirmatory tests such as toxin assays or molecular tests that detect the presence of tcdA or tcdB, the genes encoding toxins A and B, respectively. To serve as a functional and accurate screen, isolates of all ribotypes of C. difficile must carry the gluD gene and produce the enzyme. Therefore, our study was undertaken to extend current knowledge on the reactivity of a broad number of clinical isolates and ribotypes for the expression of GDH and immunoreactivity in GDH immunoassays used in algorithm testing schemes. For our analyses, we (i) evaluated isolates for the presence of gluD, (ii) compared the predicted amino acid sequences for evidence of possible antigenic variation, and (iii) screened all ribotypes for expression of GDH.

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TABLE 1 Clostridium difficile ARL and TL ribotypes evaluated in commercial GDH immunoassay

<table>
<thead>
<tr>
<th>Toxin phenotype</th>
<th>ARL or TL</th>
<th>Ribotype no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>TcdA TcdB</td>
<td>ARL</td>
<td>009, 010, 031, 032, 033, 035, 037, 038, 039, 051, 059, 071, 085, 150, 155, 211, 237, 321, 396, 399, 405, 406, 407, 409, 410</td>
</tr>
<tr>
<td>TcdA TcdB</td>
<td>ARL</td>
<td>017, 036, 110</td>
</tr>
<tr>
<td>TcdA TcdB</td>
<td>ARL</td>
<td>001, 002, 003, 005, 006, 012, 014, 015, 019, 024, 027, 043, 046, 050, 053, 054, 056, 057, 061, 066, 073, 078, 081, 103, 106, 109, 111, 116, 126, 137, 153, 154, 180, 198, 209, 220, 244, 248, 251, 274, 305, 378, 379, 389, 394, 398, 400, 408</td>
</tr>
<tr>
<td>TL</td>
<td>5028</td>
<td></td>
</tr>
</tbody>
</table>

a All isolates and all ribotypes reacted in the GDH immunoassays. No individual isolates or ribotypes gave a negative result in the assays.

b TcdA, toxin A; TcdB, toxin B.

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ily detectable levels of GDH in vitro that were at least 500-fold over the lower limit of detection in all 3 tests.

Our results show that (i) the broad range of ribotypes that we examined are uniformly gluD positive, (ii) the DNA sequences of 24 of 25 ribotypes are identical, with the lone exception being a single base substitution resulting in a conservative amino acid change that does not affect immunoreactivity, and (iii) all isolates produced in vitro levels of GDH that were readily detected by commercial tests. Collectively, these data show that GDH is highly conserved among C. difficile ribotypes and that there is no effect of ribotype on the detection of GDH produced by C. difficile in vitro, thus confirming the findings of Goldenberg et al. (1). Our findings support the interpretation that when a fecal sample contains C. difficile DNA but not GDH, it is not because of ribotypes lacking gluD or nonfunctioning gluD or antigenic variation in GDH.

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REFERENCES
