SUPPLEMENTAL MATERIALS

Supplemental Methods

C. innocuum isolation from stool. Clinical stool samples were saved by the clinical microbiology laboratory and stored at 4°C for up to 1 week prior to being aliquoted and stored at -80°C. To isolate *C. innocuum,* patient stool samples were thawed, and 200 mg aliquots were resuspended in 1 ml of saline. Sterile swabs were used to plate on pre-reduced cycloserine-cefoxitin-fructose agar with sodium taurocholate (TCCFA) with or without 1µg/ml vancomycin to further select for *C. innocuum*. Plates were then incubated for 72 h at 37°C in an anaerobic chamber (5% hydrogen, 5% CO_2, 90% N_2). Stools that did not grow *C. innocuum* underwent ethanol shock for culture enrichment. An equal volume of 95% ethanol was added, and the mixture was incubated with shaking at 220 rpm at room temperature for 45 min. Samples were then centrifuged at 2,000xg for 15 min, and pellets were washed twice with saline and plated onto TCCFA and incubated as described above. Individual colonies were then selected and re-streaked onto Blood Agar Plates (TSA w/5% sheep blood). Single colonies were then picked and grown in Tryptic Soy Broth (TSB) (Millipore Sigma, Burlington, MA) as described below. *C. innocuum* was identified by matrix-assisted laser desorption/ionization- time of flight (MALDI-TOF) using the Bruker biotyper (Bruker, Billerica, MA). Further confirmation was identified by whole genome sequencing (see below).

Bacterial isolates and culture conditions**.** All bacterial isolates and plasmids used are listed in **Table S3.** In an anaerobic chamber, *C. innocuum* isolates were first cultured on blood agar plates and incubated for 24 h at 37°C. Single colonies were re-cultured for 24-48 h when indicated. For cloning, TOPO®10 *Escherichi coli* (Life Technologies Invitrogen, Carlsbad, CA) was grown at 37°C shaking in Luria-Bertani (LB) broth with 50–100 µg/ml ampicillin, when needed for plasmid maintenance.

Strain construction. *C. innocuum* (CI) amplicon was generated by identifying the core genome from 5 publicly available whole genome sequences (NCBI accessions CP022722.1, CZBW01, AGYV01, JQIF01, and FOTN01) using Spine [1]. Primer3 v2.5.0 was used to generate primer sets from the consensus core genome. Primer sets were screened using *in silico* PCR (https://github.com/egonozer/in_silico_pcr) against all available reference and draft genome sequences and one set was selected that was found in each genome with no mismatches or indels.

The primer set was found to amplify an intergenic region upstream of the tRNA-Ser gene with locus ID G4D54_RS11630. NCBI Primer Blast was used to screen the primer set for amplification against the RefSeq representative genomes database filtered for all bacteria (taxid 2) and *Homo sapiens* (taxid 9606) with only *C. innocuum* species producing an amplicon. To further confirm our primer set was specific to *C. innocuum*, we performed *in silico* PCR against 60,664 draft genome assemblies produced from metagenomic sequencing of human gut samples from multiple experiments [2]. Our amplicon primers amplified unique nucleotide sequences only found in *C. innocuum*. The CI amplicon sequence was generated using the primers indicated in **Table S4**, and was cloned into pCR™II-TOPO® TA. PCR and sequencing confirmed the identity of vector inserts. Plasmids were introduced into TOPO®10 *E. coli* using Rapid One Shot® TOPO® TA Cloning® (Life Technologies Invitrogen, Carlsbad, CA) chemical transformation protocol. The plasmid containing CI amplicon was used as a positive control for qPCR experiments.

C. innocuum Quantitative PCR: Genomic DNA (gDNA) was extracted from stool samples (100 mg) using QIAaMP PowerFecal Pro DNA Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. Quantitative PCR (qPCR) for *C. innocuum* was performed using PowerUp™ SYBR™ Green Master Mix (Applied Biosystems™, Waltham, MA) and qPCR amplicon primers listed in **Table S4**. qPCR samples were then run on a QuantStudio 6 Flex using QuantStudio Real-Time PCR Software v1.7.1 for analysis (Applied Biosystems™, Waltham, MA).

C. difficile tcdB PCR: *C. difficile tcdB* endpoint PCR was performed on either gDNA extracted from stool samples using QIAaMP PowerFecal Pro DNA Kit or from a 2 ml bacterial culture overnight using the QIAamp BiOstic Bacteremia DNA Kit (Qiagen, Hilden, Germany) following the manufacturer’s instructions. Primers used to amplify *tcbB* are indicated in **Table S4**. Cycling Conditions: 95°C 5 min; 40 cycles of: (95°C for 1 min, 55°C for 1 min**,** 72°C for 1 min); 72°C 10 min**;** Hold at 4°C.

Whole genome sequencing and analyses

<u>Genomic DNA extraction</u>: The gDNA was extracted from single colonies cultured overnight using appropriate growth conditions as described above and the QIAamp BiOstic Bacteremia DNA Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol.

<u>Library preparation and whole genome sequencing (WGS)</u>: Libraries for Illumina sequencing were prepared using Nextera XT library kits (Illumina, Inc., San Diego, CA) or plexWell library kits (seqWell, Beverly, MA) and sequenced on the Illumina MiSeq platform to generate paired-end 300 bp reads. Reads were quality trimmed and adapters removed using Trimmomatic (v0.36) [3], and *de novo* assembly was performed using SPAdes version 3.9.1 [4]. Assembly sequences were annotated with prokka v1.14.6 [5] using the *C. innocuum* strain ATCC 14501 (NCBI accession NZ_CP048838) as reference. WGS data, analyses, and genome accession numbers are contained in **Table S5**.

<u>Phylogenomic and comparative genomics analyses</u>**:** Core genome plylogenomic relationships between sequenced *C. innocuum* isolates were determined from whole genome assemblies using kSNP v3.01 [6]. Two additional *C. innocuum* strains were included in the analyses, the ATCC reference strain 14501 [7, 8] and LC-LUMC-CI-001 (LC-CI), a strain isolated from the stool of an adult thought to have *C. innocuum*-associated diarrhea [9]. Cladogram figures were generated using EvolView [10, 11].

To identify accessory genomic elements, the core genome of the 119 assembled genome sequences was determined using Spine v0.3.2 and accessory genome sequences of each isolate were determined using AGEnt v0.3.1 [1] ClustAGE v0.8.1 [12] as used to identify unique accessory genomic elements (AGEs) and determine their distributions among the set of assembled sequences. Cramer's V statistic for association with isolates from individuals with diarrheal illness was calculated for each accessory element in R using the *rcompanion* package v2.4.1 [13].

Supplemental Results

Prevalence of C. innocuum: Adult and Pediatric Subgroup Analyses

Overall, *C. innocuum* was isolated by culture from stool from 126/300 (42%) subjects. Among these, *C. innocuum* was isolated by culture from the stool of 71/150 (47%) adults and 55/150 (36%) children (p=0.06). Children had a lower median (IQR) ct values than the adult group (24.34 [19.85-27.31] vs. 25.33 [21.58-28.29]; *p*=0.03).

Among adults, *C. innocuum* was isolated by culture more frequently among asymptomatic controls: 30/50 (60%) from subjects with AAD and 41/100 (41%) from asymptomatic controls (*p*=0.03; **Figure S1A**). However, qPCR ct values between asymptomatic and diarrheal symptomatic adults were not significantly different, with median (IQR) ct values of 24.9 (22.5-26.1) vs. 25.6 (21.4-28.7), respectively (*p*=0.42; **Figure S1C**). From subjects with AAD who were colonized with *C. difficile*, *C. innocuum* was isolated more frequently and had lower median (IQR) ct values (56% and 24.9 [21.2-27.6]) than those who were not colonized with *C. difficile* (26% [*p*=0.04]; and 27.9 [23-29.5], *p*=0.002; **Figure S1B and Figure S1D**).

Among children with or without AAD, *C. innocuum* was isolated by culture at a similar frequency: 20/50 (40%) subjects with AAD and 35/100 (35%) asymptomatic controls (*p*=0.06; **Figure S2A**). Asymptomatic controls had lower median (IQR) ct values than the diarrheal group (23.2 [18.9-26.8] vs. 25.2 [21.3-28.0], *p*=0.02; **Figure S2C**). From subjects with AAD who were colonized with *C. difficile*, *C. innocuum* was isolated at a similar frequency and had similar stool qPCR ct values (42% and 24.3 [19.9-28.1]) than those who were not colonized with *C. difficile* (28% [*p*=0.15]; and 25.7 [23.1-27.5], *p*>0.9999; **Figure S2B and Figure S2D**).

Supplemental References

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Supplemental Tables

Table S1: Demographic and clinical characteristics of included subjects in AAD and asymptomatic control pediatric and adult subgroups

	Study Cohort (n=300)	Pediatric: AAD, C. diff colonized (n=50)	Pediatric: AAD, C. diff non-colonized (n=50)	Pediatric: Asymptomatic controls (n=50)	Adult: AAD, C. diff colonized (n=50)	Adult: AAD, C. diff non-colonized (n=50)	Adult: Asymptomatic controls (n=50)
Age (y)							
Median (IQR)	20 (9-59)	11 (6-16)	12 (6-15)	6 (4-11)	58 (47-71)	60 (48-69)	20 (11-59)
Age (y) range	2-94	(2-18)	(2-18)	(2-17)	(24-94)	(22-92)	2-94
Sex (male)	155 (51.7%)	36 (72%)	25 (50%)	25 (50%)	18 (36%)	26 (52%)	25 (50%)
Race/Ethnicity							
Asian	11 (3.7%)	3 (6%)	1 (2%)	4 (8%)	1 (2%)	1 (2%)	1 (2%)
American Indian/Alaska Native	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Black	64 (21.3%)	6 (12%)	4 (8%)	8 (16%)	14 (28%)	12 (24%)	20 (40%)
Native Hawaiian/Other Pacific Islander	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
White	169 (56.3%)	25 (50%)	31 (62%)	25 (50%)	31 (62%)	32 (64%)	25 (50%)
Unknown	6 (2%)	0 (0%)	0 (0%)	1 (2%)	2 (4%)	3 (6%)	0 (0%)
Other	50 (16.7%)	16 (32%)	14 (28%)	12 (24%)	2 (4%)	2 (4%)	4 (8%)
Ethnicity							
Hispanic or Latino	57 (19%)	16 (32%)	15 (30%)	11 (22%)	6 (12%)	5 (10%)	4 (8%)
Not Hispanic or Latino	237 (79%)	34 (68%)	34 (68%)	39 (78%)	42 (84%)	42 (84%)	46 (92%)
Unknown	6 (2%)	0 (0%)	1 (2%)	0 (0%)	2 (4%)	3 (6%)	0 (0%)
Comorbidities							
Any comorbid condition	273 (91%)	48 (96%)	45 (90%)	45 (90%)	47 (94%)	46 (92%)	42 (84%)
Malignancy/stem cell transplant	95 (31 7%)	18 (36%)	19 (38%)	16 (32%)	14 (28%)	23 (46%)	5 (10%)
IBD	6 (2%)	2 (4%)	3 (6%)	0 (0%)	0 (0%)	1 (2%)	0 (0%)
Non-IBD gastrointestinal condition	34 (11.3%)	10 (20%)	9 (18%)	2 (4%)	5 (10%)	6 (12%)	2 (4%)
Solid organ transplant	32 (10.7%)	5 (10%)	4 (8%)	2 (4%)	10 (20%)	6 (12%)	5 (10%)
Other immunocompromising condition	21 (7%)	5 (10%)	1 (2%)	0 (0%)	9 (18%)	3 (6%)	3 (6%)
Cardiovascular	99 (33%)	6 (12%)	4 (8%)	7 (14%)	25 (50%)	25 (50%)	32 (64%)

Liver	7 (2.3%)	0 (0%)	1 (2%)	1 (2%)	3 (6%)	1 (2%)	1 (2%)
Kidney	32 (10.7%)	1 (2%)	4 (8%)	2 (4%)	9 (18%)	8 (16%)	8 (16%)
Pulmonary	20 (6.7%)	1 (2%)	2 (4%)	9 (18%)	0 (0%)	3 (6%)	5 (10%)
Endocrine/Diabetes	34 (11.3%)	1 (2%)	1 (2%)	0 (0%)	11 (22%)	8 (16%)	13 (26%)
Antibiotic exposure (past 30d)							
Any systemic antibiotic	263 (87.7%)	50 (100%)	50 (100%)	37 (74%)	50 (100%)	50 (100%)	26 (52%)
Third/fourth generation cephalosporin	103 (34.3%)	19 (38%)	23 (46%)	17 (34%)	13 (26%)	23 (46%)	8 (16%)
Clindamycin	16 (5.3%)	4 (8%)	3 (6%)	6 (12%)	0 (0%)	1 (2%)	2 (4%)
Fluoroquinolone	45 (15%)	2 (4%)	7 (14%)	4 (8%)	12 (24%)	18 (36%)	2 (4%)

Table S2. ***tcdB*** **Endpoint PCR of asymptomatic control subjects**

	C. difficile tcdB **PCR positive**	***C. difficile tcdB*** **PCR negative**	**Total**
C. innocuum **culture positive**	10	40	50 (50%)
C. innocuum **culture negative**	9	41	50 (50%)
Total	19 (19%)	81 (81%)	100

Association between *C. difficile tcdB* PCR and *C. innocuum* culture positivity: Cramer's V = 0.03 (no association)

Table S3. Bacterial strains and plasmids used in this study

Strain or plasmid		**Relevant genotype or description**	**Source or reference**
Strains	*E. coli*		
	One Shot® TOP10 Competent Cells	F- mcrA Δ(mrr-hsdRMS-mcrBC) φ80lacZΔM15 ΔlacX74 recA1 araD139 Δ(araleu)7697 galU galK rpsL (StrR) endA1 nupG	Life Technologies
Plasmids	pCR™II-TOPO®		Life Technologies
	pCR™II-TOPO®/CI amplicon	pCR™II-TOPO/CI-amplicon cloned; amp^R	This study

Table S4. Primers used for ***C. difficile*** **endpoint PCR and** ***C. innocuum*** **qPCR**

Primer Name	**Primer Sequence**
tcdB-F5670	CCAAARTGGAGTGTTACAAACAGGTG
tcdB-R6079A	GCATTTCTCCATTCTCAGCAAAGTA
tcdB-R6079B	GCATTTCTCCGTTTTCAGCAAAGTA
CI_amplicon F	TTTGAAGCAGACCTCTTCCG
CI_amplicon R	ATACAGCGGTATGCAGATTCC

Table S5: Accession numbers and whole genome sequencing read data for included samples

Genome Accession	Biosample Accession	Isolate ID	Study Tree Label	Trimmed Reads (bp)	Contigs (n)	Genome Assembly size (bp)	Fold coverage
JAKTIN000000000	SAMN26133632	ES-LK3N-079	Ch-Di-CDN-079	145263905	70	4267269	34.04
JAKTIO000000000	SAMN26133634	ES-LK3N-089v	Ch-Di-CDN-089	142159129	131	4680075	30.37
JAKTIP000000000	SAMN26133635	ES-LK3N-099V	Ch-Di-CDN-099	210863915	226	4926477	42.8
JAKTIQ000000000	SAMN26133637	ES-LK3N-118	Ch-Di-CDN-118	105303577	204	4626388	22.76
JAKTIR000000000	SAMN26133641	ES-LK3N-157	Ch-Di-CDN-157	70560628	161	4617537	15.28
JAKTIS000000000	SAMN26133643	ES-LK3N-18vs	Ch-Di-CDN-018	184474992	70	4432230	41.62
JAKTIT000000000	SAMN26133644	ES-LK3P-313v	Ch-Di-CDC-313	107554920	39	4321392	24.88
JAKTIU000000000	SAMN26133648	ES-LK3P-333v	Ch-Di-CDC-333	157230099	113	4670227	33.66
JAKTIV000000000	SAMN26133649	ES-LK3P-335v	Ch-Di-CDC-335	109496484	103	4621349	23.69
JAKTIW000000000	SAMN26133650	ES-LK3P-337v	Ch-Di-CDC-337	145325807	86	4575492	31.76
JAKTIX000000000	SAMN26133654	ES-LK3P-402v	Ch-Di-CDC-402	179207488	86	4279078	41.87
JAKTIY000000000	SAMN26133655	ES-LK3P-413v	Ch-Di-CDC-413	127424075	175	5042912	25.26
JAKTIZ000000000	SAMN26133656	ES-LK3P-415v	Ch-Di-CDC-415	135674149	73	4123513	32.9
JAKTJA000000000	SAMN26133657	ES-LK3P-452v	Ch-Di-CDC-452	210359527	73	4135343	50.86
JAKTJB000000000	SAMN26133658	ES-LK3P-462v	Ch-Di-CDC-462	143118540	72	4165313	34.35
JAKTJC000000000	SAMN26133661	ES-LK3P-474v	Ch-Di-CDC-474	108330895	139	4710457	22.99
JAKTJD000000000	SAMN26133662	ES-LK3P-475v	Ch-Di-CDC-475	141011252	135	4709847	29.93
JAKTJE000000000	SAMN26133663	ES-LK3P-476v	Ch-Di-CDC-476	82982610	80	4230786	19.61
JAKTJF000000000	SAMN26133664	ES-LK3P-477v	Ch-Di-CDC-477	57438175	173	4544210	12.63
JAKTJG000000000	SAMN26133665	ES-LK4-006	Ch-As-006	67218540	127	4614424	14.56
JAKTJH000000000	SAMN26133666	ES-LK4-008	Ch-As-008	63540684	220	5002814	12.7
JAKTJI000000000	SAMN26133667	ES-LK4-009v	Ch-As-009	57791925	110	4231820	13.65
JAKTJJ000000000	SAMN26133668	ES-LK4-013v	Ch-As-013	189760205	115	4550898	41.69
JAKTJK000000000	SAMN26133669	ES-LK4-017	Ch-As-017	70266001	93	4281236	16.41
JAKTJL000000000	SAMN26133670	ES-LK4-023v	Ch-As-023	90147588	76	4477892	20.13
JAKTJM000000000	SAMN26133671	ES-LK4-029v	Ch-As-029	78398554	78	4476279	17.51
JAKTJN000000000	SAMN26133675	ES-LK4-047v	Ch-As-047	115302853	196	4630186	24.9
JAKTJO000000000	SAMN26133676	ES-LK4-049s	Ch-As-049	82220052	165	5124170	16.04
JAKTJP000000000	SAMN26133677	ES-LK4-051s	Ch-As-051	132663210	184	4542123	29.2
JAKTJQ000000000	SAMN26133678	ES-LK4-057v	Ch-As-057	122058116	104	4750803	25.69
JAKTJR000000000	SAMN26133680	ES-LK4-060v	Ch-As-060	101261237	102	4370918	23.16
JAKTJS000000000	SAMN26133681	ES-LK4-063	Ch-As-063	69794702	121	4782743	14.59
JAKTJT000000000	SAMN26133685	ES-NU1-AC-001	Ad-As-001	53658042	145	4423388	12.13
JAKTJU000000000	SAMN26133686	ES-NU1-AC-004v	Ad-As-004	186369525	106	4852829	38.4
JAKTJV000000000	SAMN26133689	ES-NU1-AC-012L	Ad-As-012	90542211	130	4814669	18.8
JAKTJW000000000	SAMN26133690	ES-NU1-AC-013L	Ad-As-013	113177718	144	4766266	23.74
JAKTJX000000000	SAMN26133691	ES-NU1-AC-019v	Ad-As-019	93113280	120	4871818	19.11
JAKTJY000000000	SAMN26133692	ES-NU1-AC-020v	Ad-As-020	71792794	43	4433780	16.19
JAKTJZ000000000	SAMN26133694	ES-NU1-AC-024s	Ad-As-024	55482473	270	4704092	11.79
JAKTKA000000000	SAMN26133700	ES-NU1-AC-037v	Ad-As-037	73848451	90	4226656	17.47
JAKTKB000000000	SAMN26133701	ES-NU1-AC-039	Ad-As-039	70432411	91	4225041	16.67
JAKTKC000000000	SAMN26133702	ES-NU1-AC-041v	Ad-As-041	85989592	126	4912908	17.5
JAKTKD000000000	SAMN26133703	ES-NU1-AC-043	Ad-As-043	63897825	153	4683634	13.64
JAKTKE000000000	SAMN26133704	ES-NU1-AC-044v	Ad-As-044	86388982	126	4732861	18.25

JAKTKF000000000	SAMN26133705	ES-NU1-AC-045v	Ad-As-045	155716725	166	5053631	30.81
JAKTKG000000000	SAMN26133706	ES-NU1-AC-047v	Ad-As-047	80232308	52	4506785	17.8
JAKTKH000000000	SAMN26133707	ES-NU1-AC-050v	Ad-As-050	102226202	107	4915124	20.79
JAKTKI000000000	SAMN26133708	ES-NU1-AC-055v	Ad-As-055	123562972	73	4200827	29.41
JAKTKJ000000000	SAMN26133709	ES-NU1-AC-058v	Ad-As-058	132316410	160	5092192	25.98
JAKTKK000000000	SAMN26133710	ES-NU1-AC-060v	Ad-As-060	109117691	171	5053153	21.59
JAKTKL000000000	SAMN26133711	ES-NU1-AC-063L	Ad-As-063	116505255	99	4288226	27.16
JAKTKM000000000	SAMN26133712	ES-NU1-AC-069v	Ad-As-069	61988719	333	4982908	12.44
JAKTKN000000000	SAMN26133714	ES-NU1-N-006v	Ad-Di-CDN-006	136321473	76	4683957	29.1
JAKTKO000000000	SAMN26133719	ES-NU1-N-042V	Ad-Di-CDN-042	94250036	80	4309235	21.87
JAKTKP000000000	SAMN26133722	ES-NU1-P-011vL	Ad-Di-CDC-011	161444008	111	4458369	36.21
JAKTKQ000000000	SAMN26133723	ES-NU1-P-021v	Ad-Di-CDC-021	126855973	97	4340552	29.22
JAKTKR000000000	SAMN26133724	ES-NU1-P-023v	Ad-Di-CDC-023	108393838	158	4778707	22.68
JAKTKS000000000	SAMN26133725	ES-NU1-P-030v	Ad-Di-CDC-030	95263670	65	4321681	22.04
JAKTKT000000000	SAMN26133734	ES-NU1-P-075v	Ad-Di-CDC-075	63132375	222	4742362	13.31
JAKTKU000000000	SAMN26133740	ES-NU1-P-088s	Ad-Di-CDC-088	147480999	128	4588916	32.13
JAKTKV000000000	SAMN26133744	ES-NU1-P-098v	Ad-Di-CDC-098	144603464	120	4708720	30.7
JAKTKW000000000	SAMN26133745	ES-NU1-P-099v	Ad-Di-CDC-099	125721298	189	5116544	24.57
JAKTKX000000000	SAMN26133746	ES-NU1-P-101v	Ad-Di-CDC-101	96106006	79	4402081	21.83
JAKTKY000000000	SAMN26133747	ES-NU1-P-102v	Ad-Di-CDC-102	116502650	74	4288338	27.16
JAKTKZ000000000	SAMN26133630	LK3N-002v	Ch-Di-CDN-002	88441821	158	4820073	18.34
JAKTLA000000000	SAMN26133631	LK3N-042v	Ch-Di-CDN-042	117934124	64	4097096	28.78
JAKTLB000000000	SAMN26133633	LK3N-085v	Ch-Di-CDN-085	90928149	140	4624173	19.66
JAKTLC000000000	SAMN26133636	LK3N-102	Ch-Di-CDN-102	57099045	102	4553048	12.54
JAKTLD000000000	SAMN26133638	LK3N-119V	Ch-Di-CDN-119	97032151	64	4096925	23.68
JAKTLE000000000	SAMN26133639	LK3N-120v	Ch-Di-CDN-120	71724351	79	4258639	16.84
JAKTLF000000000	SAMN26133640	LK3N-131v	Ch-Di-CDN-131	115129240	119	4156210	27.7
JAKTLG000000000	SAMN26133642	LK3N-164V	Ch-Di-CDN-164	70877291	145	4560140	15.54
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JAKTLP000000000	SAMN26133672	LK4-030v	Ch-As-030	78765864	51	4492787	17.53
JAKTLQ000000000	SAMN26133673	LK4-037v	Ch-As-037	140531555	193	4736494	29.66
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JAKTMB000000000	SAMN26133697	NU1-AC-030v	Ad-As-030	135206510	172	5033604	26.86
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JAKTMF000000000	SAMN26133715	NU1-N-014v	Ad-Di-CDN-014	126188389	100	5007695	25.19
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JAKTMH000000000	SAMN26133717	NU1-N-024v	Ad-Di-CDN-024	82713555	229	5007713	16.51
JAKTMI000000000	SAMN26133718	NU1-N-037v	Ad-Di-CDN-037	103430746	118	4531600	22.82
JAKTMJ000000000	SAMN26133720	NU1-N-083v	Ad-Di-CDN-083	88735714	63	4238605	20.93
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JAKTMV000000000	SAMN26133737	NU1-P-083v	Ad-Di-CDC-083	97119045	152	4643782	20.91
JAKTMW000000000	SAMN26133738	NU1-P-084v	Ad-Di-CDC-084	75709735	67	4133230	18.31
JAKTMX000000000	SAMN26133739	NU1-P-086v	Ad-Di-CDC-086	107516359	92	4414456	24.35
JAKTMY000000000	SAMN26133741	NU1-P-089s	Ad-Di-CDC-089	84127968	80	4181681	20.11
JAKTMZ000000000	SAMN26133742	NU1-P-090v	Ad-Di-CDC-090	123011076	73	4214980	29.18
JAKTNA000000000	SAMN26133743	NU1-P-091v	Ad-Di-CDC-091	139462877	38	4120577	33.84
JAKTNB000000000	SAMN26133748	NU1-P-103l	Ad-Di-CDC-103	177206824	49	4340322	40.82

SUPPLEMENTAL FIGURES

Figure S1. *C. innocuum* culture positivity rates and cycle threshold values from adult subjects with AAD and asymptomatic controls.

(A-B) Percent *C. innocuum* (CI) culture positivity rates for a cohort of subjects with (panel A) diarrhea (Di) or asymptomatic controls (As), and (panel B) subjects with diarrhea who are either *C. difficile* colonized (Di-CDC) or *C. difficile* negative (Di-CDN). (C-D) Box plots of CI cycle threshold (Ct) values as determined by qPCR of (panel C) As and Di subjects, and (panel D) As, Di-CDC, and Di-CDN. Boxes show the median, 25th and 75th percentiles. The whiskers represent the maximum and minimum data values.

Ns, not significant; *p-value < 0.05 (or Bonferroni-corrected p value <0.25 for pairwise comparisons of multiple groups in panels B and D); **p-value < 0.01; ***p-value < 0.001

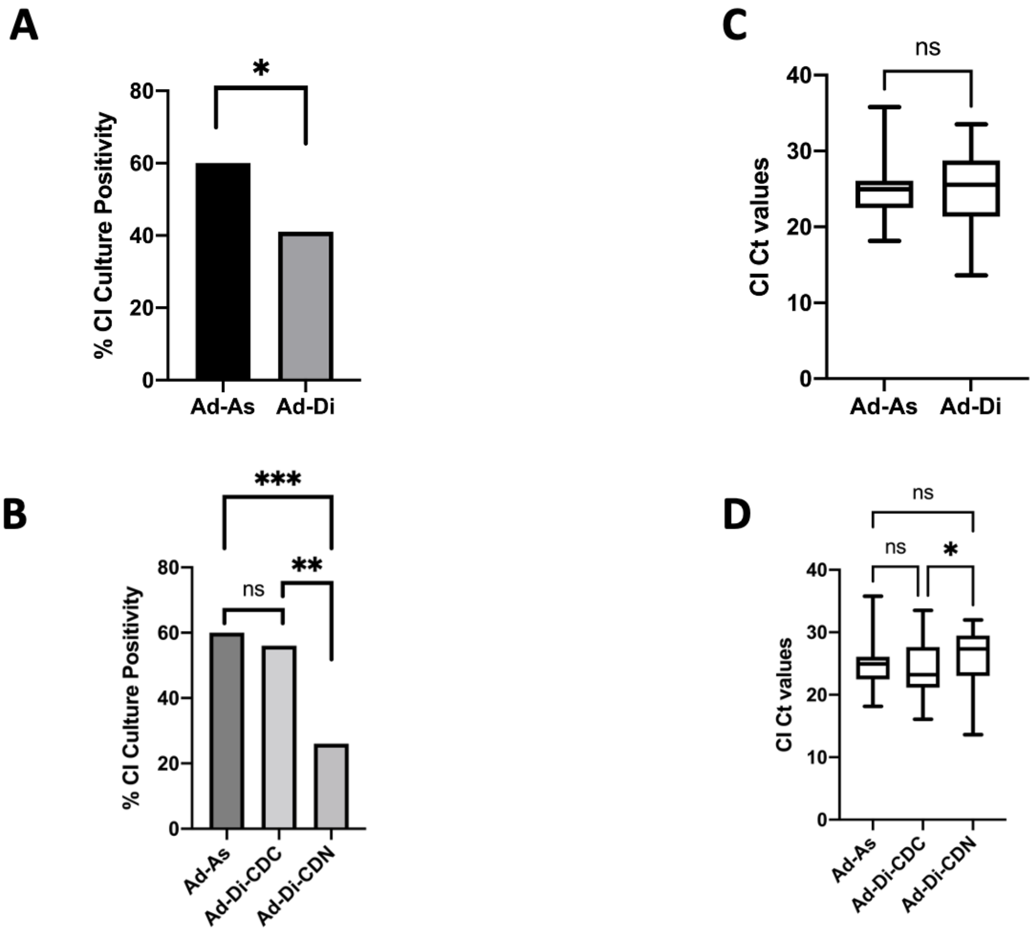

Figure S2. *C. innocuum* culture positivity rates and cycle threshold values from pediatric subjects with AAD and asymptomatic controls.

(A-B) Percent *C. innocuum* (CI) culture positivity rates for a cohort of subjects with (panel A) diarrhea (Di) or asymptomatic controls (As), and (panel B) subjects with diarrhea who are either *C. difficile* colonized (Di-CDC) or *C. difficile* negative (Di-CDN). (C-D) Box plots of CI cycle threshold (Ct) values as determined by qPCR of (panel C) As and Di subjects, and (panel D) As, Di-CDC, and Di-CDN. Boxes show the median, 25th and 75th percentiles. The whiskers represent the maximum and minimum data values.

ns, not significant; *p-value < 0.05 (or Bonferroni-corrected p value <0.25 for pairwise comparisons of multiple groups in panels B and D); **p-value < 0.01; ***p-value < 0.001

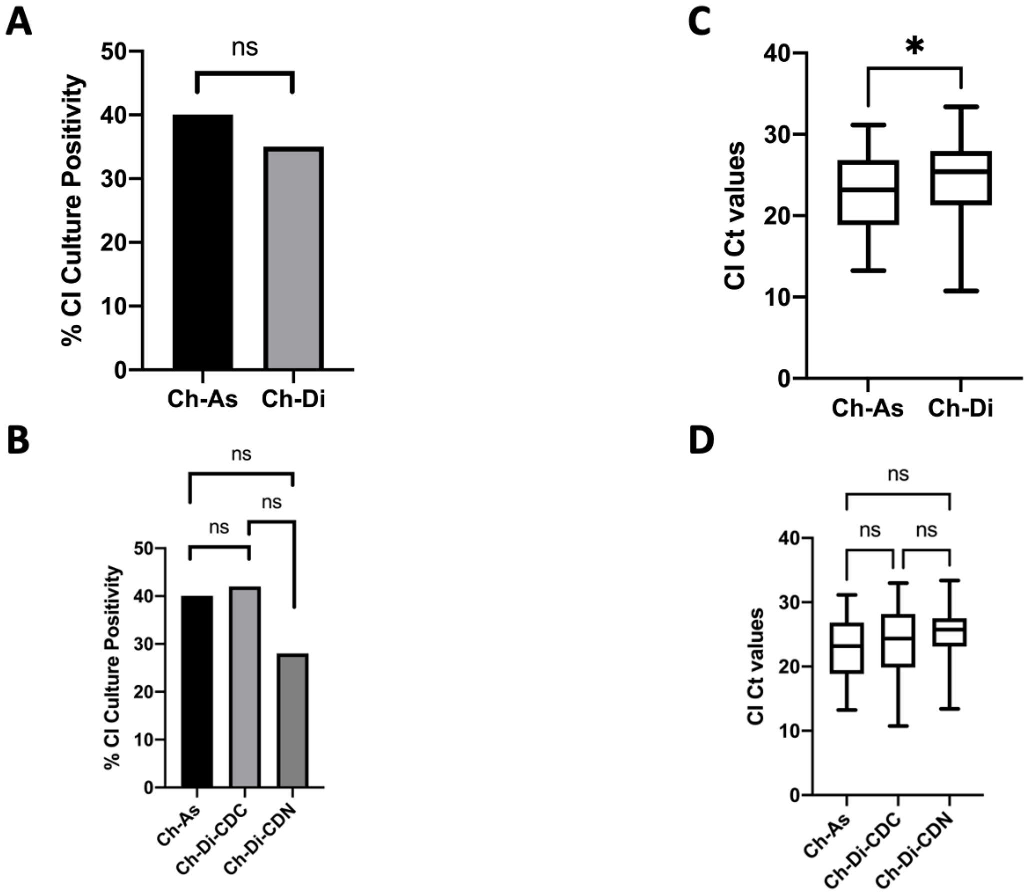

Figure S3. Cladogram of *C. innocuum* isolates stratified by age group.

C. innocuum isolates from children (n=55) and adults (n=64) are identified by pink and gray bars, respectively. The two reference strains, LC-CI [9] and CI-ATCC [8], are indicated by the black bar.