Research Article

Rapid, Simultaneous Detection of Clostridium sordellii and Clostridium perfringens in Archived Tissues by a Novel PCR-Based Microsphere Assay: Diagnostic Implications for Pregnancy-Associated Toxic Shock Syndrome Cases

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Clostridium sordellii and Clostridium perfringens are infrequent human pathogens; however, the case-fatality rates for the infections are very high, particularly in obstetric C. sordellii infections (>90%). Deaths from Clostridium sordellii and Clostridium perfringens toxic shock (CTS) are sudden, and diagnosis is often challenging. Formalin-fixed, paraffin-embedded (FFPE) tissues usually are the only specimens available for sudden fatal cases, and immunohistochemistry (IHC) for Clostridia is generally performed but it cannot identify species. A clear need exists for a rapid, species-specific diagnostic assay for FFPE tissues. We developed a duplex PCR-based microsphere assay for simultaneous detection of C. sordellii and C. perfringens and evaluated DNA extracted from 42 Clostridium isolates and FFPE tissues of 28 patients with toxic shock/endometritis (20 CTS, 8 non-CTS, as confirmed by PCR and sequencing). The microsphere assay correctly identified C. sordellii and C. perfringens in all known isolates and in all CTS patients (10 C. sordellii, 8 C. perfringens, 2 both) and showed 100% concordance with PCR and sequencing results. The microsphere assay is a rapid, specific, and cost-effective method for the diagnosis of CTS and offers the advantage of simultaneous testing for C. sordellii and C. perfringens in FFPE tissues using a limited amount of DNA.

1. Introduction

Clostridium species are ubiquitous Gram-positive, anaerobic, spore-forming bacteria that are generally found in soil and in the intestinal tract of humans and other animals. Clostridium sordellii has been reported to cause a variety of diseases including peritonitis, endocarditis, pneumonia, arthritis, cellulitis, and myonecrosis [1–4]. Fulminant toxic shock syndrome and sepsis among previously healthy persons have been described most often in cases associated with gynecologic infections and neonatal omphalitis [3, 5]. Clostridium perfringens is also responsible for a number of clinical conditions in humans ranging from acute food poisoning and enteritis to gas gangrene, enterotoxemia, and endometritis [6–8]. In the last couple of years, several studies reported pregnancy-associated fatal toxic shock syndrome cases due to C. sordellii and C. perfringens infections [8–10]. Among all C. sordellii infections reported in the literature, the overall case fatality ratio is 70%, while, for obstetric infections, it is more than 90% [8]. Pregnancy-associated C. perfringens fulminant septicemia also carries a very high mortality rate [8]. Deaths from C. sordellii and C. perfringens toxic shock (CTS) are sudden, and most patients die from hypotension and multiorgan failure within hours to a few days after the initial presentation.

A presumptive clinical diagnosis of CTS can be challenging and often confounded by nonspecific symptoms, an absence of fever, and the low prevalence of these infections
2. Materials and Methods

2.1. Samples. DNA was extracted from various Clostridium isolates \( n = 42 \) and FFPE tissues of patients with toxic shock/necrotizing endometritis \( n = 28 \) and evaluated by the duplex PCR-based microsphere assay for C. sordellii and C. perfringens. DNA samples of 42 known Clostridium isolates were obtained from the Laboratory Branch, Division of Healthcare Quality Promotion, Centers for Disease Control and Prevention (CDC). All 28 cases were submitted to the Infectious Diseases Pathology Branch (IDPB), CDC, from 2004 to 2009 for diagnostic consultation. Clinical and demographic information on the cases were collected when available from medical records. For the confirmation, identification of all the isolates and diagnosis of all the cases were also performed by conventional diagnostic methods. Clinical information as well as conventional diagnostic assay (PCR, sequencing and IHC) results of all C. sordellii and all except 2 C. perfringens cases were previously published as case reports by our group, and, in this study, these cases were used for validation of the microsphere assay [8–10, 16].

2.2. Conventional Diagnostic Methods. DNA was extracted from FFPE tissues of all the cases using the QIAamp DNA mini kit (QIAGEN) following the tissue extraction protocol as previously described [18]. The DNA samples were evaluated by C. sordellii and C. perfringens specific conventional PCR assays targeting the lethal toxin and alpha toxin genes and sequencing, as we described before [8]. To monitor the quality of extraction and to verify the presence of amplifiable DNA, samples were also tested for the amplification of the house-keeping gene human beta-globin [19]. FFPE tissues of all the cases were analyzed by routine hematoxylin-eosin (H&E) and special stains to evaluate histopathological changes and IHC for Clostridia species was performed to localize Clostridia antigens in deparaffinized, rehydrated 3 \( \mu \)m sections of tissue samples, as we described previously [20]. For the diagnosis of C. sordellii and C. perfringens PCR and IHC negative cases, PCR and/or IHC assays for other suspect bacterial agents were also performed [20–25].

2.3. PCR-Based Microsphere Assay

2.3.1. PCR Primers, Oligonucleotide Capture Probes, and Probe Coupling to Microspheres. The primers, probes sequences, gene targets, and amplification product sizes are summarized in Table 1. The primers and probes were synthesized in the Biotechnology Core Facility, CDC, Atlanta, GA. The reverse primers were biotinylated. The probe sequences were labeled at the 5’ end with an amino-modified 6-carbon linker and covalently conjugated to individual carboxylated microspheres (Luminex Corp., Austin, TX) using a carbodiimide coupling procedure [26]. A Beckman Coulter Z2 (Hialeah, FL) was used to count conjugated microsphere stocks to determine concentration. Conjugated microspheres were stored in the dark at 4°C in TE buffer. For each probe, a corresponding 5’ biotinylated, complementary oligonucleotide was designed for testing the specificity and effectiveness of microsphere-probe conjugation.

2.3.2. PCR Amplification. DNA samples of all the cases and isolates were evaluated by the microsphere assay. Initially, phospholipase C genes of C. sordellii and C. perfringens were amplified using a High Fidelity PCR Kit (Roche), 0.3 \( \mu \)M of DNA samples of 42 known Clostridium species make them difficult to isolate [11, 12]. In addition, for the fatal cases, involving sudden death, unavailability of appropriate specimens in adequate amount poses further challenge. Formalin-fixed, paraffin-embedded (FFPE), archival autopsy or biopsy tissues are often the only specimens available for the fatal cases, and they are unsuitable for microbial methods. For FFPE tissues, generally tissue-based diagnostic methods such as histopathology, special stains, and immunohistochemistry (IHC) are used; however, these methods cannot identify specific Clostridium species.

In the last couple of years, we reported detection of C. sordellii and C. perfringens in FFPE tissues of several fatal obstetrical cases using the conventional, agent-specific toxin genes PCR assays and sequencing [8–10]. These assays can identify species; however, the conventional PCRs and sequencing can be time-consuming, laborious, and expensive. In addition, to perform multiple conventional PCR assays, an adequate amount of patient specimen is required, which is often limiting in cases of sudden death. All the above-mentioned factors highlighted the need to develop a rapid, sensitive, and specific multiplex diagnostic assay such as multiplex PCR. However, traditional multiplex PCR/real time PCR can be challenging because amplification conditions for multiple targets are often incompatible and the combination of several primers, probes, and reagents in a single reaction generally results in loss of sensitivity and specificity for each of the individual target species. In addition, primer dimer formation and spectral overlap of fluorescent labels when using TaqMan or Molecular Beacons may compromise the ability to interpret quantitative data [13]. Luminex xMAP (multianalyte profiling) technology (Luminex Corp., Austin, TX) addresses these issues via application of microsphere-based suspension arrays and allows for multiplexing. Multiplexed microsphere-based detection of bacteria using the Luminex platform has been described previously [14, 15].

The objective of this study was to develop a rapid duplex PCR-based microsphere assay that can simultaneously detect C. sordellii and C. perfringens in different clinical specimens, including FFPE tissues, and to evaluate its application as a potential diagnostic tool for pregnancy-associated toxic shock syndrome cases in comparison with other tissue-based diagnostic methods, including IHC and conventional PCR.
each primer (final concentration), and 5 μL of template DNA sample in a 50 μL final reaction volume, following the manufacturer’s instructions. Amplification was carried out on a GeneAmp 9700 thermocycler (Applied Biosystems), using the following cycle conditions: 94°C/2 minutes; 35 cycles of 94°C/20 seconds, 60°C/30 seconds, and 72°C/30 seconds, followed by a final extension of 72°C/5 minutes.

2.3.3. Hybridization of PCR Amplicons to Microsphere Conjugated Probes and Detection. For hybridization, nonpurified biotinylated PCR products were hybridized to the probe-microsphere complexes under optimized reaction conditions in a 96 conical well PCR plate (Costar no. 6509). Each sample was run in triplicate with six reaction blanks per plate. The probe-conjugated microspheres were pulse-vortexed and sonicated for at least 40 seconds, and a working microsphere mixture was prepared by adding calculated volumes of both sets of conjugated microspheres (C. sordellii and C. perfringens) to 1.5X TMAC buffer (4.5 M tetramethyl ammonium chloride, 75 mM Tris-HCl, pH 8.0, 6 mM EDTA, and 0.15% sarkosyl) to achieve a concentration of 1500 microsphere per set per well. The mean age for the C. sordellii patients was 24 years and 33 years for the C. perfringens patients. All cases, except one C. sordellii case, were fatal. Of 10 C. sordellii cases, 8 were medical abortion cases, 1 was a spontaneous abortion case, and 1 had no pregnancy association but was associated with a gynecological procedure. Of 8 C. perfringens cases, 3 were medical abortion, 4 were postpartum cases, and 1 was a spontaneous abortion case. Of 42 isolates included in the study, 9 each were isolates of C. sordellii and C. perfringens, sequenced, and IHC assay, and the duplex microsphere assay for all the CTS cases are listed in Table 2. The mean age for the C. sordellii patients was 24 years and 33 years for the C. perfringens patients. All cases, except one C. sordellii case, were fatal. Of 10 C. sordellii cases, 8 were medical abortion cases, 1 was a spontaneous abortion case, and 1 had no pregnancy association but was associated with a gynecological procedure. Of 8 C. perfringens cases, 3 were medical abortion, 4 were postpartum cases, and 1 was a spontaneous abortion case. Of 42 isolates included in the study, 9 each were isolates of C. sordellii and C. perfringens, sequenced, and IHC assay, and the duplex microsphere assay for all the CTS cases are listed in Table 2.

3. Results
Organism specific PCR and sequencing identified C. sordellii in 10 cases, C. perfringens in 8 cases, and mixed infection of C. sordellii and C. perfringens in 2 cases of CTS. In the non-CTS cases, PCR and/or IHC assays detected C. difficile in 2 cases and Streptococcus pyogenes, Streptococcus agalactiae, Staphylococcus aureus, Neisseria meningitides, Neisseria gonorrhoeae, or Bacillus cereus were identified in one case each. Demographic and clinical information, pathologic findings, results of conventional diagnostic assays, including organism specific PCR, sequencing, and IHC assay, and the duplex microsphere assay for all the CTS cases are listed in Table 2.

The duplex microsphere assay correctly identified C. sordellii and C. perfringens in all 18 known isolates of C. sordellii and C. perfringens (9 each). All 24 other Clostridia isolates were negative by the assay (Table 3). Figure 1 shows the MFI readings for the C. sordellii and C. perfringens isolates. The MFI readings for non-clostridia isolates (negative controls) ranged from 0 to 58. Thus, the microsphere assay showed 100% analytical specificity for C. sordellii and C. perfringens. The detection limits of the microsphere and conventional PCR assays were determined by preparing serial

Table 1: Oligonucleotide primers and probes used in the duplex microsphere assay.

| Primer                  | Sequence (5’-3’)        | Gene target                  | Product size (bp) |
|------------------------|-------------------------|------------------------------|------------------|
| **Clostridium sordellii** primers and probes |                          |                              |                  |
| CSP09-F primer         | TGG GAT GAT TGG GAT TAT TCA G | Phospholipase C of C. sordellii (Csp) |                  |
| CSP09-BR primer        | TCA GTT CCT GCA TAT TCA TTG T  | Phospholipase C of C. sordellii (Csp) | 176 bp          |
| CSP09 probe            | AGA AGC GAT AAA AAA TTC TCA A  | Phospholipase C of C. sordellii (Csp) |                  |
| **Clostridium perfringens** primers and probes |                          |                              |                  |
| PL3-F primer           | AAG TTA CCT TTG CTG CAT AAT CCC | Phospholipase C of C. perfringens (plc) | 283 bp          |
| PL7-BR primer          | ATA GAT ACT CCA TAT CAT CCT GCT  | Phospholipase C of C. perfringens (plc) |                  |
| CP1 probe              | TTT AGC AAA ACC TCT TG      | Phospholipase C of C. perfringens (plc) |                  |

Table 2: Oligonucleotide primers and probes used in the duplex microsphere assay.
dilutions of *C. sordellii* and *C. perfringens* isolate DNA for testing by the assays. The detection limit for the conventional PCR assays was 2 ng/µL for the starting DNA sample, while the detection limit of microsphere assay was 1.4 ng/µL. Thus, clinical sensitivity of the duplex microsphere assay was slightly higher than the single-plex conventional PCR assays.

The microsphere assay also accurately detected *C. sordellii* and *C. perfringens* in FFPE tissues of all confirmed CTS cases (10 *C. sordellii*, 8 *C. perfringens*) as summarized in Tables 2 and 4. Figure 2 shows the MFI readings of these CTS cases. The assay was also able to identify both *C. sordellii* and *C. perfringens* in 2 mixed infection cases. FFPE tissues from 8 patients with non-CTS bacterial infections were negative with MFI readings ranging from 0 to 23. These results demonstrate that the microsphere assay was 100% concordant with the conventional PCR and sequencing results.

Table 2: Demographic, clinicopathological findings, and case-by-case comparison of IHC, PCR, and microsphere assay results.

| Case no. | Age | Association with pregnancy | Outcome | Tissue tested | Pathological findings                                      | Clostridia IHC | *C. sordellii* PCR | *C. perfringens* PCR | Microsphere assay |
|----------|-----|----------------------------|---------|---------------|-----------------------------------------------------------|----------------|-------------------|-------------------|-------------------|
| 1        | 18  | Medical abortion           | Fatal   | Uterus        | Necrotizing endomyometritis                                | Positive       | Positive          | Negative          | Positive for *C. sordellii* |
| 2        | 22  | Medical abortion           | Fatal   | Uterus        | Necrotizing endomyometritis                                | Positive       | Positive          | Negative          | Positive for *C. sordellii* |
| 3        | 34  | Medical abortion           | Fatal   | Uterus        | Necrotizing endomyometritis                                | Positive       | Positive          | Negative          | Positive for *C. sordellii* |
| 4        | 21  | Medical abortion           | Fatal   | Uterus        | Necrotizing endomyometritis                                | Positive       | Positive          | Negative          | Positive for *C. sordellii* |
| 5        | 26  | Medical abortion           | Fatal   | Endometrium   | Necrotizing endomyometritis                                | Positive       | Positive          | Negative          | Positive for *C. sordellii* |
| 6        | 25  | Spontaneous abortion      | Non-fatal | Placenta     | Severe acute chorioamnionitis                              | Positive       | Positive          | Negative          | Positive for *C. sordellii* |
| 7        | 32  | No association             | Fatal   | Uterus        | Necrotizing endomyometritis                                | Positive       | Positive          | Negative          | Positive for *C. sordellii* |
| 8        | 18  | Medical abortion           | Fatal   | Unknown       | Necrotizing endomyometritis                                | Positive       | Positive          | Negative          | Positive for *C. sordellii* |
| 9        | 29  | Medical abortion           | Fatal   | Uterus        | Necrotizing endomyometritis                                | Positive       | Positive          | Negative          | Positive for *C. sordellii* |
| 10       | 21  | Medical abortion           | Fatal   | Decidual and chorionic tissue | Acute inflammation and necrosis | Positive       | Positive          | Negative          | Positive for *C. sordellii* |
| 11       | 24  | Medical abortion           | Fatal   | Uterus        | Necrotizing endomyometritis                                | Positive       | Negative          | Positive          | Positive for *C. perfringens* |
| 12       | 28  | Medical abortion           | Fatal   | Uterus        | Necrotizing endomyometritis                                | Positive       | Negative          | Positive          | Positive for *C. perfringens* |
| 13       | 40  | Postpartum                 | Fatal   | Uterus        | Acute endomyometritis                                      | Positive       | Negative          | Positive          | Positive for *C. perfringens* |
| 14       | 41  | Postpartum                 | Fatal   | Uterus        | Necrotizing endomyometritis                                | Positive       | Negative          | Positive          | Positive for *C. perfringens* |
| 15       | 37  | Medical abortion           | Fatal   | Uterus        | Necrotizing endomyometritis                                | Negative       | Negative          | Positive          | Positive for *C. perfringens* |
| 16       | 26  | Postpartum                 | Fatal   | Uterus        | Necrotic and hemorrhagic uterus                             | Negative       | Negative          | Positive          | Positive for *C. perfringens* |
| 17       | 41  | Spontaneous abortion      | Fatal   | Uterus        | Endomyometritis and sepsis                                 | Positive       | Negative          | Positive          | Positive for *C. perfringens* |
| 18       | 32  | Postpartum                 | Fatal   | GI            | Necrotizing enteritis                                      | Positive       | Negative          | Positive          | Positive for *C. perfringens* |
| 19       | 16  | Medical abortion           | Fatal   | Appendix      | Acute appendicitis and peritonitis                         | Positive       | Positive          | Positive          | Positive for both |
| 20       | 40  | No association             | Fatal   | Cervix        | Acute cervicitis and endometritis                          | Positive       | Positive          | Positive          | Positive for both |
Table 3: Microsphere assay results of *Clostridium* species isolates.

| *Clostridium* species | Number of isolates tested | Number of positive isolates |
|-----------------------|---------------------------|----------------------------|
|                       |                           | For *C. sordellii* | For *C. perfringens* |
| *C. sordellii*        | 9                         | 9/9 (100%)         | 0/9 (0%)           |
| *C. perfringens*      | 9                         | 0/9 (0%)           | 9/9 (100%)         |
| *C. difficile*        | 4                         | 0/4 (0%)           | 0/4 (0%)           |
| *C. butyricum*        | 5                         | 0/5 (0%)           | 0/5 (0%)           |
| *C. septicum*         | 5                         | 0/5 (0%)           | 0/5 (0%)           |
| *C. sporogenes*       | 5                         | 0/5 (0%)           | 0/5 (0%)           |
| *C. tetani*           | 5                         | 0/5 (0%)           | 0/5 (0%)           |

Table 4: Microsphere, PCR, and IHC assay results of CTS cases.

| *Clostridium* cases (no. of cases) | Positive for *C. sordellii* | Microsphere assay | Positive for *C. perfringens* | PCR** | Microsphere assay |
|-----------------------------------|-----------------------------|-------------------|-------------------------------|-------|-------------------|
| *C. sordellii* (n = 10)           | 10                          | 10                | 10                            | 10    | 0                 |
| *C. perfringens* (n = 8)          | 6                           | 0                 | 0                             | 0     | 8                 |
| *C. sordellii* and *C. perfringens* coinfection (n = 2) | 2 | 2 | 2 | 2 | 2 |

*IHC assay is specific for Clostridia but cannot speciate further.
**Conventional PCR assay targeting the phospholipase C gene of *C. sordellii* and *C. perfringens*.

The *Clostridium* IHC was positive in all except for 2 *C. perfringens* cases. Figure 3(a) illustrates the hemorrhage, inflammation, and necrosis of the endometrium and abundant Gram-positive bacilli in a positive *C. sordellii* case. IHC also detected Clostridial antigens inside the inflammatory cells present in the necrotic endometrial tissues (Figure 3(b)) and in myometrial blood vessels of the positive CTS cases.

4. Discussion

This study describes the development of a duplex PCR-based microsphere assay using a suspension array technology, the Luminex xMAP, for rapid, simultaneous detection of *C. sordellii* and *C. perfringens*. Although DNA assays developed on the Luminex platform have been used for identification and genotyping of infectious agents such as *Mycobacterium*, *Escherichia coli*, *Salmonella*, *Cryptosporidium*, *Listeria*, *Candida*, and various respiratory bacteria and viruses [14, 15, 26–29] in serum and other clinical specimens, to our knowledge, this represents the first report of the application of this technology for the diagnosis of *Clostridium* infections, particularly in the FFPE archival tissues. This duplex microsphere assay can have important implications for the premortem and postmortem diagnosis of pregnancy-associated CTS cases.

In recent years, *C. sordellii* and *C. perfringens* have been found to be associated with severe or fatal toxic shock syndrome in both postpartum and postabortive women [8–10]. Although pregnancy and gynecologic infections due to *C. sordellii* and *C. perfringens* are rare, they progress so rapidly that death often precedes diagnosis. Early confirmatory diagnosis before the development of irreversible toxic shock is critical for implementation of treatment measures and support of epidemiologic investigations. However, presumptive diagnosis of CTS often relies on vague clinical manifestations and is quite challenging. Also, the isolation of *Clostridium* species is very difficult. Only molecular approaches guarantee identification to the species level, which is important for the selection of treatment as well as for the identification of the source of infection [9]. Generally, several diagnostic assays for different organisms have been performed on the premortem biopsy samples for differential diagnosis, but selecting individual assays in sequential order can delay diagnosis and treatment as well as prolong hospitalization. On the other hand, simultaneous testing by individual assays for each organism can be expensive and requires large volumes of patients’ samples, which is usually difficult to obtain, particularly for premortem diagnosis. Even for the fatal cases, autopsies are often not performed and the FFPE archival biopsy tissues are the only specimens available. The microsphere assay addresses these issues and is particularly useful for the rapid, accurate, and simultaneous identification of *C. sordellii* and *C. perfringens* in FFPE tissues using only a small volume of starting material. Another added advantage of this assay is the low cost. The total cost of one test using this approach is less than 20 cents excluding costs for lab personnel and equipment. On the other hand, the traditional PCR and automated DNA sequencing methods cost at least $4 per test.

In this study, we compared conventional PCR assays and microsphere assay results for various Clostridia stock
strains (isolates) and FFPE tissue specimens from confirmed pregnancy or gynecologic procedure-associated CTS cases to establish analytic sensitivity and specificity of the assay. For the microsphere assays, direct DNA hybridization was used in conjunction with short probes and TMAC buffer to produce a stringent assay. Tetramethyl ammonium chloride (TMAC) binds to the A-T rich regions of the genome and significantly reduces the difference in the melting temperature between the A-T and G-C pairs. The phospholipase C gene was targeted for the primers and probes because of its presence in all types and strains of C. sordellii and C. perfringens. Our results clearly demonstrate the high level of clinical sensitivity and specificity of the microsphere assay for the pathogens examined with 100% concordance with the conventional PCR assay results for all clinical cases and isolates. In addition, the microsphere assay was able to detect mixed infections of C. sordellii and C. perfringens and showed no cross-reactivity with any other organisms tested. The average MFI value for the C. sordellii cases was 1645 and for C. perfringens cases was 1567. No correlation was seen between the MFI values of C. sordellii and C. perfringens cases and the duration of illness, type of infection (medical abortion versus postpartum), IHC results, or any other clinical feature.

We also performed histopathological evaluation and Clostridia IHC on the tissues to define the pathology and to detect Clostridial antigens in the areas of inflammation and pathology. This information is particularly important because Clostridium species are present in the vaginal tract of 4–18% of healthy women [9, 30, 31]. We detected Clostridial antigens by IHC in the areas of pathology, particularly in the necrotic endometrial tissue in all confirmed CTS cases, except for 2 C. perfringens cases. In these IHC negative cases, MFI values of the microsphere assay were quite good (above 1200) and the conventional PCR assays were also positive. The reason for this discrepancy may be the lower sensitivity of the IHC assay in comparison to the PCR and microsphere assay or clearance of antigens by the host immune system.

5. Conclusions

The duplex microsphere assay is a rapid, sensitive, specific, and cost-effective method for the diagnosis of pregnancy-associated CTS cases and offers the advantage of simultaneously testing for C. sordellii and C. perfringens in FFPE tissues using a limited amount of DNA. The assay can be an excellent diagnostic tool for the identification of mixed infections. This technology also allows for further multiplexing of additional pathogens associated with toxic shock syndrome that could be of great value for epidemiologic investigations. In addition, the combination of the microsphere assay and IHC for the analysis of Clostridial DNA and antigens together in the tissues of fatal cases can provide an insight into the disease pathogenesis, improve detection, and have important implications for the diagnosis of CTS. Early recognition and confirmatory diagnosis of CTS, along with an aggressive surgical approach and appropriate antimicrobial therapy, can
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Figure 3: (a) Abundant-gram-positive bacilli in the necrotic endometrial tissues (Gram’s stain). (b) Clostridial antigens (red staining) inside the inflammatory cells present in the necrotic endometrial tissues (IHC using polyclonal anti-clostridium species antibody).

decrease the mortality associated with this syndrome that occurs primarily among young, otherwise healthy women.

Disclaimer

The findings and conclusions in this paper are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

Conflict of Interests

The authors cite no relationships or support, which might be perceived as constituting a conflict of interests.

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