Clostridium sordellii lethal toxin gene is not detectable by PCR in the intestinal flora of infants who died from sudden infant death syndrome or other causes

Infection caused by Clostridium sordellii translocating from the gastrointestinal tract has been reported to cause septic shock, often resulting in fatality (Abdulla & Yee, 2000). Cases summarized by Abdulla & Yee (2000) involved patients who had underlying medical conditions; however, more recently several cases have been described that occurred in previously healthy women who underwent medically induced abortion (Fischer et al., 2005) or shortly after delivering a baby (vaginal or Caesarean section) (Golde & Ledger, 1977; Soper, 1986; Hogan & Ireland, 1989; McGregor et al., 1989; Sosolik et al., 1996; Bitti et al., 1997; Rorbye et al., 2000; Sinave et al., 2002). Pregnancy and childbirth have been suggested to predispose a small number of women to acquire C. sordellii in the vaginal tract, where the acidic pH of the vagina enhances the cytopathic effects of its toxins (Fischer et al., 2005). The fastidious anaerobic growth, variable staining characteristics and complex biochemical profiles of clostridia probably contribute to low numbers of reports of non-lethal C. sordellii infection (Fischer et al., 2005). It is not known whether an infant born to a mother asymptatically colonized by C. sordellii acquires the organism at birth through the ingestion of vaginal flora, as has been shown for other organisms such as Escherichia coli (Bettelheim & Lennox-King, 1976).

C. sordellii produces lethal toxin (LT) a highly potent single-chain protein toxin of 250 kDa molecular mass that acts intracellularly by catalysing the glycosylation of small GTPases. When LT is injected intraperitoneally into mice, the adherens junctions of lung vascular tissue break down, resulting in the extravasation of blood into the thoracic cavity (Geny et al., 2007). Notable clinical features of C. sordellii toxic shock include an absence of fever and rash, refractory tachycardia, hypotension, diarrhoea and a relatively short time course (<1 day from hospitalization to death) (Fischer et al., 2005). These are not unlike the observations made prior to sudden infant death syndrome (SIDS) death, in particular where the event was recorded on memory monitors to include a cardiogenic event preceded by bradycardia (Poets et al., 1999). The organism has only been isolated from the blood in 1 of 14 cases of fatal toxic shock syndrome reported in the literature (Fischer et al., 2005), and it is recognized that toxin produced from a localized site can cause a shock-like death without bacteremia (Rorbye et al., 2000; Sinave et al., 2002).

We designed an experiment to explore the possibility of colonizing intestinal C. sordellii contributing to SIDS, possibly via toxin absorption into the bloodstream by a similar mechanism to infant botulism, which has previously been shown to account for a percentage of SIDS deaths (Arnon et al., 1978; Bohnel et al., 2001).

This study was approved by the Research Ethics Committee of the Children, Youth & Women’s Health Service, Australia. Testing was done retrospectively on stored material from previous investigations conducted in South Australia, where such material was available. Small portions of intestinal contents (both small and large intestine for each infant) from 50 SIDS and 13 non-SIDS comparison infants who died between 1989 and 1994 in South Australia were collected at autopsy into sterile containers and immediately stored at −80 °C until required. SIDS diagnoses retrospectively matched the criteria specified by the 1991 definition for SIDS (Willinger et al., 1991). The median age of SIDS infants was 4 months, the interquartile range was 3.65 months and the proportion of male infants was 46%. All post-mortem investigations were conducted within 48 h of death. Samples were frozen only once and were not thawed prior to this investigation. Samples had not been stored anaerobically, so a small portion (<1 g) of intestinal contents or faeces was collected from the middle of the sample, i.e. from a part of the sample that had not been in contact with air during storage, and cultured in cooked meat broth medium [12.5 % cooked meat medium, 2.7 % (w/v) Schaedler’s broth made up to the desired volume in deionized water and autoclaved] containing 0.07 μg neomycin ml⁻¹ at 30 °C for 72 h. We incubated the cultures at 30 °C to optimize toxin production by clostridia (Hatheway, 1979). The culture supernatant was stored at −80 °C for future toxin assays on any samples that tested positive by PCR. Cells from 500 μl culture were pelleted and resuspended in sterile saline to χ₀₉₀₀ 0.1, then heated at 100 °C for 10 min to lyse the cells. Cell debris was pelleted and the lysate stored at −20 °C until required. American Type Culture Collection C. sordellii strain ATCC 9714 was used as a control strain as it was shown by Voth et al. (2006) to possess the LT gene and express the toxin under appropriate conditions. The suitability of the storage and culture conditions for clostridia has been confirmed previously by testing the crude lysate samples for two common anaerobic flora Clostridium innocuum and Bacteroides thetaiotaomicron. Fifty-four per cent of the samples were positive for either species (A. R. Highet, data not shown). Possession of the LT gene by the control strain was confirmed by sequencing the amplification products (Institute of Medical and Veterinary Science Sequencing Centre, Adelaide, Australia).
It was necessary to determine whether the organism at low frequency in an intestinal sample could be detected by PCR after 72 h culture in cooked meat media broth with neomycin. Broths were inoculated with a single infant’s intestinal contents sample (20 μl) and 100 μl of an aliquot of a serial dilution of C. sordellii ATCC 9714 suspended in sterile saline. Another 100 μl aliquot of the dilution suspension was plated onto brain heart infusion (BHI) agar [4.5 % (w/v) BHI, 1.8 % (w/v) yeast extract, 0.0019 % (w/v) (autoclaved) vitamin K, 0.19 % haemin, 4.76 % (v/v) defibrinated horse blood] and incubated for 72 h at 37 °C in an anaerobic chamber. The results indicated that when two organisms were present in the dilution suspension (i.e. two colonies grew on the BHI plate) LT could be detected in the crude lysate made from a spiked cooked meat broth culture (Fig. 1). A broth that was not inoculated with C. sordellii suspension (i.e. with intestinal contents only) gave a negative PCR result.

Detection of the LT gene in samples used the published primer pair CLS-F1 and CLS-F2, which are based upon the sequence of C. sordellii deposited under GenBank accession number X82638 (Fischer et al., 2005), to screen the crude culture lysate samples. Cycling conditions with a Hybaid OmniGene thermocycler were as follows: initial denaturation at 94 °C for 120 s, 35 cycles of 94 °C for 30 s, 54 °C for 40 s and 72 °C for 70 s, then final extension at 72 °C for 120 s. Each PCR contained: 0.1 μM MgCl₂, 1 × colourless GoTag Flexi buffer, 0.005 μM dNTP mix, 0.1 μM each primer of the pair, 0.5 units GoTag DNA polymerase (Promega), 2 μl crude lysate, and water to a total volume of 25 μl. All amplification products were visualized using 2 % agarose gel stained with ethidium bromide. A positive result yielded a 250 bp product.

None of the SIDS or comparison crude culture lysate samples tested positive for LT by PCR. Anecdotally, a negative result was also obtained from intestinal samples that came from a deceased infant who was excluded from the study on the basis of age (>12 months), but who in the post-mortem investigation had C. sordellii isolated from heart blood. Interestingly, the original cause of death was reported to be SIDS-like with thymic petechial haemorrhages. The results of this investigation suggest that intestinal colonization by LT toxigenic C. sordellii is unlikely to contribute to SIDS. However, based on these results alone we cannot completely exclude the role of C. sordellii bacteraemia or toxemia in SIDS.

Clostridium botulinum neurotoxin has been found to account for a percentage of SIDS deaths (Arnon et al., 1978) but not for SIDS from South Australia (Byard et al., 1992). Accordingly C. sordellii LT may account for a proportion of SIDS deaths in other populations.

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**Amanda R. Hightet,1,2 Catherine S. Gibson3 and Paul N. Goldwater1,2**

1Department of Microbiology and Infectious Diseases, SA Pathology, Women’s & Children’s Hospital, 72 King William Road, North Adelaide, South Australia, Australia

2Discipline of Paediatrics, School of Paediatrics and Reproductive Health, University of Adelaide, North Adelaide, South Australia, Australia

3Discipline of Obstetrics and Gynaecology, School of Paediatrics and Reproductive Health, University of Adelaide, North Adelaide, South Australia, Australia

**Correspondence:** Amanda R. Hightet (amandahightet@adelaide.edu.au)


