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*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 bacteraemia (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. 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) extract, 0.0019 % (w/v) (autoclaved) agar] [plated onto brain heart infusion (BHI) agar].

A serial dilution of the sample (i.e. with intestinal contents also obtained from intestinal samples that could be detected in the same culture lysate samples tested positive for LT. 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 toxicigenic 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.

Clotidium 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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**(Image 107x644 to 306x715)**

**Fig. 1.** Agarose gel electrophoresis of amplification products. Lanes 1 and 6, pUC19/Hpall DNA molecular mass marker; lane 2, positive control ATCC 9714; lane 3, spiked culture; lane 4, unspiked culture; lane 5, no-template control.


