Germination of spores of *Clostridium difficile* strains, including isolates from a hospital outbreak of *Clostridium difficile*-associated disease (CDAD)

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We were unable to reproduce germination results reported in our previous study (Paredes-Sabja *et al.*, 2008) using *Clostridium difficile* spores prepared as described by Wilson *et al.* (1982) and Wilson (1983). Unfortunately, PCR analyses of spores used in our previous study (Paredes-Sabja *et al.*, 2008) revealed that the spore suspensions of *C. difficile* were cross-contaminated with *Clostridium perfringens*, and thus the phenotype observed might not be related to *C. difficile* spores. Consequently, we revisited some of the germination assays with pure *C. difficile* spores of strains CD630 and 196. Germination was calculated by measuring the percentage of phase dark spores by phase-contrast microscopy. Results showed that fewer than 20% of spores of both strains became phase dark when germinated with water, 100 mM KCl in 25 mM Tris/HCl (pH 7.4) or 100 mM sodium phosphate (pH 6.0) for 60 min at 40 °C (Fig. 1). However, the majority (~75%) of CD630 and 196 spores became phase dark after 60 min of incubation at 40 °C in sodium taurocholate and glycine (Fig. 1), consistent with results reported elsewhere (Sorg & Sonenshein, 2008). Collectively, these results indicate that *C. difficile* spores germinate poorly with KCl or sodium phosphate at pH 6.0, and are able to germinate with sodium taurocholate and glycine.

**References**


**Fig. 1.** Germination of *C. difficile* spores. Heat-activated (60 °C, 20 min) spores of strains CD630 (grey bars) and 196 (black bars) were incubated for 60 min at 40 °C with: H₂O, sterile distilled water; ST+Gly, 1% sodium taurocholate and 1.3 mM glycine in 25 mM Tris/HCl (pH 7.0); KCl, 100 mM KCl in 25 mM Tris/HCl (pH 7.4); Pi, 100 mM sodium phosphate (pH 6.0). The percentage of phase dark spores was determined with phase-contrast microscopy by counting at least 200 spores.