Title: Genotyping and phylogenetic analysis of enteroaggregative *Escherichia coli* (EAEC) strains isolated from extraintestinal infections

Autores: Nery, D. R.¹; Lara, F. B. M¹, Araujo, M. L.¹ Maria, P. O.¹, Faria-Junior C.², Pereira, A. L.¹

Instituição: ¹UnB-FCE - Universidade de Brasília – *Campus* Ceilândia (Centro Metropolitano, conjunto A, lote 01, Brasília – DF); ²Laboratório Central de Saúde Pública do Distrito Federal

Abstract

Escherichia coli strains have evolved acquiring virulence genes that have contributed to the adaptation of pathogenic strains to different infection sites in humans. The diversity of behaviors showed by E. coli strains is supported by a high genetic plasticity that allows the transfer and maintenance of a diversity of virulence genes among E. coli strains. Enteroaggregative E. coli (EAEC) is a heterogeneous category of pathogenic strains classically associated with acute and persistent diarrhea. Here, three EAEC strains (pCVD⁺, *aggR*⁺) isolated from extraintestinal infections (two strain recovered from urinary tract infection and one from bacteremia) were genotyping and submitted to phylogeny analysis along with contemporaneous E. coli strains isolated from UTI in the same hospital. Twenty one E. coli strains, including the EAEC strains, were tested for the presence of 6 virulence markers of UPEC (focA, pap, vat, fyuA, chuA and yfcV), 2 of MNEC (cnf and sfa) besides csgA (curli fimbriae) and ag43. Amplification and sequencing of the genes dinB (DNA polymerase) and icdA (isocitrate dehydrogenase) were employing in the phylogenetic analysis. dinB-icdA concatenated sequences (966 base-long sequences) were used in bootstrap analysis (with 1000 replications) in order to infer phylogenetic relationship among strains that was illustrated as a maximum likelihood tree. The EAEC strains (pCVD⁺ and $aggR^+$) were tested positive for the presence of UPEC virulence markers: fyuA⁺chuA⁺pap⁺csgA⁺ag43⁺pCVD⁺aggR⁺ in EAEC strain 1.1; fyuA⁺vat⁺focA⁺pap⁺csgA⁺ag43⁺pCVD⁺aggR⁺ EAEC 1.2; strain and in *fyuA*⁺*chuA*⁺*pap*⁺*csgA*⁺*p*CVD⁺*aggR*⁺ in EAEC strain 1.3. Among the other 18 strains isolated from urine, all were tested positive for virulence markers of uropathogenic E. coli (UPEC). These strains showed typical genetic array predictive for UPEC strain (6 strains typed as fyuA⁺chuA⁺csgA⁺; 4 typed as $fyuA^{\dagger}chuA^{\dagger}pap^{\dagger}csgA^{\dagger}$; 3 typed as $fyuA^{\dagger}yfcV^{\dagger}chuA^{\dagger}pap^{\dagger}csgA^{\dagger}$; 3 typed as $fyuA^{\dagger}yfcV^{\dagger}chuA^{\dagger}csgA^{\dagger}$; 1 typed as $yfcV^{\dagger}chuA^{\dagger}pap^{\dagger}csgA^{\dagger}$; and 1 typed as $fyuA^{\dagger}yfcV^{\dagger}chuA^{\dagger}vat^{\dagger}focA^{\dagger}pap^{\dagger}sfa^{\dagger}cnf^{\dagger}csgA^{\dagger}$). In phylogenetic tree, heteroathogenic EAEC strains 1.1 and 1.3 compose a large cluster along with 7 UPEC strains. Branching of this cluster was supported by a bootstrap value of 98. In this cluster, 6 of out 7 strains displayed the combination $fyuA^+chuA^+pap^+csgA^+$ as shared gene set. Our data showed that heteropathogenic strains displaying hybrid genotype (EAEC/UPEC) can cause extraintestinal infections.

Palavras-chaves: heteropathogenic strain, genotyping, phylogenetic, UPEC

Agência Fomento: FAP-DF – custeio 193.000.019/2012.