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. The UV spectrometer method was used, and the results showed that the content of DNA nucleotides (A, T, G and C) of Vero cells after infection with *E. coli clbA+* did not significantly different. These findings suggested that *E. coli* producing colibactin toxin did not limit to phylogenetic group B2 and may increasingly be detected in other phylogenetic groups in the future.

INTRODUCTION

E. coli is a normal flora commonly found in intestines of human and animal, environments, and contaminated food. *E. coli* is classified on phylogenetic groups including A, B1, B2, and D. *E. coli* phylogroup B2 is the most prevalent in carrying the *pks* island gene, which is responsible for producing coibactin toxin. When *E. coli* producing colibactin toxin infect into the human body, they can synthesize and release coibactin through direct membrane diffusion. Coibactin then binds to DNA strands, leading to DNA interstrand cross-linking (ICL) and DNA double-strand breaks (DSB).

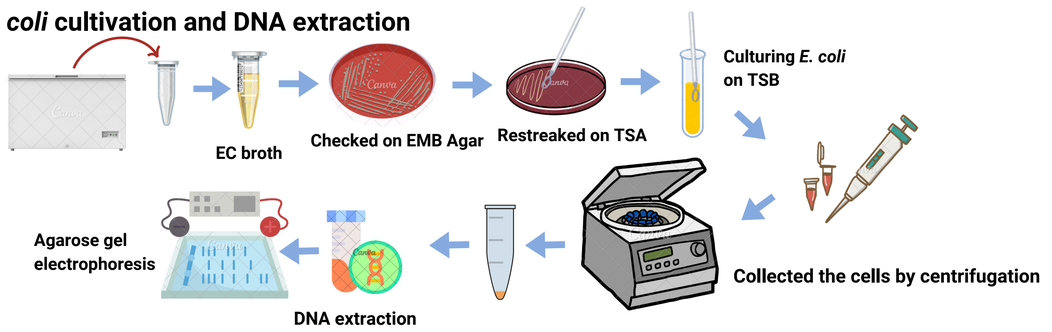
OBJECTIVES

- To identify the phylogenetic groups of *E. coli* isolates from food samples
- To detect *E. coli* encoding colibactin gene
- To study the pathogenicity of *E. coli* producing colibactin toxin on mammalian cells

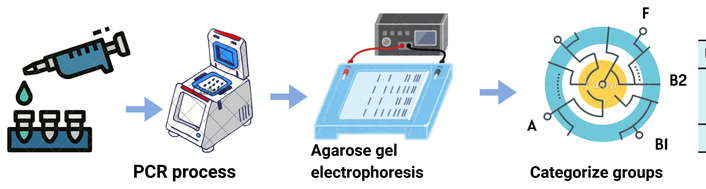
METHODOLOGY

E. coli Classsification

- ***E. coli* cultivation and DNA extraction**



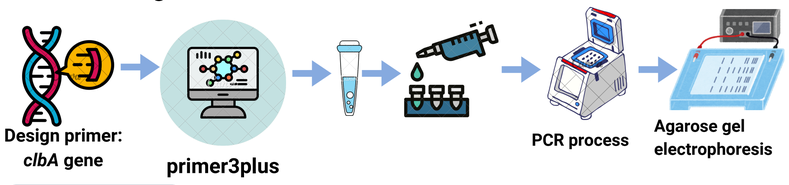
- **Phylogenetic groups of *E. coli* isolates by Multiplex PCR**



Multiplex PCR cycle

	Temperature (°C)	Time (min)	Cycle
Initial denaturation	95	3	1
Denaturation	95	0.45	45
primer extension	72	0.45	
Final extendion	72	5	1

- **Colibactin gene detection in *E. coli* isolates**

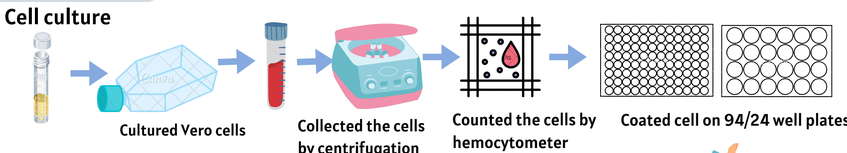


Colibactin gene PCR cycle

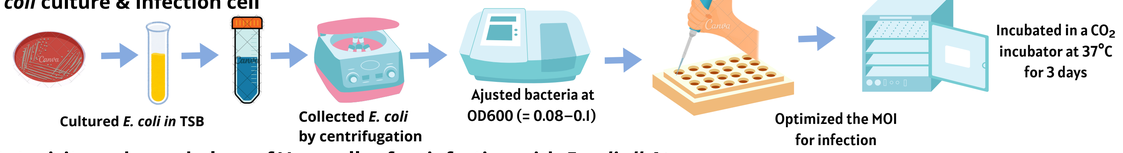
	Temperature (°C)	Time (min)	Cycle
Initial denaturation	94	2	1
Denaturation	94	0.3	30
Annealing	58	0.3	
extension	72	0.3	
Final extendion	72	7	1

Infection Part

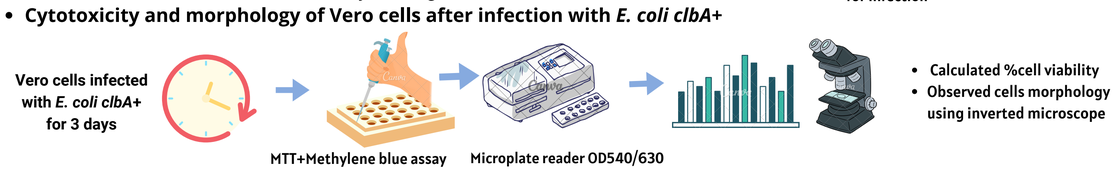
- **Cell culture**



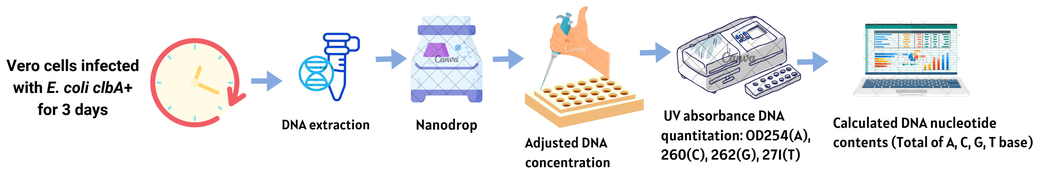
- ***E. coli* culture & Infection cell**



- **Cytotoxicity and morphology of Vero cells after infection with *E. coli clbA+***



- **DNA nucleotide differences of Vero cell after infection with *E. coli clbA+***



RESULTS

- **Phylogenetic groups of *E. coli* isolates from food samples**

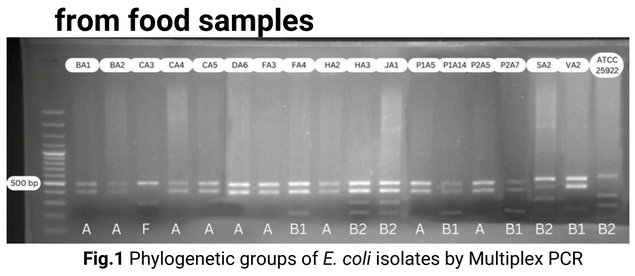


Fig.1 Phylogenetic groups of *E. coli* isolates by Multiplex PCR

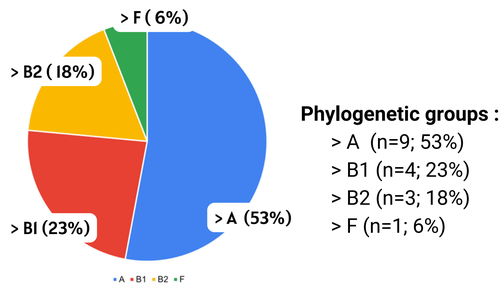


Fig.2 % Phylogenetic groups of *E. coli* isolates (n=18)

- **Colibactin gene detection in *E. coli* isolates**

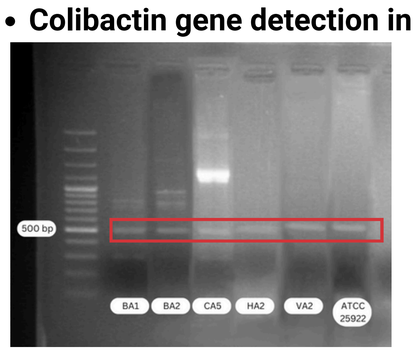


Fig.3 *clbA* gene detected from *E. coli* isolates by PCR

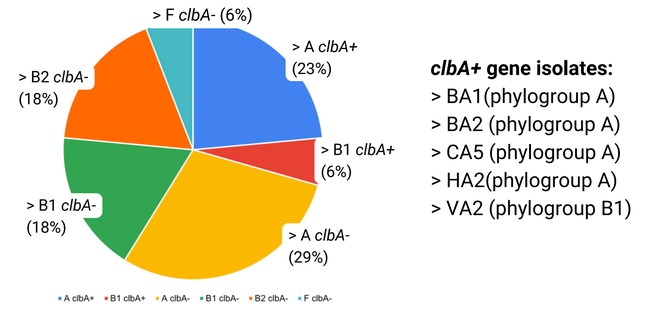


Fig.4 Total isolates of *E. coli clbA+* gene (n=18)

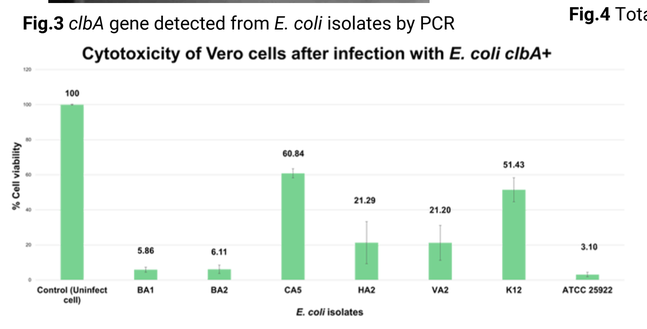


Fig.5 % Cell viability of Vero cell after infection with *E. coli clbA+*

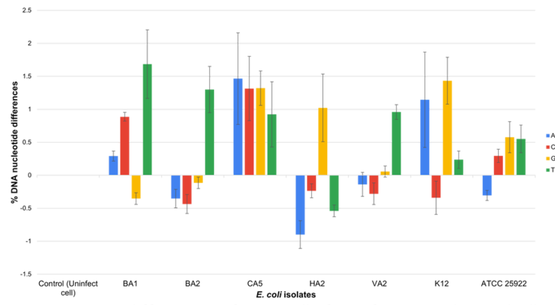


Fig.6 % DNA differences of Vero cell after infection with *E. coli clbA+*

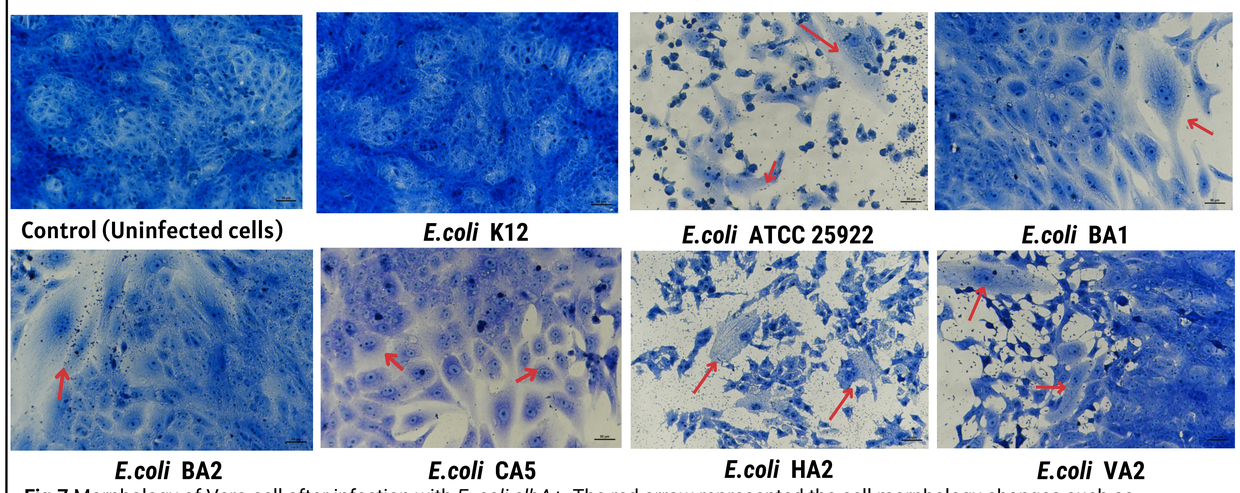


Fig.7 Morphology of Vero cell after infection with *E. coli clbA+*. The red arrow represented the cell morphology changes such as multinucleated cells, cellular enlargement, and vacuolation cells.

CONCLUSION

- The phylogenetic classification of *E. coli* isolates from food samples (n=17) revealed the following prevalence distribution including A, B1, B2, and F groups.
- *E. coli* carrying the *clbA* gene could detect in phylogenetic groups of A and B1.
- Vero cells after infection with *E. coli clbA+* exhibited cytotoxicity and cellular changes including multinucleation, cellular enlargement, and vacuolation.
- Total DNA nucleotides (A, T, G, C) of Vero cells after infection with *E. coli clbA+* did not significantly different that determined by UV absorbance DNA quantitation.

References

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- Du L, Song J. Delivery, structure, and function of bacterial genotoxins. Virulence. 2022 Dec;13(1):1199-1215. doi: 10.1080/21505594.2022.2097417. PMID: 35795909; PMCID: PMC9341358.

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