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Investigating the Prevalence of PK Island in *Enterobacteriacea*

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ABSTRACT

The Enterobacteriaceae family has a pathogenicity island that leads to increase in pathogenicity. Colibactin is a genotoxin that induces DNA double-strand breaks that may lead to carcinogenesis and is produced by Escherichia coli strains harboring the pks island. During study the detection of colibactin occurs by molecular method (polymerase chain reaction) followed by bacterial identification and DNA extraction. The result of study shows the bacterial E.coil is the most bacterial isolate found in enterobacteriacea sample used in study followed by Klebsiella contain the four genes that expressing of colibactin including (ClbA, ClbB, ClbQ and ClbN) representing the PK island.

Keywords: Enterobacteriacea, Colibactin gene (K gene), Pathogenicity island: PK island.

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INTRODUCTION

A genomic island called polyketide synthase or pks, which included colibactin (Clb) genes, was described in B2 Nissle 1917 and B2 *E. coli* strains associated with meningitis, septicemia, and urinary tract infection (ExPEC) as well as in other *Enterobacteriaceae* including *Klebsiella pneumoniae*, *Citrobacter koseri*, and *Enterobacter aerogenes* [1,2]. In vitro characterization of pks-encoding *E. coli* demonstrated megalocytosis (or progressive enlargement of cells and nuclei without mitosis), activation of G2 checkpoint and induction of cell cycle arrest and DNA double-strand breaks [1]. Strains encoding other cyclomodulins (bacterial effectors and toxins that obstruct the cell cycle) including cytotoxic necrotizing factor (CNF), cytolethal distending toxin (CDT), and cycle inhibiting factor (Cif) are also able to induce megalocytosis [3, 4].

The non-ribosomal peptide/polyketide hybrid colibactin is a secondary metabolite found in a variety of bacterial species of the family *Enterobacteriaceae*. The colibactin biosynthetic machinery is encoded by a 54-kb large polyketide synthase (pks) or clb genomic island[5], which includes 19 genes. The largest part of the island consists of a section of overlapping or closely spaced genes: clbB to clbL and clbN to clbQ, which are aligned on the same strand and code for components of the biosynthesis complex. The colibactin assembly line is supplemented with a dedicated transporter, encoded by clbM, and a resistance-conferring protein encoded by clbS [6,7]

Recently, the structure of the colibactin molecule has been proposed [7, 8-10]. Yet, the biological role of colibactin is still under discussion. Colibactin can interfere with the progression of the eukaryotic cell cycle,

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presumably by cross-linking DNA resulting in double strand DNA breaks in genomic instability in eukaryotes [5, 11, 12]. The ability to produce colibactin has been described to increase the pathogenic potential of the producing bacteria and to promote colorectal cancer development [13], but has also been related to beneficial effects to the host [14-15].

Initially, the pks island has been described in extraintestinal pathogenic E. coli to be chromosomally inserted into the asnW tRNA locus in close proximity to another tRNA(Asn) gene-associated pathogenicity island, the so-called “high pathogenicity island” (HPI). The HPI harbors an additional polyketide determinant coding for the metallophore yersiniabactin biosynthetic machinery [16]. As members of the Enterobacteriaceae are generally not known as archetypal secondary metabolite producers the origin of the pks island remains to be further investigated. Interestingly, the colibactin determinant has also been detected as part of an “integrative and conjugative element” (ICE) in different enterobacteria. This ICE also integrates near a tRNA(Asn) locus into the bacterial chromosome and commonly carries the yersiniabactin gene cluster [17].

MATERIAL AND METHODS

Sample collection

The bacterial sample was collected from Al-sadder medical city in AL-Najaf province from 2 January to 1 April , we collect 20 sample from different type of enterobacteriacea family to detect the presence of PK island in bacterial species.

Bacterial identification

The enterobacteriacea identification occurred by vitek-2-compact system.

Molecular study

Bacterial DNA was extracted by DNA extraction kit from geneaid \UK and the primer design from Bioneer /Korea company for research for the following gene (ClbA,ClbQ,ClbB and ClbN) as shown in **Table 1 ,2** (18).

Table (1) The primer sequences and PCR conditions of PKs.

Target gene	P	Primer sequence (5′-3′)	bp	PCR condition
clbA	F	CTAGATTAT CCGTGGCGA TTC	1000	94 ºC 1min 94 ºC 30Sec 58 ºC 30Sec 30x 72 ºC 1min 72 ºC 5 min
	R	CAGATACAC AGATACCAT TCA		
clbQ	F	TTATCCTGTTAGCTT TCG TTC	850	
	R	CTTGTATAGTTACACAACTATTTC		

Table (2) The primer sequences and PCR conditions of colibactin.

Target gene	P	Primer sequence (5′-3′)	Bp	PCR condition
clbB	F	GATTTGGAT ACTGGCGAT AAC CG	579	95 ºC 5min 95 ºC 30Sec 55 ºC 30Sec 30x 72 ºC 1min 72 ºC 5 min
	R	CCATTTCCC GTTTGAGCA CAC		
clbN	F	GTTTTGCTC GCCAGATAGTCA TTC	733	95 ºC 5min 95 ºC 30Sec 55ºC 30Sec 30x 72 ºC 1min 72 ºC 5 min
	R	CAGATACAC AGATACCAT TCA		

RESULT AND DISCUSSION

Bacterial identification

The result for bacterial identification show from 20 sample we found 15 specimen was enterobacteriacea from different species while 5 was non growth, also the most enterobacteriacea found wes the E.coli followed by Klebsiella as shown in table 2, the result of study was conflicted with another study such as study for Oliero, M., *et al* .,2021(19).

Table 2 : Bacterial identification during study.

Enterobacteriacea type	Number of isolates
E.coli	**11**
Klebsiella	**4**

Molecular detection

The current study gene found in a bacterial sample after PCR detection. We performed electrophoresis in Agarose gel for detect the band for each gene as shown in **Figure 1** , during another study from (Wami, H., *et al* .,2021) (20) we found result that are consistent with the results of the current study.

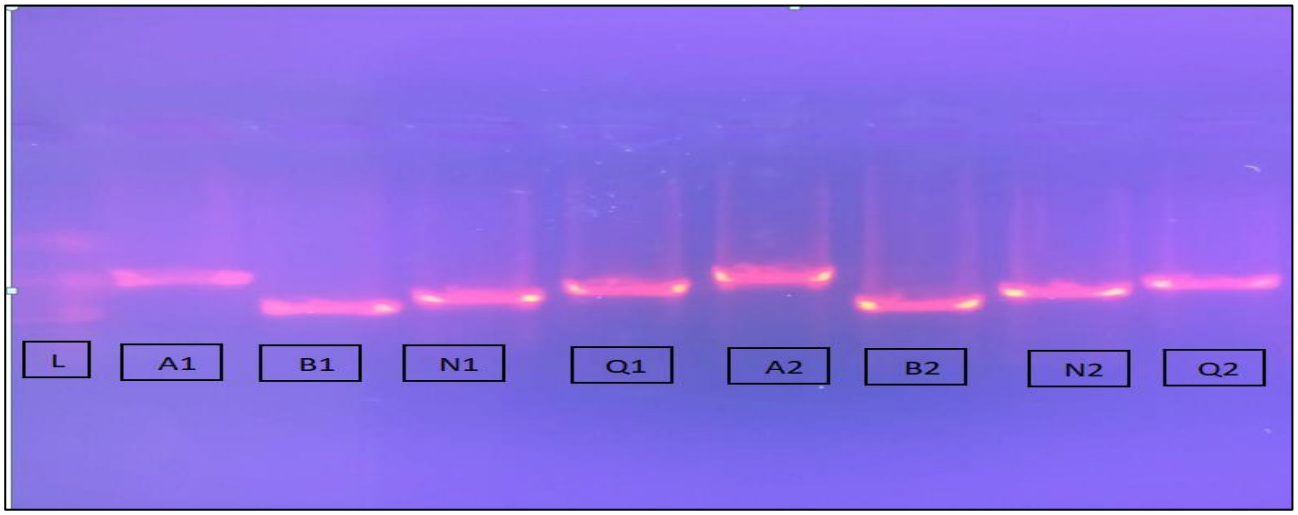

Figure 1: Gel electrophoresis for e.coli amd klebsiella to detect 4 gene type found in PK island

L: Ladder , (A1:ClpA , B1:Clp B, N1:Clpn ,Q1:Clpq) for E.coli, And (A2: ClpA , B2:Clp B, N2:Clpn ,Q2:Clpq) for Klebsiella.

CONCLUSION

During research, the e.coli is the high percentage of enterobactericea followed by other bacterial type such as klebsiella and the two types of bacteria have the gene responsible for the pathogenicity island PK in enterobacteriaceae.

Ethical approval

This study was approved by the Committee on Publication Ethics at Al-Sader Medical City in Najaf province.

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