

THE IMPACT OF ENTEROAGGREGATIVE *Escherichia coli* AND ITS VIRULENCE GENES IN A CASE-CONTROL STUDY OF MALNOURISHED CHILDREN IN THE NORTHEAST OF BRAZIL

Alexandre Havt¹, Ila Fernanda Nunes Lima¹, Pedro Henrique Quintela Soares de Medeiros¹, Marco Antônio de Freitas Clementino¹, Ana Karolina Silva dos Santos¹, Noélia L. Lima¹, Alessandra Di Moura², Álvaro M. Leite², Richard L. Guerrant^{1,3}, Aldo Ângelo Moreira Lima^{1,3}

¹ Institute of Biomedicine for Brazilian Semi-arid, Federal University of Ceará, ² Institute for the Promotion of Nutrition and Human Development, Fortaleza, Ceará, Brazil; ³ Center for Global Health, University of Virginia, Charlottesville, Virginia, USA

Medium-term impact of enteroaggregative *E. coli* (EAEC) colonization on children development has been described, highlighting the contribution of EAEC to childhood malnutrition, regardless of the presence of diarrhea. Our main goal was to investigate if EAEC could impact children nourishment and to identify any trait of EAEC virulence genes (VRGs) that could be associated with malnutrition. A case-control study was sited in Fortaleza-CE with children aging 6-24 months. Cases were defined by weight for age Z-score (WAZ) ≤ -2 and controls were enrolled following the criteria of WAZ > -1.99 . Stools were collected from 353 enrolled children and they were cultured in MacConkey agar plates, which were examined for flat, lactose fermenting colonies. DNA from five colonies that morphologically resembled *E. coli* was extracted by boiling method, followed by a PCR reaction that used EAEC specific primers for the genes *aaiC* and *aatA*. Samples were considered positive when presented either one or both diagnostic genes. Positive samples were further analysed by five multiplex PCRs to identify 20 EAEC VRGs. We used classification and regression tree (CART) analysis to investigate the correlation of specific combinations of VRGs. From 353 enrolled kids, 152 were cases and 201 were controls. The prevalence of EAEC was 40,79%. We found statistical association with samples that presented both diagnostic genes among cases (P value = 0.0179). Among all 20 VRGs, the presence of *aafC* was found associated with cases (P = 0.0033). CART analysis showed two sets of clusters that were associated with malnourished kids and other two that were associated with controls. Samples presenting *agg4A*, *aggR*, but missing *aafC* and *agg3/4C* (P= 0.0186); and samples showing *sigA* but lacking *aafC*, *agg4A* and *shiA* (P= 0.0239) were associated with malnourished children. However, samples that showed *aap* and *shiA*, but missed *aafC*, *aag4A*, *sigA* and *sepA* (P = 0.0116); and samples lacking *aafC*, *agg4A*, *sigA*, *aap*, and *shiA* (P= 0.0009) were found associated with nourished children. In conclusion, EAEC samples that presented the usher assembly for fimbria subtype II, codified by the gene *aafC*, were most found among malnourished children. However, the presence of the gene *sigA*, that codifies a *Shigella* Ig-A like protease homolog, was also important when *aafC* was absent.

Keywords: malnutrition, enteroaggregative *E. coli*, virulence profile

Financial Support: Bill & Melinda Gates Foundation, FIC/NIH, CNPq