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Glutamate Dehydrogenase Is Highly Conserved among *Clostridium difficile* Ribotypes

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***gluD* was highly conserved and glutamate dehydrogenase (GDH) was readily expressed *in vitro* by all 77 *Clostridium difficile* ribotypes assayed. All ribotypes, including ARL 002, ARL 027, and ARL 106, were reactive in assays that detect *C. difficile* GDH.**

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.

(This work was presented in poster format at the 111th General Meeting of the American Society for Microbiology, New Orleans, LA, 20 to 24 May, 2011.)

We assayed 104 isolates of *C. difficile*, representing 77 ribotypes, including 25 nontoxigenic ribotypes. Seven isolates were European. One was from South America. Ninety-five were North American. One was from Australasia. Most were collected between 2004 and 2011, although some were well-known laboratory isolates from the late 1970s. There was no date for 7 isolates. We included fluoroquinolone-resistant isolates associated with outbreaks, in particular ARL 027 and ARL 001, as well as fluoroquinolone-resistant isolates not associated with outbreaks. These 77 ribotypes account for 98% of the >1,500 ribotyped isolates from our culture collection. Toxin phenotypes were established by specific immunoassays and were confirmed by PCR (Table 1). With only minimal changes, our PCR ribotyping methods were those of Stubbs et al. (2) at the Anaerobe Reference Laboratory (ARL), Cardiff, Wales. Isolates were given an ARL ribotype number or, if no control was available, a TechLab (TL) number.

The gene *gluD* was detected by PCR with a forward primer (5′-TGTCAGGAAAAGATGTAAATGTCTTCGAG-3′) which crossed the *gluD* start codon and a reverse primer (5′-TTAGTAC CATCCTCTTAATTTCATAGCTTC-3′) that crossed the stop codon. Based on online sequence data for *gluD* in *C. difficile* strain 630, the amplicon was predicted to be 1,287 bp. We sequenced $gluD_{69\text{-}1218}$ from the 25 most abundant ribotypes among our isolate collection (ARL 001, 002, 003, 009, 010, 012, 014, 015, 017, 027, 033, 036, 039, 043, 053, 054, 056, 057, 078, 106, 110, 126, 154, 251, and 274). The predicted amino acid sequences encompassing the entire glutamine-binding domain and 226 of the 231 residues comprising the NADP-binding domain were analyzed using ClustalW2 (EMBL-EBI). Broth cultures were tested for GDH production with the C. Diff Chek-60, C. Diff Quik Chek, and C. Diff Quik Chek Complete tests (TechLab, Inc.). An additional quantitative in-house immunoassay was used to measure GDH levels.

TABLE 1 *Clostridium difficile* ARL and TL ribotypes evaluated in commercial GDH immunoassays[a]

Toxin phenotype[b]	ARL or TL	Ribotype no.
$TcdA^-$ $TcdB^-$	ARL	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
$TcdA^-$ $TcdB^+$	ARL	017, 036, 110
$TcdA^+$ $TcdB^+$	ARL	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
	TL	5028

[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.

All 77 ribotypes carried *gluD*. Each template generated a single amplicon of the predicted size with no apparent size differences between amplicons. Twenty-four of 25 predicted amino acid sequences of $GDH_{23\text{-}406}$ generated from $gluD_{69\text{-}1218}$ DNA sequences were identical. Three isolates of ribotype ARL 054 were the exceptions. Each had the same $G_{585}T$ substitution, causing a predicted $Val_{217}Leu$ substitution, a conservative shift from one nonpolar amino acid with a hydrophobic side chain to another. The amino shift did not affect immunoreactivity, as demonstrated by the finding that all 3 isolates reacted in GDH immunoassays as well as isolates lacking the amino acid shift. All ribotypes expressed read-

Received 27 October 2011 Returned for modification 29 November 2011
Accepted 24 January 2012
Published ahead of print 1 February 2012
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doi:10.1128/JCM.05600-11
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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.

ACKNOWLEDGMENTS

We are grateful to Val Hall and the Anaerobe Reference Laboratory, Cardiff, Wales, for validating our ribotyping.

T.D.W. and D.M.L. are part-owners of TechLab, Inc. R.J.C., K.N.W., L.C., and A.M.L. are employees of TechLab, Inc.

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