

P1748

Paper Poster Session

Microbial pathogenesis and virulence

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

Mahmoud Agena*¹, Michael Loughlin¹, Stephen Forsythe²

¹*Nottingham Trent University, School of Science and Technology, Nottingham, United Kingdom*

²*Nottingham Trent University, Pathogen Research Group, School of Science and Technology, Nottingham, United Kingdom*

Background: The neonatal stage is considered to be the most critical period with regards to infection with death about 38% of children aged below five years.

Recently, the genus Cronobacter, a member of family Enterobacteriaceae was classified as an emerging opportunistic pathogen responsible for systemic bacteraemia, meningitis and necrotizing enterocolitis (NEC) in neonates with a high rate of morbidity and mortality. The nonmalignant human intestinal cell line (H4) cells were used in this study to investigate the potential threat of this genus to new-born's life.

Material/methods: Seven isolates presenting the Cronobacter spp. i.e. *C. sakazakii*, *C. malonaticus*, *C. turicensis*, *C. muytjensii*, *C. condimentii*, *C. universalis* and *C. dublinensis* of Nottingham Trent University collection, were chosen to investigate the ability of these organisms to attach and invade the H4 cell line. Bacterial isolates were co-cultured with the cell line for period of 3hrs. The cells were then lysed with 1% triton-X 100 and the number of attached bacteria were determined using Miles and Misra method. For internalized bacteria, H4 cells were incubated for another 60 min with media containing 100mg/ml gentamicin and then plated on Tryptone Soya Agar. Cytotoxicity of the selected species was also examine and Trypan blue method was used

Results: Our findings indicate that all of the selected strains were able to attach to H4 with levels ranged from 5.03% of the inoculum presented by *C. sakazakii* to 34.78% which was showed by a *C. malonaticus*. Invasion results also indicated that most of the type species were able invade H4 cells, and *C. malonaticus* was the highest invasive strain (0.39%) while the least was *C. condimentii* (0.01%). Interestingly, *C. condimentii* which was the least invasive strain show the highest cytotoxicity with about 6-folds of the blank

Conclusions: This is the first report of attachment and invasion of Cronobacter genus to H4 cells, which meant that there is no published data to compare with, for this cell line. However, the high levels of cytotoxicity, adhesion and invasion of some strains observed in this assay, 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 incidence of Cronobacter infection in neonates in neonatal intensive care units and mainly NEC. Further investigation is needed to better

understand the infectious mechanisms of the emergent pathogen Cronobacter to help safeguard the precious lives of new-borns.