Supplementary data

Suppl. Table 1 Clinical isolates analyzed in this study

Strain Name	Ribo-type	Strain Source	[TcdA] in culture (ng/ml)	[TcdB] in culture (ng/ml)	Actox EC_{50} (ng/ml)	Bezlotox EC_{50} (ng/ml)	Actox % max inh.	Bezlotox % max inh.
M106	001	Gerding	1	6	TTL[a]	7.1	N/D	100
M147	001	Gerding	30	3	91	8.5	77	96
M186	001	Gerding	122	12	78	9.9	96	95
05A07CD0025	001	Miller	210	19	85	8.9	93	103
03A09CD0010	001	Miller	265	25	101	9.7	84	98
08ACD0012	001	Miller	305	20	82	10	91	98
15ACD0027	001	Miller	236	22	101	10.2	90	106
140	001	TGC	183	10	112	11.8	107	97
41	001	TGC	484	41	106	9.1	95	85
M027	002	Gerding	121	7	35.5	6.4	92	103
26ACD0042	002	Miller	305	60	201	17	99	100
48	002	TGC	525	42	275	4.4	96	101
7	003	Wilcox	556	12	84	20	103	99
8	003	Wilcox	592	11	88	11	101	89
04ACD0004	003	Miller	360	18	140	1.9	107	106
121	012	TGC	45	2	72	6.8	79	106
128	012	TGC	111	8	45	10	81	102
17	012	Wilcox	14	1	TTL	32	TTL	89
18	012	Wilcox	11	1	TTL	7.4	TTL	105
158	017	TGC	BLOQ[c]	32	TTL	1.1	TTL	104
52	017	TGC	BLOQ	49	TTL	0.8	TTL	104
25	017	Wilcox	BLOQ	74	TTL	1.9	TTL	116
26	017	Wilcox	BLOQ	56	TTL	1.6	TTL	101
138	023	TGC	13	4	TTL	20	TTL	88
160	023	TGC	19	2	TTL	23	TTL	95
BI17	027	Gerding	N/D[b]	N/D	3690	212	104	92
BI6	027	Gerding	N/D	N/D	9230	222	103	82
M041	027	Gerding	697	231	3310	380	92	85
M125	027	Gerding	70	28	3420	251	81	95
01A07CD0051	027	Miller	512	758	2970	254	77	96
01A07CD0001	027	Miller	555	922	2580	374	67	98
15A07CD0002	027	Miller	848	1591	3180	582	86	89
20ACD0009	027	Miller	902	1186	N/D	242	N/D	89
04ACD0081	027	Miller	503	579	N/D	609	N/D	88
19A08CD0009	027	Miller	868	1199	N/D	190	N/D	89
11A09CD0045	027	Miller	285	299	N/D	220	N/D	85
07B09CD0002	027	Miller	712	1415	6630	717	101	92
14A10CD0012	027	Miller	288	397	N/D	221	N/D	80
07ACD0110	027	Miller	1319	1955	3050	561	80	92
33	027	TGC	116	54	2680	558	79	80
89	027	TGC	339	197	2500	323	91	81

40	027	Wilcox	1002	383	3660	176	98	88
41	027	Wilcox	1086	552	3370	378	90	90
M137	053	Gerding	386	17	68	5.6	101	99
58	063	Wilcox	34	6	N/D	24	N/D	90
59	063	Wilcox	44	7	N/D	17	N/D	90
M129	077	Gerding	246	7	141	7.7	99	99
M196	077	Gerding	21	4	80	26	74	98
M220	078	Gerding	392	37	2590	183	72	96
26B10CD0003	078	Miller	216	68	3600	134	80	94
09A10CD0018	078	Miller	204	48	2160	180	77	95
169	078	TGC	1164	218	3580	67	72	96
73	078	TGC	240	38	3000	97	75	88
66	078	Wilcox	349	90	3840	140	75	98
67	078	Wilcox	627	112	2920	183	78	102
46	081	Wilcox	1138	34	119	9.6	92	92
23A08CD0002	087	Miller	2185	658	248	46	105	104
01A08CD0078	087	Miller	1112	249	122	21	110	104
M111	106	Gerding	37	1	35	9.5	70	93
M192	106	Gerding	32	11	51	4.6	75	105
173	106	TGC	827	33	230	5.7	93	97
73	106	Wilcox	167	7	48	41	91	90
74	106	Wilcox	167	5	32	7.5	87	95
47	198	Wilcox	364	145	3130	429	87	82
48	198	Wilcox	333	82	4900	300	78	102
M015	014	Gerding	47	18	108	31	80	100
M121	014	Gerding	54	5	135	19	82	99
19A07CD0003	014	Miller	165	95	183	31	80	92
20A10CD0007	014	Miller	1032	413	53	9.4	99	90
123	014	TGC	320	16	256	11	89	96
49	014	TGC	346	15	279	7.3	95	110
DJNS 10-010	Smz	Kato	85	27	89	8.2	81	106
MRY 04-0409 (TR01)	Smz	Kato	499	36	37	11	88	91
JND 12-006	Smz	Kato	406	29	107	14	98	95
JND 11-133	Smz	Kato	585	39	73	27	90	98
GAI 97660 (KO20)	Smz	Kato	464	30	43	25	86	97
JND 09-149	Trf	Kato	BLOQ	74	TTL	1.4	N/D	105
DJNS 10-031	Trf	Kato	BLOQ	52	TTL	1.9	N/D	101
DJNS 10-001	Trf	Kato	BLOQ	93	TTL	2.3	N/D	92
DJNS 09-017	Trf	Kato	BLOQ	62	TTL	1.8	N/D	101
TR15	Trf	Kato	BLOQ	50	TTL	2	N/D	92

[a]TTL = Toxin Too Low to evaluate

[b]N/D = Not determined

[c]BLOQ = Below Limit of Quantitation

Suppl. Table 2 Predicted effects of TcdB amino acid differences on bezlotoxumab/TcdB binding

Ribotype	Residue change[a]	Location	Impact[b]	Predicted effect on Fab/TcdB binding
027	Ile1876Val	Epitope-1	neg	Limited direct contact with Fab but may reduce favorable hydrophobic interactions within TcdB
	Asp1939Gly	Epitope-1	neg	Loss of interaction with Trp102 of Fab heavy chain
	Gly1944Ala	Epitope-1	neg	Introduces VDW clash at Tyr1905 of TcdB which may affect binding to Fab
	Val1946Ile	Epitope-1	neg	Introduces VDW clash with Asn101 of Fab heavy chain
	Pro1960Thr	Epitope-1	neg	Introduces VDW clash within TcdB which combined with Val1946Ile causes steric clashes with Fab.
	Glu1961Asp	Epitope-1	neg	Weakens H-bond and hydrophobic interactions with Tyr32 of Fab heavy chain
	Val2007Ser	Epitope-2	0	Limited contact with Fab and no change to toxin structure
	Glu2033Ala	Epitope-2	neg	Loss of salt bridge with Arg59 and of favorable hydrophobic interactions with Trp33 of Fab heavy chain
019	Same changes as 027	See above	See above	See above
	Gln1890Pro	Epitope-1	0	Limited contact with Fab and no change to toxin structure
036	Same changes as 027	See above	See above	See above
	Asp2093Asn	Epitope-2	pos	Strengthens electrostatic interactions with Tyr32 of Fab heavy chain
078	Phe1905Leu	Epitope-1	neg	Introduces VDW clash with Asn101 of Fab heavy chain
	Asp1939Glu	Epitope-1	neg	Introduces VDW conflict with Trp102 of Fab heavy chain when combined with mutation Phe1905Leu .
	Glu2033Ser	Epitope-2	neg	Loss of salt bridge with Arg59 and of hydrophobic interactions with Trp33 of Fab heavy chain. This effect is less severe than Glu2033Ala change in 027 Ribotype.
063	Same changes as 078	See above	See above	See above
	Tyr2009His	Epitope-2	neg	Limited direct contact with Fab but may affect toxin stability through loss of H-bonds with Glu2053 and Asp2034
017	Asp1939Glu	Epitope-1	pos	Strengthens favorable polar interaction with Trp102 of Fab heavy chain

[a]Compared with 087 TcdB sequence

[b]0 = limited/none; neg = interferes with binding or stability; pos = favorable to binding or stability