Interaction of Cronobacter seven type species with non-malignant human foetal primary small intestinal cell line (H4) cells

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Background

The genus *Cronobacter* is comprises of 7 species: *C. sakazakii, C. malonaticus, C. turicensis, C. muytjensii, C. dublinensis, C. condimenti and C. universalis* (Iversen *et al*, 2008). *Cronobacter* spp. are involved in neonatal diseases including systemic infection, meningitis, and necrotizing enterocolitis especially in premature infants. Published data indicates that *C. sakazakii and C. malonaticus* are more likely related to clinical sources, while others are most likely non-clinical (Holy and Forsythe, 2014). Although many of human epithelial cells were extensively investigated, the only non-transformed human epithelial cells being used in investigation of host/pathogen interaction is a H4 cells, which derived from the small intestine of foetuses aged from 20 to 22 weeks of gestation.

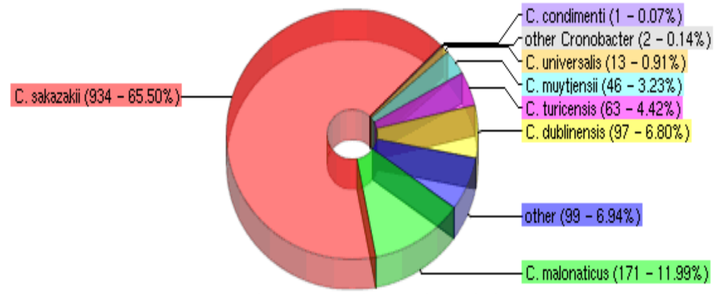

Fig. 3 *Cronobacter* isolates breakdown
http://pubmlst.org/perl/bigsdb/bigsdb.pl?db=pubmlst_cronobacter_isolates

Results

All of the *Cronobacter* type species were able to attach to H4 cells with levels ranged from about 2% to 34.78% showed by *C. turicensis* and *C. malonaticus* respectively (Figure 1). Invasion results was varied from 0.49% to 0.01% presented by strains *C. malonaticus* and *C. condimenti* respectively (Figure 2). However, most of strains were more adhesive to H4 than Caco-2 cells. Although *C. condiment* was the least invasive strain it was the highest cytotoxic with about 6-folds of the blank(Figure 4). Conversely, some of these isolates revealed less virulence when co-cultured with Caco-2 Cells, for example, *C. condiment* showed only cytotoxicity of 1.4-folds of the blank. Furthermore, the inflammatory response of H4 cells to the investigated species was slightly varied and IL-8 produced by of H4 in response to *C. sakazakii* and *C. muytjensii* was extremely higher than that created by caco-2 cells (Figure 5).

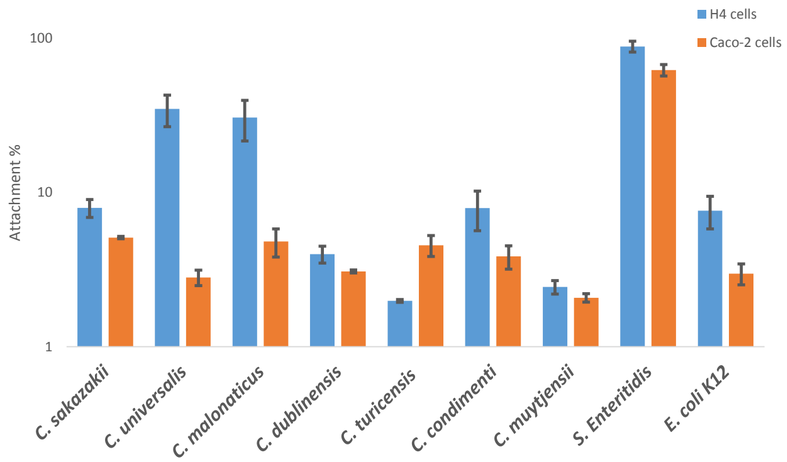

Fig. 1 Attachment of *Cronobacter* type species to the neonatal H4 cell line compared with Caco-2 cells

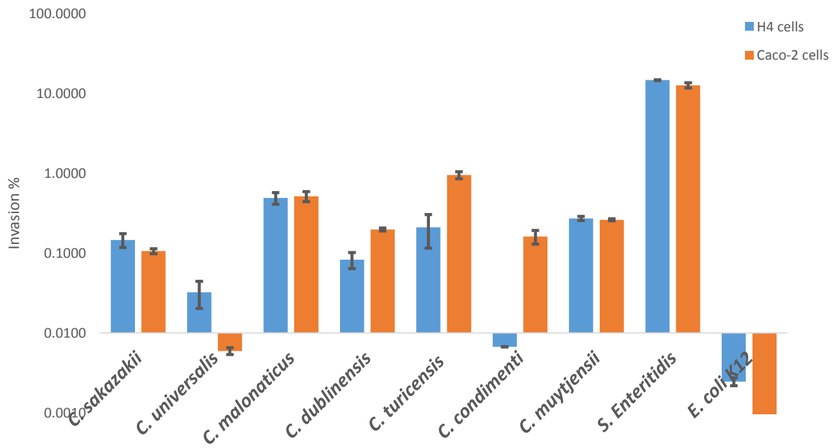

Fig. 2 invasion of the *Cronobacter* seven type species into Neonatal H4 cell line compared with Caco-2 cells

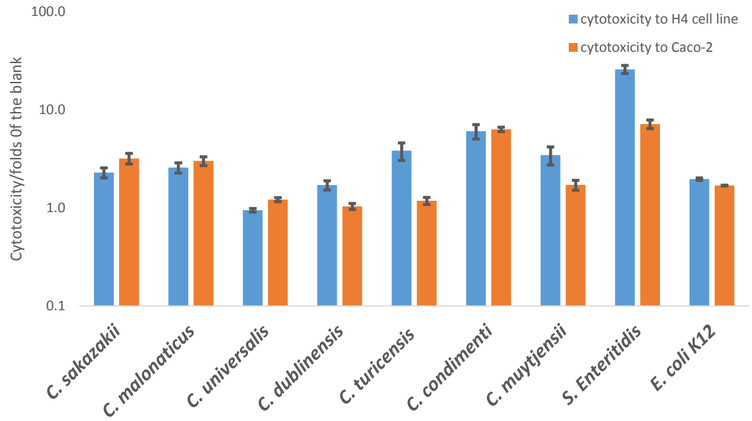

Fig.4 Cytotoxicity of *Cronobacter* seven type species to H4 and Caco-2 cells

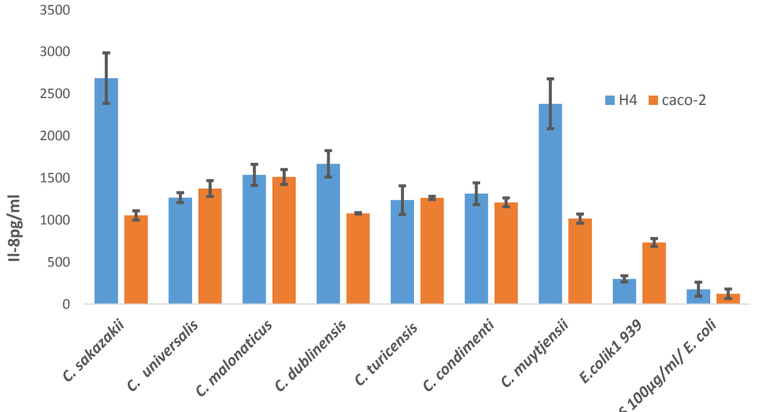

Fig. 5 IL-8 produced by H4 and Caco-2 cell lines in response to *Cronobacter* infection

Discussion

The high levels of, adhesion and inflammatory behaviour of some investigated strains observed in these assays, might reflect the lack of neonatal cells to identify and suppress the pathogens attack, as a result of the immaturity of innate immune system of the newborns. This may explain the increase of the incidence of *Cronobacter* infection in neonates in neonatal intensive care units and mainly NEC, since the inflammatory response of H4 especially to *C. sakazakii* was considerably higher than that displayed by Caco-2. To the best of our knowledge, this is the first work of interaction of *Cronobacter* seven species with H4 cells, which mean that there is no published data to compare with, for this cell line. However, further investigation is needed to better understand the infectious mechanisms of the emergent pathogen *Cronobacter* to help safeguard the precious lives of new-borns.

26th ECCMID Amsterdam, Netherlands 9 – 12 April 2016
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