

Supplementary Figures for:

Novel Clade C-I *Clostridium difficile* strains escape diagnostic tests, differ in pathogenicity potential and carry toxins on extrachromosomal elements

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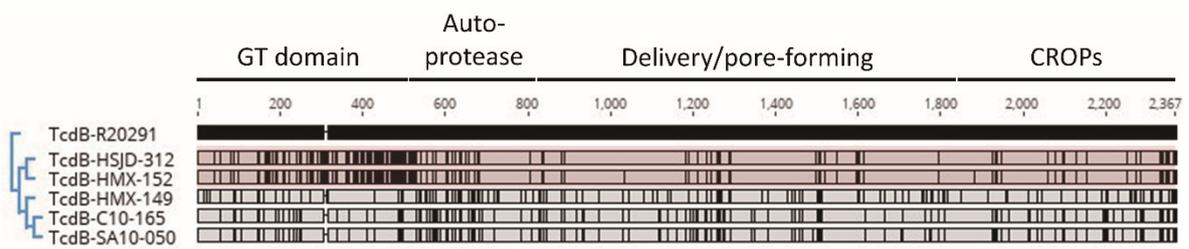
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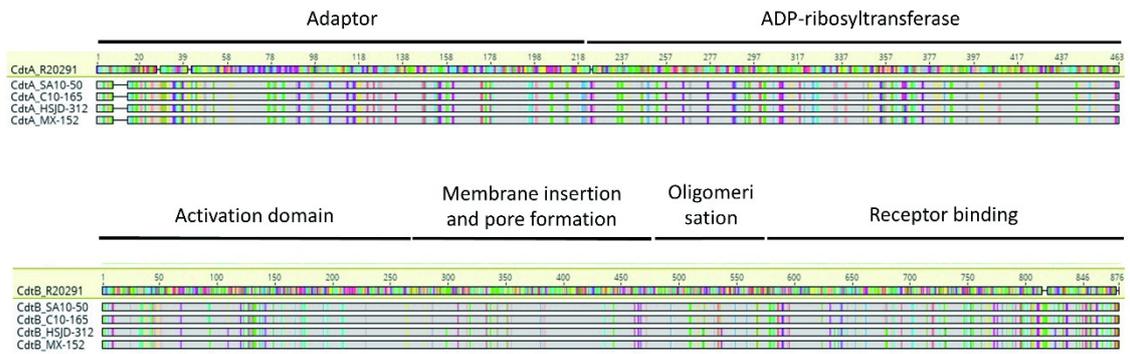
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Identity (%)						
TcdB	R20291	SA10-050	C10-165	HMX-149	HSJD-312	HMX-152
R20291	100	94	94	94	92	92
SA10-050	94	100	100	94	91	91
C10-165	94	100	100	95	91	91
HMX-149	94	94	95	100	90	90
HSJD-312	92	91	91	90	100	100
HMX-152	92	91	91	90	100	100

S.Fig 1. Alignment and % identity of predicted TcdB sequences from Clade C-I strains. The TcdB sequence of the Clade 2 strain R20291 was used as a reference.

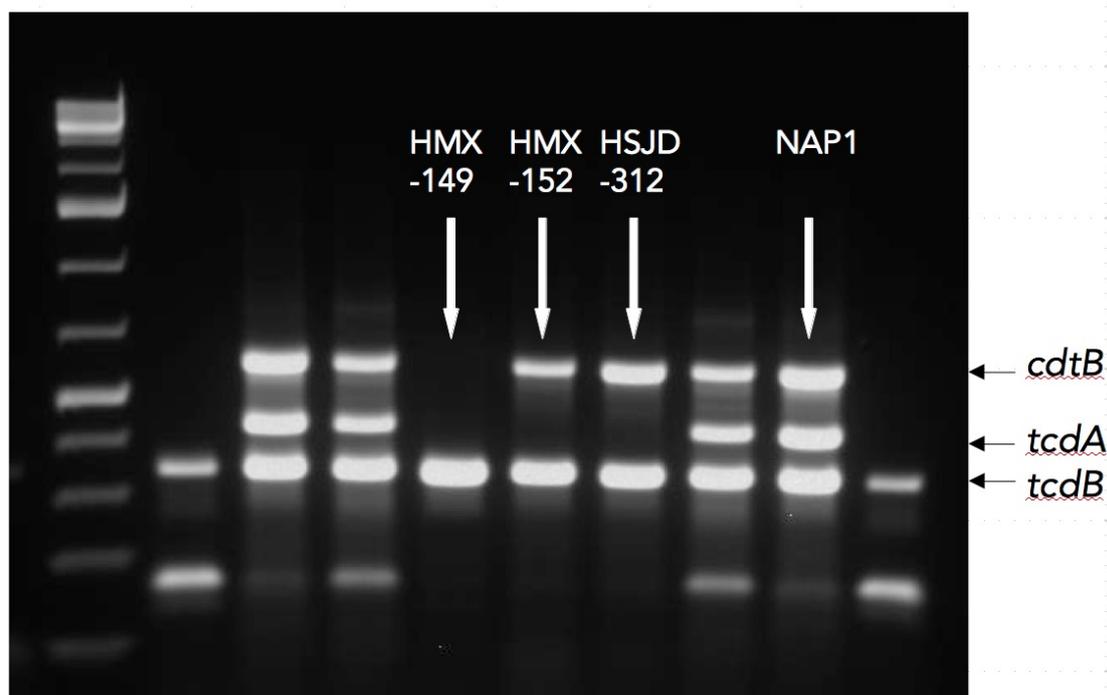


% identity						% identity					
CdtA	R20291	SA10-050	C10-165	HSJD-312	HMX-152	CdtB	R20291	SA10-050	C10-165	HSJD-312	HMX-152
R20291		71	71	71	71	R20291		81	81	80	80
SA10-050	71		99	99	99	SA10-050	81		100	98	98
C10-165	71	99		100	100	C10-165	81	100		98	98
HSJD-312	71	99	100		100	HSJD-312	80	98	98		100
HMX-152	71	99	100	100		HMX-152	80	98	98	100	

S.Fig 3. Alignment and % identity of predicted CdtA and CdtB sequences from Clade C-I strains. The CdtA and CdtB sequences of the Clade 2 strain R20291 were used as references.

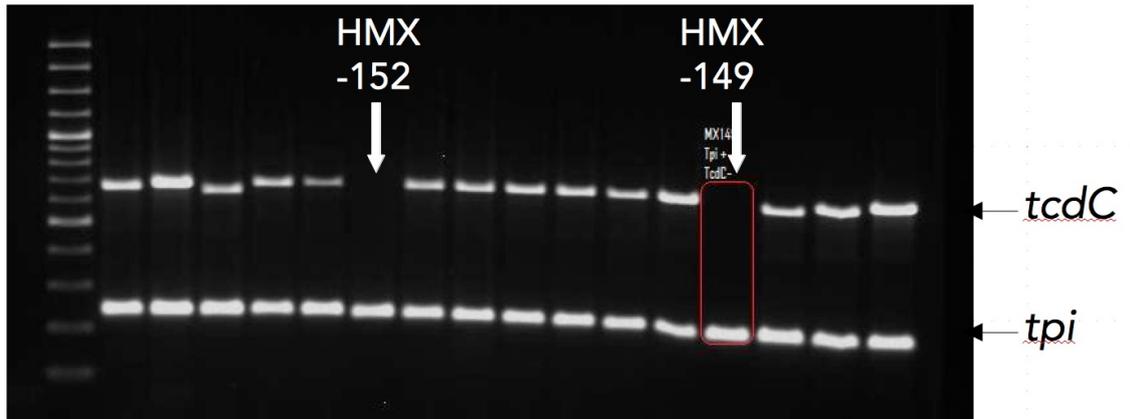
A**B**

S.Fig 4. Unedited images of the rapid immunochematographic test for detection of the GDH antigen and TcdA/B (A) and the molecular test for detection of a *tcdA* fragment (B) shown in Figure 1. (CQ)91: HMX-149; (CQ)92: HSJD-152; (CQ)93: HSJD-312, Ctl+: NAP1 strain

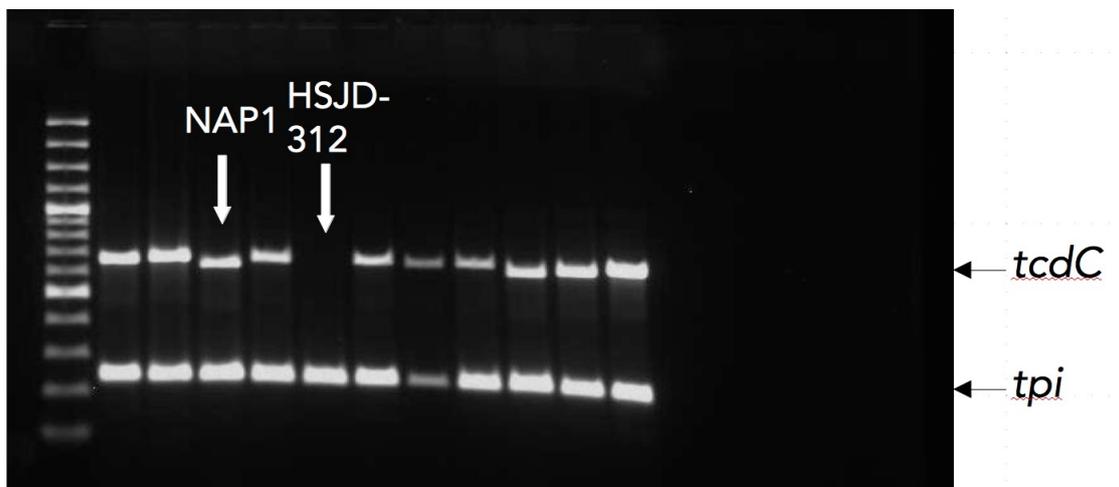


S.Fig 5. Unedited image of an agarose gel loaded with PCR-amplified *cdtB*, *tcdA*, and *tcdB* fragments for HMX-149 (lane 5), HMX-152 (lane 6), HSJD-312 (lane 7), and a control NAP1 strain (lane 9). A 1 kb ladder appears in Lane 1. Only lanes 5-7 and 9 were used in Figure 1.

A

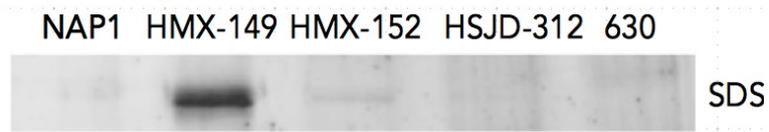


B

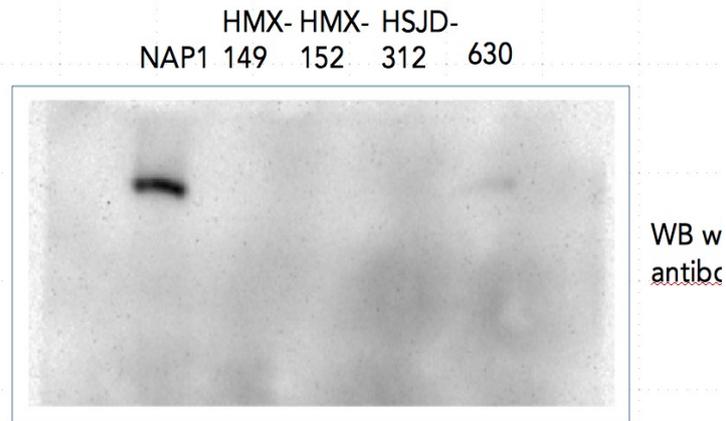


S.Fig 6. Unedited images of agarose gels loaded with PCR-amplified *tcdC* and *tpi* for HMX-152 and HMX-149 (A) or a control NAP1 strain and HSJD-312 (B). A 1 kb ladder appears in the first lane of both gels. Only lanes 7 (HMX-152) and 14 (HMX-149) from panel A and lanes 4 (NAP1) and 6 (HSJD-312) from panel B appear in Figure 1.

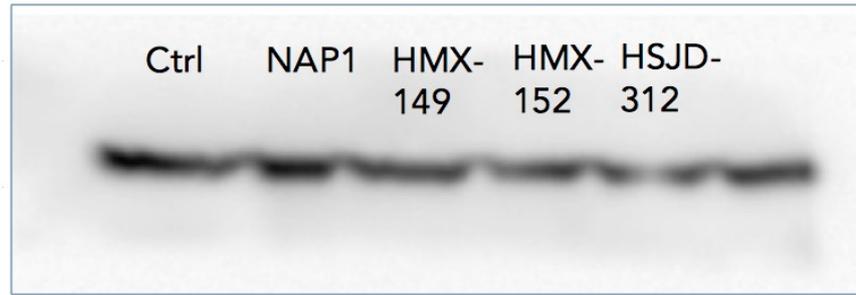
A



B



S.Fig 7. Unedited images of the SDS-PAGE (A) transferred to a PVDF membrane (B) for detection of TcdB by Western Blotting in a control NAP1 strain, HMX-149, HMX-152, HSJD-312, and the reference strain 630 (from left to right). These unedited images were used in Figure 5.



S.Fig 8. Unedited chemiluminescence image of a PVDF membrane probed with an anti-B-actin antibody as load control in the detection of glycosylated RhoA. Lysates from HeLa cells were intoxicated with cell-free supernatants from a NAP1 strain (lane 2), HMX-149 (lane 3), HMX-152 (lane 4), and HSJD-312 (lane 5). Unintoxicated cells were tested in parallel as a control (Ctrl, lane 1). Lanes 1-5 were used in Figure 5. The signal from anti-RhoA antibody is weaker and must therefore be detected using film. The corresponding film was damaged and is not available.