Supplemental Table

Protein antigens	Sequence	Note			
Class 5a antigens	ongin				
CfaE		dectoCfaE (reference)			
CIAL					
	$(\Pi 10407)$	Adhasin domain (residues 22,202) of CtoF			
		Auriesin domain (residues 23-202) of Clae			
		Dilin demain (regidues 202, 202) of CtoE			
CIAE PD		Plin domain (residues 203-383) of Crae			
	$(\Pi 10407)$	CfoE mutant P67A			
CIAE/ROTA	(U10107)				
	$(\Pi 10407)$	CfaE has an allelia variation of T011 in the			
CIAE/1911	CFA/I(10FZ)				
$C_{f_0} E / (120) /$		CFA/I diffile Stidii 10F2			
CIAE/A120V					
	(VV 54437 A- 1)	CFA/I allelic Strain WS4437A-1			
CfaE/0142R	CFA/I	CfaE has an allelic variation of O142R in the			
	(SM 1344)	CFA/L allelic strain SM 1344			
CfaE/R181A		CfaE mutant $R181\Delta$			
	(H10/07)				
CfaE/R182A	$(\Gamma \Gamma 0 + 07)$	CfaE mutant R1824			
CIAL/ICIOZA	(H10407)				
CofD	(110407)	deau-CofD			
CSID					
	(DANG IU-				
	OP	dearaCouD			
CSUD	(14)	USC19CSUD			
Class 5h antigens	(1100294A)				
	CS17	dectoCebD (reference)			
0300	(\//\$6788A)				
	(W30700A)	Adhasin domain (rasiduas 10,205) of CabD			
CSDD AD	(1/106700/1)	Adhesin domain (residues 19-205) of CSDD			
	(W30700A)	CabD has allelia variations of			
CSDD E20736A	(E20729A)				
	(E20730A)	IN025/5741/104IN/L03R/1144A/1145IN/12931			
	0047	In the CS17 allelic strain E20738A			
CSDD LSN139		USDD has allelic variations of L851/H144A in			
	(LSN02-	the CS17 allelic strain LSN139			
	013966/A)				
USDD/184N/L85R		USDD mutant 184N/L85R			
0 1 D // 1 / / 1 / / /	(WS6788A)				
CsbD/H144A/Y145N	CS17	CsbD mutant H144A/Y145N			
	(WS6788A)				

Table S1. Table of antigens used in the ELISA.

CsbD WS4240A	CS17 (WS4240A)	CsbD has allelic variation of L85I in the CS17 allelic strain of WS4240A				
CooD	ČS1 (E24377A)	dsc15CooD				
Class 5c antigens	· · · ·					
CotD	CS2 (C91f)	dsc19CotD (reference)				
CotD AD	CS2 (C91f)	Adhesin domain (residues 19-205) of CotD				
CotD/R69A	CS2 (C91f)	CotD mutant R69A				
CotD/T87A	CS2 (C91f)	CotD mutant T87A				
CotD/K183A	CS2 (C91f)	CotD mutant K183A				
CotD/R184A	CS2 (C91f)	CotD mutant R184A				

Supplemental Figures

Figure S1. Anti-CfaE Mab ELISA to evaluate responses to various class 5 ETEC fimbrial adhesin variants. The responses to the immunogen CfaE were highlighted in black bars. The bars and error bars represent the respective mean OD values and standard deviations of at least two repeated assays. The dashed lines represented the limit of detection in the anti-CfaE Mab ELISA assays, which equals the sum of average background of PBS buffer and three times of the standard deviation. The mutated residue was recognized as a hotspot residue within the epitope when the mean OD value of the CfaE mutant was lower than the limit of detection.



A. anti-CfaE Mab P8D10 ELISA

* CsbD E20738A has allelic variations of N62S/S74T/T84N/L85R/H144A/Y145N/Y293H comparing to CsbD in the reference strain W S6788A.

B. Anti-CfaE P6C11 ELISA



C. Anti-CfaE Mab P6H4 ELISA



D. Anti-CfaE Mab P10A7 ELISA



E. Anti-CfaE Mab P5C7 ELISA



F. Anti-CfaE Mab P2E11 ELISA



G. Anti-CfaE Mab P3B2 ELISA



H. Anti-CfaE Mab P13A7 ELISA



I. Anti-CfaE Mab P1F9 ELISA



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Figure S2. Anti-CsbD Mab ELISA to evaluate responses to various class 5 ETEC fimbrial adhesin variants. The responses to the immunogen CsbD were highlighted in black bars. The bars and error bars represent the respective mean OD values and standard deviations of at least two repeated assays. The dashed lines represented the limit of detection in the anti-CsbD Mab ELISA assays, which equals the sum of average background of PBS buffer and three times of the standard deviation. The mutated residue was recognized as a hotspot residue within the epitope when the mean OD value of the CsbD mutant was lower than the limit of detection.



A. Anti-CsbD Mab P7C2 ELISA

* CsbD E20738A has allelic variations of N62S/S74T/T84N/L85R/H144A/Y145N/Y293H comparing to CsbD in the reference strain W S6788A.

B. Anti-CsbD Mab P9A5 ELISA



C. Anti-CsbD Mab P2H6 ELISA



D. Anti-CsbD Mab P6G1 ELISA



E. Anti-CsbD Mab P2A9 ELISA



F. Anti-CsbD Mab P1F7 ELISA



G. Anti-CsbD Mab P9E11 ELISA



H. Anti-CsbD Mab P7F9 ELISA



I. Anti-CsbD Mab P5A12 ELISA



J. Anti-CsbD Mab P9D12 ELISA



K. Anti-CsbD Mab P7F12 ELISA



Figure S3. Anti-CotD Mab ELISA to evaluate responses to various class 5 ETEC fimbrial adhesin variants. The responses to the immunogen CotD were highlighted in black bars. The bars and error bars represent the respective mean OD values and standard deviations of at least two repeated assays. The dashed lines represented the limit of detection in the anti-CotD Mab ELISA assays, which equals the sum of average background of PBS buffer and three times of the standard deviation. The mutated residue was recognized as a hotspot residue within the epitope when the mean OD value of the CotD mutant was lower than the limit of detection.



A. Anti-CotD Mab P7F6 ELISA

* CsbD E20738A has allelic variations of N62S/S74T/T84N/L85R/H144A/Y145N/Y293H comparing to CsbD in the reference strain WS6788A.

B. Anti-CotD Mab P3F4 ELISA



C. Anti-CotD Mab P6B8 ELISA



D. Anti-CotD Mab P3D11 ELISA



E. Anti-CotD Mab P9A10 ELISA



F. Anti-CotD Mab P9G7 ELISA



G. Anti-CotD Mab P2B8 ELISA



H. Anti-CotD mAb P12A2 ELISA



Figure S4. Additional anti-CotD mAb ELISA to evaluate responses to specific CotD adhesin mutants. The responses to the immunogen CotD were highlighted in black bars. The bars and error bars represent the respective mean OD values and standard deviations of at least three repeated assays. The dashed lines represented the limit of detection in the anti-CotD Mab ELISA assays, which equals the sum of average background of PBS buffer and three times of the standard deviation. The mutated residue was recognized as a hotspot residue within the epitope when the mean OD value of the CotD mutant was lower than the limit of detection.



A. Anti-CotD mAb P7F6 ELISA

B. Anti-CotD mAb P3F4 ELISA



C. Anti-CotD mAb P6B8 ELISA



D. Anti-CotD mAb P3D11 ELISA



E. Anti-CotD mAb P9A10 ELISA



F. Anti-CotD mAb P9G7 ELISA



G. Anti-CotD mAb P2B8 ELISA



H. Anti-CotD mAb P12A2 ELISA



Figure S5. Multiple sequence alignment of CfaE from CFA/I H10407 strain, CsbD from CS17 WS6788A strain, and CotD from CS2 C91f strain. Residues with allelic variations and site-directed mutations are highlighted with black boxes. The numbers at the top of the sequences indicate amino acid positions in CfaE starting from the leader sequence (not shown). The numbers on the right side of the sequences indicate amino acids positions starting from the leader sequences (not shown) of CfaE, CsbD, and CotD, respectively.

	26	36	45	54	64	74	84	94	
			1	<mark> .</mark>		 · · · · · · · · 		1 1	
CfaE/H10407	ADKNPGSEN	MINTIGPHD	RGGSSPIY	NILNSYLTAYNO	SHHLYDRM	SFLCLSSONTI	LNGACPSSDA	PGTATIDG	96
CsbD/WS6788A	-GRYPETTVGNLT	SFOAPRODI	RSVOSPIY	NIFTNHVAGYSI	SHNLYDRI	VFLCTSSSNPV	NGACPTIG-	-TSGVOYG	33
CotD/C91f	OSWHTNVEAGSIN	TESIGPID	RSAAASYPAH	YIFHEHVAGYNE	DHSLFDRM	TFLCMSSTDAS	KGACPIGE-	-NSKSSOG	96
•								~	
	104	114	124	133	141	150	160	170	
			<mark> </mark>	<mark> </mark> .	<mark> </mark>	<mark> </mark>		1 1	
CfaE/H10407	ETNITLOFTEKRSI	LIKRELOIK	JYKOFLFKNA	NCPSKLALM	ISSHFOCNR	E-OASGATLSI	LYIPAGELNK	LPFGGVWN 1	172
CsbD/WS6788A	TTTITLOFTEKRSI	LIKRNINLA	JNKKPIWENO	SCDTSN-LMVLN	ISKSWSCGH	YGNANGTLLNI	LYIPAGEINK	LPFGGIWE 1	172
CotD/C91f	ETNIKLIFTEKKSI	LARKTLNLK	JYKRFLYESD	RCIHYVDKMNLM	SHTVKCVG	S-FTRGVDFTI	LYIPOGEIDG	LLTGGIWE 1	175
							~		
	180	187	197	207	217	227	237	247	
	and constructions		[[[].		[]		Lecelee	
CfaE/H10407	AVLKLNVKRR	DTTYGTYT:	INITVNLTDK	GNIQIWLPOFKS	NARVDLNL	RPTGGGTYIGE	RNSVDMCFYD	GYSTNSSS 2	249
CsbD/WS6788A	ATLILRLSRYGEVS	SSTHYGNYT	VNITVDLTDK	GNIOVWLPGFHS	SNPRVDLNL	HPIGNYKYSGS	SNSLDMCFYD	GYSTNSDS 2	252
CotD/C91f	ATLELRVKRH	DYNHGTYK	VNITVDLTDK	GNIOVWTPKFHS	DPRIDLNL	RPEGNGKYSGS	SNVLEMCLYD	GYSTHSOS 2	252
				~				~	
	257	267	277	287	297	306	316	326	
			1 1	1				1	
CfaE/H10407	LEIRFODDNSKSDO	KFYLKKIN	DSKELVYTL	SLLLAGKNLTPT	INGOALNIN	-TASLETNWNE	RITAVTMPEI	SVPVLCWP	328
CsbD/WS6788A	MVIKFODDNPTYS	EYNLYKIG	G-TEKLPYAV	SLLMGEKIFYPV	NGOSFTIN	DSSVLETNWNE	RVTAVAMPEV	NVPVLCWP	331
CotD/C91f	IEMRFODDSOTGNI	EYNLIKTG	EPLKKLPYKL	SLLLGGREFYPN	NGEAFTIN	DTSSLFINWNE	RIKSVSLPOI	SIPVLCWP	332
	~ ~ ~						~		
	336	346	356	366	376				
				1		1			
CfaE/H10407	GRLOLDAKVKNPE	AGOYMGNIK:	ITFTPSSOTL	DNKOVEKNITVT	ASVDPVID	LLO 383			
CsbD/WS6788A	ARLLLNADVNAPD	GOYSGOIY	ITETPSVENL	DNKOVEKNITVE	RASVDPKLD	LLO 386			
CotD/C91f	ANLTEMSELNNPE	AGEYSGILN	VTFTPSSSSL	DNKOAEKNITVI	TASVDPTID	LMO 387			
						COLUMN 20			

Figure S6. Anti-CfaE Mab ELISA to evaluate responses to two domains of CfaE. The responses to the immunogen CfaE were highlighted in black bars. The bars and error bars represent the respective mean OD values and standard deviations of at least two repeated assays. The dashed lines represented the limit of detection in the ELISA assay, which equals the sum of average background of PBS buffer and three times of the standard deviation.

