Appendix 1.

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Appendix 2. Additional Details on Methods

Culture methods: Specimens for culture were collected using Dacron® swabs [VWR, Radnor, PA], placed immediately in Port-A-Cul™ transport media [Beckton Dickinson, Cockeysville, MD] and shipped the same day for overnight delivery. Culture specimens were plated ≤48 hours after collection on Brucella anaerobe blood agar (BR) plate (Hardy Diagnostics, Santa Maria, CA), then placed in chopped meat carbohydrate (CMC) broth and both incubated at 37°C under anaerobic conditions ≥48 hours. The second swab was placed in 1.8 ml saline and frozen at -80°C. After incubation, the CMC broth was held at room temperature in ambient air for ≥7 days in order to approximate starvation conditions and induce spore production. The BR plate was immediately examined for colonies suggestive of *C. sordellii* and *C. perfringens*. Suspected isolates were Gram stained, tested for hydrogen peroxide production (15% catalase), spot indole production, lipase and lecithinase activity as previously described (1,2). All isolates were frozen at -80°C in 0.5 ml litmus milk. A 1 ml aliquot of broth was removed from the CMC tube to a sterile tube containing 1 ml of 95 % ethanol. This mixture was incubated for at ≥1 hour, then subcultured to a BR plate which was incubated anaerobically at 37 degrees C ≥48 hours and examined for the specific isolates.

PCR Methods: Two LightCycler® (Roche Diagnostics, Indianapolis, IN) assays utilizing species-specific primers and probes targeting the sialidase genes for *C. sordellii* and *C. perfringens* were used (see Appendix 3 below). Assays were validated using *C. sordellii* ATCC 9714 and *C. perfringens* ATCC 13124, as well as additional *C. sordellii* and *C. perfringens* from the CDC collection, and other *Clostridium* species to ensure specificity. PCR specimens were collected with flocked swabs placed in modified AMIES media [eSwab Collection and Transport System, Fischer Scientific, Waltham, MA] and kept frozen at -20°C. AMIES liquid was centrifuged and DNA was extracted from the pellet using the High Pure PCR Template Preparation Kit (Roche Diagnostics). 20 µl assays were run using FastStart DNA Master Hybridization Probes Kit (Roche Diagnostics) using primers, probes and conditions indicated (see Appendix 3 below). 16s positive and negative controls were performed with every run. Melting curve peaks (Tm) were interpreted alongside the positive and negative controls: *C. sordellii* generates a Tm of 55-56° C and a characteristic left shoulder, and *C. perfringens* generates a broader peak with a TM of 52-55° C and a right shift (3-6).

Specimen collection: For vaginal samples, without using a speculum, swabs were inserted into the introitus about 1 inch and then rotated around the vaginal wall about 5 times. For the rectal samples, swabs were inserted one to two cm

into the rectum and withdrawn. Labial abnormalities were noted, and observations of the cervix and vagina were documented only if speculum and/or bimanual examinations were performed as part of the participant's routine care.

Models for Predictors: We used generalized estimating equations with robust standard error estimation and exchangeable correlation structures to account for clustering of the observations by study site to explore predictors for the presence of *C. sordellii* and *C. perfringens* at baseline and at time two. We ran bi-variate analyses of socio-demographic characteristics, behaviors and laboratory results for the presence of each species at baseline and at the second visit. We explored any variable that was statistically significant in the bivariate analyses to include in the adjusted models, using a critical alpha value of 0.05 for *C. perfringens* and 0.10 for *C. sordellii*, given the low prevalence, and thus reduced power for *C. sordellii* analyses. We tested for correlations between covariates and selected variables to include based on model fit and biological plausibility. We included age in all adjusted models regardless of statistical significance; when model fit allowed, we also included race/ethnicity and /or education regardless of statistical significance as key socio-demographic characteristics. For analyses of the presence of these clostridia at the second visit, we ran two models: one for all women who attended a second visit and one for women who had received an abortion at baseline in order to test variables associated with the abortion procedure. We included measures on the use of antibiotics between baseline and the second visit in all adjusted models of abortion patients at time two, regardless of statistical significance, given its use after medical abortions in response to fatalities from these bacteria. These analyses were exploratory and include multiple comparisons, thus the p-values in the adjusted models should be interpreted with caution. Using a Bonferonni-adjustment, p-values less than 0.001 are considered significant.

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Appendix 3. Primers and Probes Used in the Study

Gene target	Primer/ probe name	Sequence (5'-3')	Final concentration	Expected size / Tm	Cycling conditions:
C. sordellii sialidase	*C. sordellii* - SF	TCA GCA TGT AAT ATC AAT GGT AT	0.5 uM	55-56 F	95C for 10 min; 45 cycles (95C for 7 sec, 53C for 5 sec, 72C for 15 sec); followed by meltcurve analysis
	C. sordellii - SR	CCA TTA TCA TCA GAA TAA ACC CAT	1 uM		
	SordSialFlu	TAA AAG CAC GGA TAA TGG ACA AAA CA--FL	0.1 uM		
	SordSialRed	LC640-GGG ACT ATA AAA CAG TAA TGG AAA ATG A--PH	0.1 uM		
C. perfringens sialidase	*C. perfringens* - 408	CCA AAT GAT TTA TTC TGA TGA C	0.5 uM	52-55 F	
	C. perfringens - 579	GGA AAT TTG TGC TGG CAT A	1 uM		
	PerfSialFlu	CAA GTA ATA CAA TTG GAT GGC TAG G --FL	0.1 uM		
	PerfSialRed	LC640-GGA GTT GGC TCA GGT ATT GTA ATG G—PH	0.1 uM		
16s amplification control (real-time PCR)	16S - P1	AGA GTT TGA TCC TGG CTC AG	1 uM	525 bp	95C for 10 min; 45 cycles (95C for 7 sec, 62C for 7 sec, 72C for 25 sec); followed by meltcurve analysis
	16S - P3	TTA CCG CGG CTG CTG GCA	1 uM		
C. sordellii lethal toxin (*tcsL*)	TCSL-F	ATG AAC TTA GTT AAC AAA GCC CAA	0.25 uM	251 bp	95C for 5 min; 35 cycles (95C for 30 sec, 60C for 90 sec, 72C for 90 sec); 68C for 10 min.
	TCSL-R	AAT ACT TCC ATA GTT AGA TAT TCT TTA	0.25 uM		
C. sordellii hemorrhagic toxin (*tcsH*)	TCSH-F	GTT GCC AGG GAT ACA ACC AGA	0.25 uM	526 bp	
	TCSH- R	GGC ACG AGC TTC TGG CAT A	0.25 uM		

C. sordellii phospholipase C (*csp*)	CSP-F	TAA AGA TGC AGT AGC TAA TAA GGA TTT	0.25 uM	223 bp	
	CSP-R	TTC CTG AAA TTT GAT CTT CTG AAA CC	0.25 uM		
C. perfringens alpha toxin (*cpa*)	CPA-PF	GTT GAT AGC GCA GGA CAT GTT AAG	0.25 uM	402 bp	
	CPA-PR	CAT GTA GTC ATC TGT TCC AGC ATC	0.25 uM		
C. perfringens beta toxin (*cpb*)	CPB-PF	ACT ATA CAG ACA GAT CAT TCA ACC	0.25 uM	236 bp	
	CPB-PR	TTA GGA GCA GTT AGA ACT ACA GAC	0.25 uM		
C. perfringens epsilon toxin (*etx*)	ETX-PF	ACT GCA ACT ACT ACT CAT ACT GTG	0.25 uM	541 bp	
	ETX-PR	CTG GTG CCT TAA TAG AAA GAC TCC	0.25 uM		
C. perfringens perfringlolysin O (*pfo*)	SFO-PF	GCA GTA GCA AAC AAT GAG AAA AAA G	0.25 uM	356 bp	
	SFO-PR	TTA TGT TCT TGT GCA TCT CCT CC	0.25 uM		
16s amplification control (conventional PCR)	16S-PS13	GGA GGC AGC AGT GGG GAA TA	0.06 uM	1062 bp	
	16S-PS14	TGA CGG GCG GTG TGT ACA AG	0.06 uM		

*FL: fluorescein; LC 640: Light Cycler Red 640; PH: phosphate