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

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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, agg4A, 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

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