Molecular detection of some virulence gene associated with pathogenicity of *Klebsiella pneumoniae*

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Abstract:-

In this study, only seven isolates of *Klebsiella pneumoniae* were subjected for detection espB, cesT/eae, and CNF1 genes in their genome.It was seen that esp gene was not found in all isolates understudy, whereas cytotoxic necrotizing factor-1(cnf1) was detected in four isolates of *Klebsiella pneumoniae*. Moreover, three isolates were shown to possess cesT/eae gene in their genome. The presence of such genes will provide the bacteria to invade and evade the host immune system and also will interfere with wound healing as a result of cnf1 production.

Introduction:-

Many virulence genes have been sequenced in enterobacteriaceae to promote their pathogenicity such as espB, cesT/eae, and cytotoxic necrotizing factor-1(CNF1).

The virulence determinants required for the induction of the attaching–effacing (A/E) lesion in entropathogenic *E. coli* are encoded in a 35.6-kb pathogenicity island, denoted LEE (for locus of enterocyte effacement) (1). LEE-encoded genes have been divided into three functional regions: the *esc* (extended-spectrum cephalosporin) and *sep* genes, which code for a type III secretion-translocation system (2); the *tir*(translocated intimin receptor), *cesT* (chaperon for type III secretion of tir) , and *eae* genes (intimate attachment to epithelial cell), coding for the proteins involved in intimate attachment (3); and the *esp* genes (intimate secreted protein), which encode effector proteins that are involved in the formation of a translocon for delivering effector molecules to the host cell (4). The *cesT* gene may encode a chaperone protein and is highly conserved in all the A-E positive strains, probably because it contains the transcriptional start of *eae* (5). *On the other hand, cesT*, previously known as *orfU*, encodes for a chaperone that is required for the stable secretion of Tir (translocated intimin receptor), it was demonstrated that *tir, cesT*, and *eae* constitute an operon and that *orf19* is a monocistronic unit (1). The *espB* gene has been sequenced in several strains, including human enteropathogenic E. coli (EPEC) (6), human and bovine enterohaemoragic E. coli (EHEC) (7), and rabbit EPEC (8). Comparison of the deduced amino acid sequences has revealed that, like intimins, EspB are highly variable proteins. The variability in genes encoding proteins that interact directly with the host, in contrast to other LEE encoded proteins, which are highly conserved, suggests that these variable proteins are subject to selection for evasion of the host immune system (9). EspB protein is essential for signal transduction in host cells and AE lesion formation (10). However its function has not been elucidated. Mutations in the *espB* gene eliminate the formation of the A-E lesion (11).

Besides CNF-1 is a toxin produced by uropathogenic *E. coli* strains that mediates its effects via the activation of the Rho GTPases. CNF-1 can either hinder cells from apoptosis or even induce apoptosis depending on the cell type and the dose. Moreover, by influencing the phagocytosis and adherence of polymorphonuclear leukocytes, CNF-1 could facilitate the growth of bacteria in the urinary tract. In addition, CNF-1 can enhance the secretion of proinflammatory

cytokines in human uroepithelial cells. Despite many in vitro effects, there have been conflicting results about the role of CNF-1 in vivo (12).

In a study conducted by (13) who found CNF-1 was able to interfere with wound healing in intestinal epithelial monolayers.

After mechanical injury, it was found that CNF-1 blocks epithelial wound repair within 48hours (14). This effect was characterized by cell elongation and filopodium formation on leading edge, in association with permanent phosphorylation of focal adhesion kinase via Rho activation (15).In addition to the promotion cell spreading cnf1 protects cell from experimentally induced rounding up and detachment and improves the ability of cells to adhere to each other and to extracellular matrix by modulating the expression of proteins related to cell adhesion (16).

This study was carried out to detect the presence of espB, cesT/eae, and CNF1genes in local isolates of *Klebsiella pneumoniae*.

Materials and methods:-

Patients:

A total 65 samples, only seven isolates of *Klebsiella pneumoniae* were obtained from patient with burn wound infection by standard bacteriological methods. All samples were obtained from patients or individuals who admitted to Al-Hilla Surgical Teaching Hospital in Babylon city during the period from 10/2011 to 1/2012.

Bacterial identification:

The samples were processed on MacConkey agar and were incubated at 37c° overnight. The identification of gram negative bacteria was performed by standard biochemical methods (catalase test, oxidase test, and IMViC test, capsule test) according to Bergy's Manual for Determinative Bacteriology (17).

DNA extraction for gram negative bacteria:

DNA extraction was carried out according to the genomic DNA purification kit supplemented by manufactured company (promega, USA) (cat# A 1120) with modification in step (3), which involved putting in temperature (100 C°) for 10min instead of 80 C° for 5min.

Detection of some pathogenicity islands markers by PCR:

The primers and PCR conditions used to amplify genes encoding virulence factors with PCR are listed in table (1). The primers for the LEE includes *cesT/eae* gene and espB, as well as the primer specific for the CNF1. Each 25µl of PCR reaction contained 2.5µl of each upstream and downstream primer, 2.5µl of free nuclease water, 5µl of DNA extraction and 12.5µl of master mix. The PCR amplification product were visualized by electrophoresis on 1% agarose gels for 45min at 60v. the size of the amplicons were determined by comparison to the 100 bp allelic ladder (promega, USA) (cat# G 2101).

Table (1):- Primers sequences and PCR condition to detect LEE & CNF1

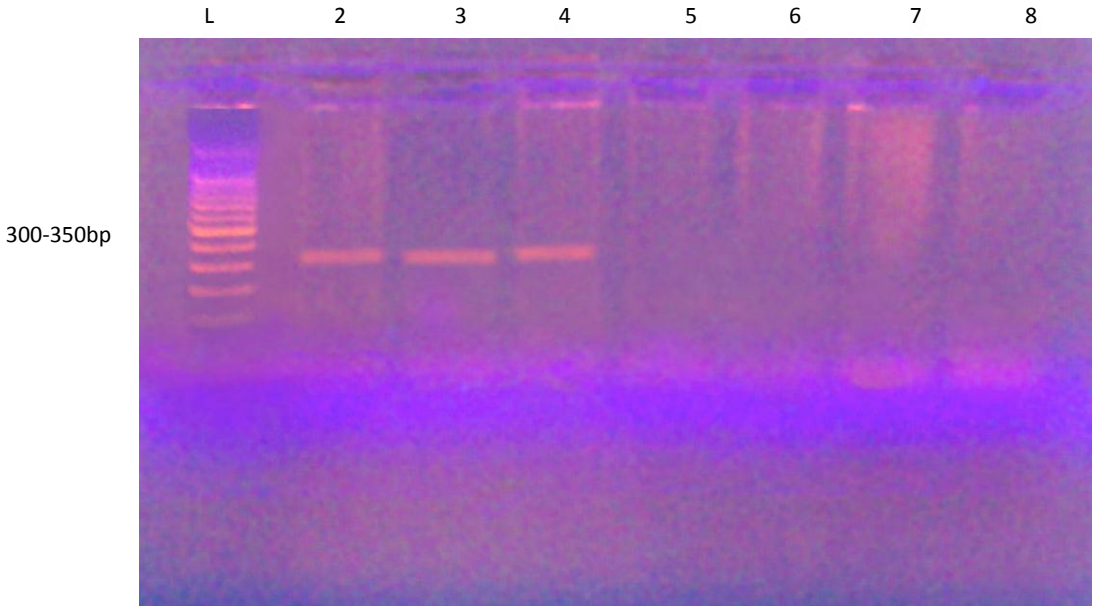
Genes	Primer sequence (5′-3′)	Size of product bp	PCR condition	Reference
cesT/eae F *cesT/eae* R	GTTTGCAGAGAATGGTGGCCC TAGCTTATGCTTGTGCCGGGT	333	94ºC 4min 1x 90ºC 1min 56ºC 1min 30x 72ºC 1min 72ºC 5min 1x	1
espB F *espB* R	GCCGCTCTGATTGGTGGTGCT TGGCGTTGAACCGGAAATCCT	387	94ºC 4min 1x 94ºC 1min 56ºC 1min 30x 72ºC 1min 72ºC 5min 1x	1
CNF1 F *CNF1* R	AAGATGGAGTTTCCTATGCAAGGAG CATTCAGATCCTGCCCTCATTATT	410	94ºC 4min 1x 94ºC 1min 60ºC 1min 30x 72ºC 1min 72ºC 7min 1x	18

Company of primers is alpha (USA).

Results and Discussion:-

A total of 65 wound swabs, only seven isolates of *Klebsiella pneumonia* were identified, according to the cultural, biochemical behavior. These seven isolated were subjected to study the presence of three important virulence genes; include espB, cesT/eae, and CNF1 genes by using PCR technique.

it was shown that the espB gene was not detected in all *Klebsiella pneumoniae* isolates. This may indicates that there is no sequence homology of this gene in *E. coli* with that in *Klebsiella pneumoniae* because espB is conserved at high frequency in *E. coli* as mentioned by (19). On the other hand cesT/eae is present in only 3 isolates as shown in fig (1).



Fig(1):-Gel electrophoresis of PCR of *cesT/eae* (chaperon type III secretion for tir/ intimate attachment to epithelial cell) amplicon product. L: ladder; 2, 3, 4,5,6,7,8: no. of isolates

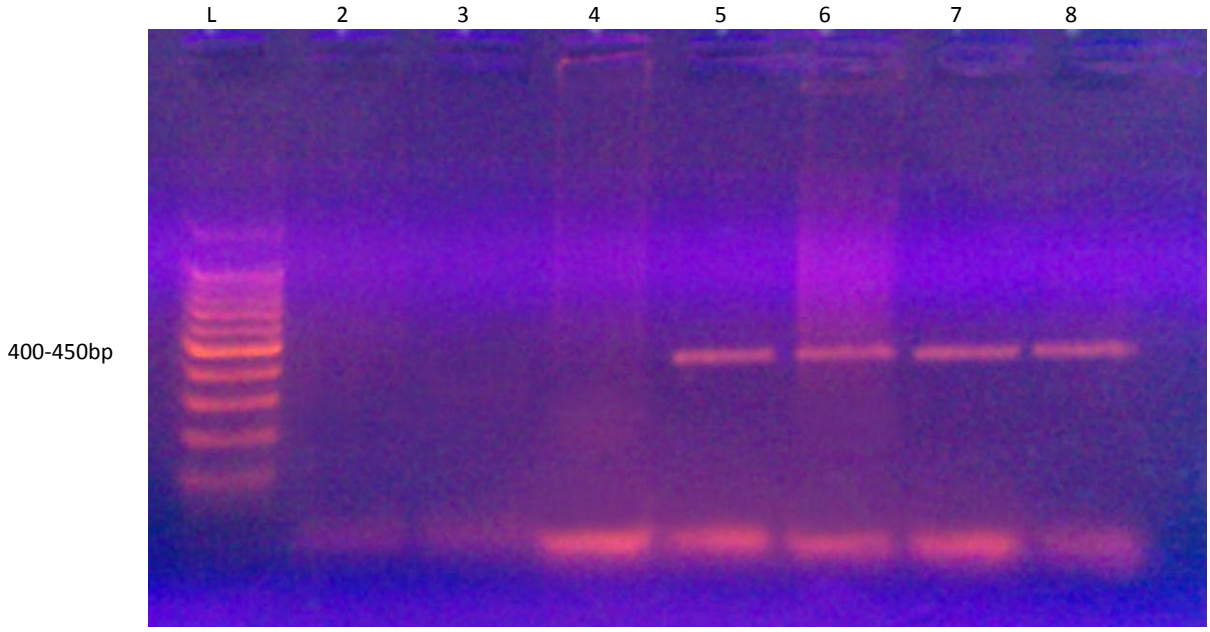
There are several possible scenarios regarding the origin and evolution of the LEE. On the other hand, the observation that the organization and functions of the LEE are conserved among studied *E. coli* strains (20) and that the locus has a different G-C proportion to the rest of the chromosome (1) suggests that this group of genes was transferred as a cassette from some other bacterial species and was inserted as a whole in the *E. coli* chromosome. Also, there is increasing evidence of horizontal transfer of some genes of the LEE, recombination within this locus (21) as well as recombination with other pathogenic genes (22). These later observations suggest that this locus was assembled by multiple independent recombination events (23). the regulation of the *tir-cesT-eae* operon (henceforth referred as the *LEE5* operon), whose expression is directed by a promoter located upstream of the *tir* gene.

Also cnf1 was detected amplification by using cnf1 primer in klebseilla genome, it was observed that cnf1 was exist in only 4 isolates as shown in fig (2). The presences of this gene may be attributed to horizontal gene transfer in which the genetic markers are transferred through many mechanisms mostly by conjugation or transformation .

So, according to that the production of cnf1 will render such bacteria to grow and survive at the site of infection due to its ability to prevent the healing of wound through its effect on polymorphonuclear leukocytes.

(24) observed that cnf1 is mostly found among entric bacteria associated with urinary and digestive tract infections and the present study suggests that the source of *Klebsiella pneumoniae* isolates from wound infections due to contamination the wounds with urine or stool containing this bacteria.

The mechanisms of action of cytotoxic necrotizing factor-1(CNF-1) involve the Rho-dependent rearrangement of the cytoskeleton in eukaryotic cells with a complex of consequences (12).



Fig(2):-Gel electrophoresis of PCR of cytotoxic necrotizing factor-1 *cnf1* amplicon product. L: ladder; 2, 3, 4,5,6,7,8: no. of isolates

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الخلاصة:-

في هذه الدراسة, تم عزل وتشخيص سبع عزلات من بكتريا *Klebsiella pneumoniae* حيث تم التحري على احتواء العزلات للجينات espB, cesT/eae, and cnf1 . وقد وجد ان جميع العزلات غير حاوية على espB في حين وجد ان ثلاث عزلات فقط حاوية على cesT/eae . كما وجد ان اربع عزلات فقط حاوية على cnf1 جين. كما لوحظ ان وجود هذه الجينات في البكتريا يزيد من قابليتها على غزو وتجنب الجهاز المناعي ويزيد من قابليتها على حدوث الاصابة وبالأخص وجود جينات cnf1.