

Title : Phylogenetic Classification and Pathogenicity Study of *Escherichia coli* Producing Colibactin Toxin in Mammalian Cells

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ABSTRACT

Escherichia coli strains efficient in producing the colibactin toxin are predominantly found in phylogenetic group B2. However, recent studies have reported its presence in other groups, such as A and B1, with an increasing trend. This study aims to classify the phylogenetic groups of *E. coli* isolates obtained from food and to screen for *E. coli* encoding the *clbA* gene. Additionally, the pathogenicity of *E. coli* producing colibactin toxin was to evaluate on mammalian cells. In this study, *E. coli* (17 isolates) was isolated from food samples such as pork, chicken, beef tongue, fish, pork intestines, pork liver and crab sticks. Multiplex PCR was used to classify *E. coli* isolates obtained from food samples (n = 18). Among these, phylogenetic group A was the most prevalent (n=9; 53%), followed by B1 (n=4; 23.5%), B2 (n=3; 17.6%), and F (n=1; 5.9%). PCR detection of the *clbA* gene identified five positive isolates (29.4%), four belonging to group A (23.5%) and one to group B1 (5.9%) that revealed *E. coli* encoding colibactin toxin. Moreover, the pathogenicity of *clbA*-positive *E. coli* isolates was studied the Infectivity in Vero cells and determined the cytotoxicity by the MTT colorimetric assay and the methylene blue staining assay. After infection with *E. coli* (MOI=400), *E. coli* BA1 exhibited the highest toxicity in Vero cells, with a cell viability of 5.86% compared to uninfected cells. This was followed by BA2 (6.11%), VA2 (21.20%), HA2 (21.29%), and CA5 (60.84%). Morphological analysis of infected cultured cells under a microscope revealed multinucleation, cellular enlargement, and vacuolation. Additionally, DNA extraction was performed to preliminarily assess DNA alterations in infected cultured cells. It was also conducted to investigate potential mutations in the DNA of these cells. These findings suggest that *E. coli* producing colibactin toxin is not limited to phylogenetic group B2 and may increasingly be detected in other phylogenetic groups in the future.

Keywords: Colibactin, *E. coli*, Infection, Phylogenetic