WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 6.805
Volume 5, Issue 3, 175-187. Research Article ISSN 2277– 7105

MOLECULAR STUDY FOR SOME VIRULENCE FACTORS OF *ACINETOBACTER BAUMANNII* ISOLATED FROM PATIENTS WITH WOUND INFECTION IN HILLA CITY

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Article Received on
01 Jan 2016,

Revised on 21 Jan 2016,
Accepted on 11 Feb 2016

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ABSTRACT

This study is devoted to the isolation and identification of *A.baumannii*; indeed seven isolates of *A.baumannii* have been isolated and identified out of 100wound swab collect from 100 patients with wound infection, The isolates obtained have been cultured in selective media and biochemical identified. Besides, some virulence factors have been detected by such molecular techniques as polymerase chain reaction PCR and it has been found that only 2(28%) of isolates gave positive result for *cnf1* at 498bp, 4(57.1%) of them gave positive result for *kps* at 272bp, 2(28.5%) of them gave positive result for *PAI* at 930bp, 5(71.4%) of them gave positive result for *ibeA* at 170bp, 1(14.2%) of them gave positive result for *sfa* at 240bp, 4(57.1%) of them gave positive result for *iutA* at 300bp.

KEYWORDS: *A.baumannii, cnf1, kps, ibeA.*

1.1. INTRODUCTION

The genus *Acinetobacter* comprises a complex heterogeneous group of bacteria, many of which are capable of causing a range of opportunistic pathogen. However, *Acinetobacter baumannii*, are important nosocomial pathogens, often associated with epidemic outbreaks of infection, that are only rarely found outside of a clinical setting. These organisms are frequently pandrug-resistant and are capable of causing substantial morbidity and mortality in patients with severe underlying disease, both in the hospital and in the community.[1]

Acinetobacter species have been implicated in a wide range of infections, particularly in critically-ill patients with impaired host defenses. These infections include pneumonia, skin

and soft-tissue infections, wound infections, urinary tract infections, meningitis and bloodstream infections nosocomial infections and hospital outbreaks have been attributed mainly to *A. baumannii*, particularly in the intensive care unit (ICU)setting.[2]

In the past, *Acinetobacter* was considered to be an organism of low virulence. The occurrence of fulminant community-acquired *Acinetobacter pneumonia* indicates that these organisms may sometimes be of high pathogenicity and cause invasive disease. Studies on *Acinetobacter* virulence factors are still at an elementary stage. Non-specific adherence factors, such as fimbriae, have been described in *Acinetobacter* and it is known that, under iron-deficient conditions, bacterial growth can be accompanied by the production of receptors and iron-regulated catechol siderophores, which will, in turn, favour bacterial growth and the expression of virulence factor.[4]

The ability of *A. baumannii* to grow as biofilm on abiotic surfaces plays an important role in causing nosocomial infections, due to the surface colonization of hospital equipment and indwelling medical devices, such as urinary catheters, central venous catheters (CVCs), endotracheal tubes, etc.[5]

Pathogenicity islands carry genes encoding one or more virulence factors, including, but not limited to, adhesins, toxins, or invasins. They may be located on a bacterial chromosome or may be transferred within a plasmid.[2]

Also the ability of *A.baumannii* to produce *cnf1* which Induces the formation of actin stress fibers and membrane ruffling, necrosis *cnf1* is internalized via receptor- mediated endocytosis upon binding to acell surface receptor.[6]

ibeA is an important virulence factor that contributes to *E. coli* K1 invasion of both intestinal epithelial cells and BMECs in vitro and in vivo *E. coli* bacteraemia-sepsis is thought to arise from the gastrointestinal tract. Human and animal studies suggest that the development of sepsis and meningitis is correlated with the magnitude of bacteraemia.[1]

Iron uptake is the outer membrane protein responsible for the uptake of ferric aerobactin and composed of a polypeptides with molecular mass of 78,223Da, Aerobactin can efficiently sequester ferric iron from transferrin allowing the growth of microorganisms in host tissues and body fluid, Role of aerobactin system as a virulence factor in the extraintestinal infections, also apportunistic strains of *E. coli* and different members of Enterobacteriaceae.[7]

The S fimbriae were discovered as a group of fimbriae among pyelonephritogenic *E. coli* strains which recognized neuraminic acid (sialic acid) - containing structures other than mannosides or P antigens on human erythrocytes and were termed the S fimbriae based on their receptor specificity, that is, their specific binding to sialylgalactosides.[6]

Aims of study

1-Isolation and identification of *Acinetobacter baumannii* from wound infection.

2-Detection of some important virulence genes like *(cnf1, ibeA, iutA, kpsMT, PAI, sfaS,).*

2.2. Patients and samples

Wound swabs were collected from (100) patients suffering from wound and burns infection from both sexes who admitted to AL-Hilla teaching hospital during the period from (Fabruary 2015 to May 2015). Process took the sample by touch the wound by sterile swab and put the swab in plastic cover to prevent contamination and after that take the swab to lab and culturing on cultural media for identification.

2.3. primers sequences

The primers sequences and PCR condition that used in study are listed in table (1-1).

Table (1-1) The primers sequences and PCR condition.

Genes name	Primer sequence (5´- 3´)	Size BP	Codition	Refrences
Cnf1	Sense – AAGTGGAGTTTCCTATGCAGGAG Antisense – CATTCAGTCCTGCCCTCATTATT	**498**	**95c°for 12min 25cycle 94c°for 30s 63c°for30s 68c°for 3min 72c°for 10min**	Braun & Vidotto, 2004
IbeA	Sense – AGGCAGGTGTGCGCCGCGTAC Antisense – TGGTGCTCCGGCAAACCATGC	**170**	**95c° for12min 25 cycle 94c°for 30s 63c°for 30s 68c°for 3min 72c°for10min**	
SfaS	Sense- GTGGATACGACGATTACTGTG Antisense CCGCCAGCATTCCCTGTATTC	**240**	**95c°for 12min 25 cycle 94c°for30s 63c°for30s 68c°for 3min 72c°for 10min**	
PAI	Sense- GGACATCCTGTTACAGCGCGCA Antisense	**930**	**95c°for 12min 25cycle 94c°for 30s**	

	TCGCCACCAATCACAGCCGA		**63c°for30s 68c°for3min 72c°10min**	
KPSMTII	Sense- GCGCATTTGCTGATACTGTTG Antisense- CATCCAGACGATAAGCATGAGCA	**272**	**95c°for 12min 25cycle 94c°for 30s 63c°for30s 68c°for3min 72c°for10min**	
iutA	Sense – FGGCTGGACATCATGGGAACTGG Antisense – CGTCGGGAACGGGTAGAATCG	**300**	**95c°for 12min 25cycle 94c° for 30s 63c°for30s 68c°for 3min 72c° for 10min**	

RESULTS AND DISSCUSION

3.1. Isolation and Identification of *Acinetobacter baumannii*

During the study period from February, 2015 to May, 2015, the results revealed that 100 swabs of wound infection were taken. The samples were collected from patients admitted to AL_Hilla Teaching hospital.

Out of 100 swab 7 isolates of *Acinetobacter baumannii* were isolated and identification according to specific biochemical test and api 20 system.

3.2. Molecular detection

3.2.1. Genes responsible for virulence factors

3.2.1.1. cytotoxic necrotizing factor *(cnf1)* gene

A total of[7] isolates of *A.baumannii*, it was found that two (28%) isolates contain this gene with base pair 498 when compart with allelic leader as shown in figure (1-1).

The result in this study will agree with Momtaz *et al*., 2015[10], who detect this gene in 35% in *A. baumannii* isolated from different clinical samples.

Cnf1 belongs to the so-called dermonecrotic toxins family, including *cnf1*, *cnf2* from *E. coli*, *cnfy* from *Yersinia pseudotubercolosis* and the dermonecrotic toxin DNT from Bordetella, all sharing considerable functional and amino acid homologies.[9]

The presence of *cnf*1gene of *E. coli* in other gram negative cells may be attributed to horizontal gene transfer in which the genetic markers are transferred through many mechanisms such as conjucation, transformation and transduction.[11]

So, according to that the production of *cnf1* will render such bacteria to grow and survive at the site of infection due to *cnf1* ability to prevent the healing of wounds through its effect The production of *cnf1* by the bacteria associated with post-operative wound infection was seen to be important that rendering this factor as virulence factor through its ability to increase the functional features of PMNL such as superoxide generation and adherence on epithelial cells but significantly decrease their phagocytic features.[12]

Petkovsek[13], found that *cnf1* gene were found with prevalences 30% in *E. coli*isolates from skin and soft tissue infections.

And Al-Amean *et al*., 2011[14], found the *cnf1* gene is distributed among enteric bacteria isolated from wound samples.

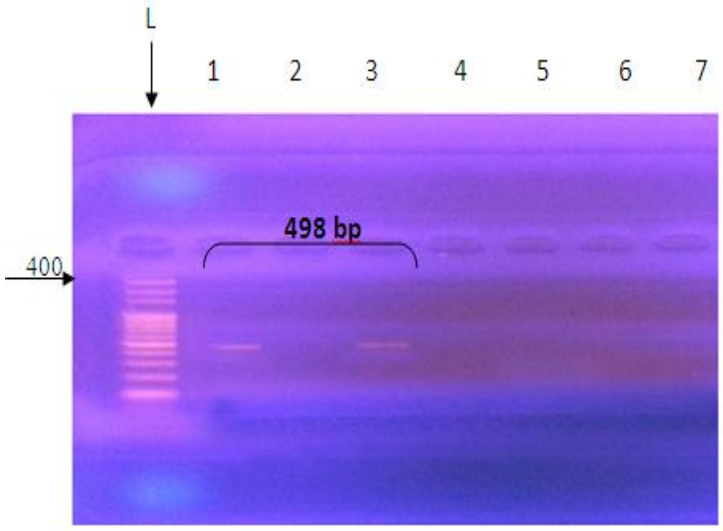

Figure (3-1) Gel electrophoresis of PCR products of *cnf1* gene.

3.2.1.2. Iron up takeA (*iutA*) gene

Primers of aerobactin gene were used for detecting the presence of *iutA* gene in *Acinetobacter* isolates. It has been found that four isolates (57.1%) of *Acinetobacter* contain the *iutA* gene with the length of (300) base pairs. The amplicon was detected in gel electrophoresis and compared with allelic ladder as shown in figure.[1-2]

This result agrees with Landgraf *et al*., 2012.[16] who have observed that this gene was present in only 38% in *A. baumannii* isolates, which isolated from wound swab.

While Momtaz *et al*., 2015[10] detect this gene in 19% in *Acinetobacter* isolates, isolated from different clinical sites.

Iron uptake is the outer membrane protein responsible for the uptake of ferric aerobactin and composed of a polypeptides with molecular mass of 78,223Da, aerobactin can efficiently sequester ferric iron from transferrin allowing the growth of microorganisms in host tissues and body fluid, role of aerobactin system as a virulence factor in the extraintestinal infections, also apportunistic strains of *E.coli* and different members of enterobacteriaceae.[7]

Braun and Vidotto, 2004[16], demonstrate that there are no member of this gene detect in *A.baumannii* strains isolated from urine samples, suggesting that may be presence of a different type of siderophore mechanism genes rather the *iut A* gene.

While Koga *et al*., 2014[17], refer in his study that *iutA* was the most commonly virulence gene, present in 65% of the tested isolates in *E. coli* isolated from blood samples.

Also, in Poland, Kaczmarek *et al*., 2012.[18] Found *iutA* gene found in approximately with percentage 35.8% in *E. coli* different clinical sites.

The strain that have negative results in this study may be due to it contain other gene responsible for aerobactin production like (*iucA*), (*iucB*) and *(iucD*).

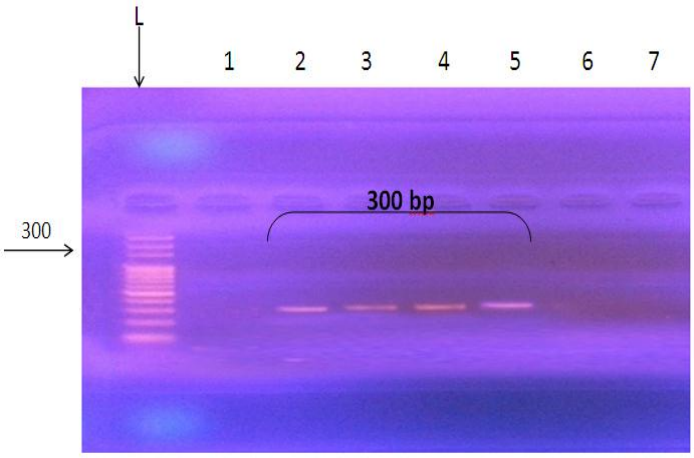

Figure. (3-2) Gel electrophoresis of PCR products of *iut A* gene.

3.2.1.3. Capsule polysaccharides *(kpsMT*II) gene

Atotal of (7) isolates of *A.baumannii*, it was found that four (57.1%) isolates contain this gene with base pair 272 when compare with allelic leader as shown in figure (3-10).

Kps are high-molecular-weight polymers made up of oligosaccharide units that are polymerized to form a long chain. The repeat units are composed of different sugars that are joined together via specific linkages, with each linkage catalyzed by a specific glycosyltransferase. In *A. baumannii*, enzymes for synthesis of the oligosaccharide units are encoded by the K locus, which is located between the *fkpA* and *lldP* genes (Kenyon and Hall, 2013(19).

This locus includes genes for the synthesis of activated sugar precursors, glycosyltransfer, modification, repeat-unit translocation across the inner membrane, repeat-unit polymerization to form the carbohydrate polymer and capsule export (Kenyon and Hall), 2013 (19).

Rocka *et al*., 2011(20), demonstrated that the K1 capsule from the *A. baumannii* strain was necessary for optimal growth in human ascitic fluid and survival in human serum as well as in a rat tissue infection model. The active protection of the capsule allows bacterial resistance to the bactericidal activity of the complement.

AL-warid and AL-thahab, 2014(21) detect the presence of Capsule in 6/11 (54%) in *Acinetobacter baumannii*is isolated from different clinical site.

Also this study agree with, Abed *et al*., 2011(22), who found this gene with percentage 72% in 50 uropathogenic Escherichia coli (UPEC) isolated from children with urinary tract infections were genotypically characterized by polymerase chain reaction (PCR) assay.

While the result in this study disagree with, mohajeri *et al*., 2013(23). In Iran who did not find the relevant gene *kps* gene in his study, who isolate *Acinetobacter baumannii* from urine, blood and sputum.

This study disagree with, Park *et al*., 2012(24), who detect this gene *kps* with percentage 82% in children infected with urinary tract infection.

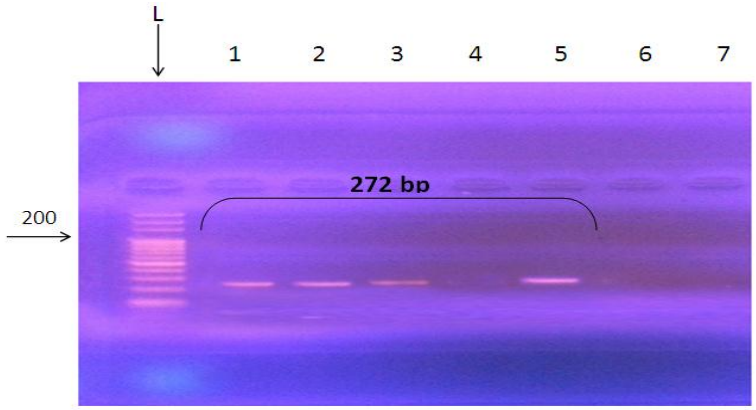

Figure. (3-3) Gel electrophoresis of PCR products of *kpsMT*II gene.

3.2.1.4. Pathogenecity island (PAI)gene

A total of (7) isolates of *A.baumannii*, it was found that two (28.5%) isolates contain this gene with base pair 930 when compart with allelic.

Pathogenicity islands carry genes encoding one or more virulence factors, including, but not limited to, adhesins, toxins, or invasins. They may be located on a bacterial chromosome or may be transferred within a plasmid (2).

This study disagree with Firoozeh, 2014(25). who found that a total of 150 UPEC (Uropathogenic *E. coli*) isolates which isolated from pyelonephritis patients, contain 59 (81.9) of this gene and from cystitis patients 33 (42.3) Of the, 130 (86.7%).

Neamati, 2015(26). Refer that a total of 150 *E. coli* strains were isolated from urine samples in patients admitted to the various wards of Shahid Beheshti Hospital Kashan, Iran, were analyzed. Found that *PAI* gene detected in (61.3%) of total isolates.

The low percentage of detection this gene in this study may be due to *PAI* often are unstable and delete with distinct frequencies. Virulence functions encoded by certain *PAI* are lost with a frequency that is higher than the normal rate of mutation. Genetic analyses showed that such mutations are caused not by defects in individual virulence genes within the *PAI* but, rather, by loss of the large portions of a *PAI* or even the entire *PAI*. These mutations can be observed during cultivation of pathogens in vitro, but they are also found in isolates obtained from infected individuals, for example during persistent infections. This indicates that such *PAI* have an intrinsic genetic instability(27).

No previous study refer the presence of this gene in *Acinetobacter baumannii*, but many study refer the presence this gene in *E.coli*, so the two isolates contain this gene in this study may attributed to gene transfer accrued between these related genus(28).

PAI are part of that flexible gene pool. Sequencing of entire bacterial genomes revealed a more ubiquitous occurrence of such islands than was previously thought and this represents a paradigm of more general genetic entities that are present in the genome of many bacteria. Therefore, the designation "pathogenicity islands" has been extended to "genomic islands," which can encode a wide range of functions. Most genomic islands carry genes useful for the survival and transmission of (Koga *et al*., 18) microbes.

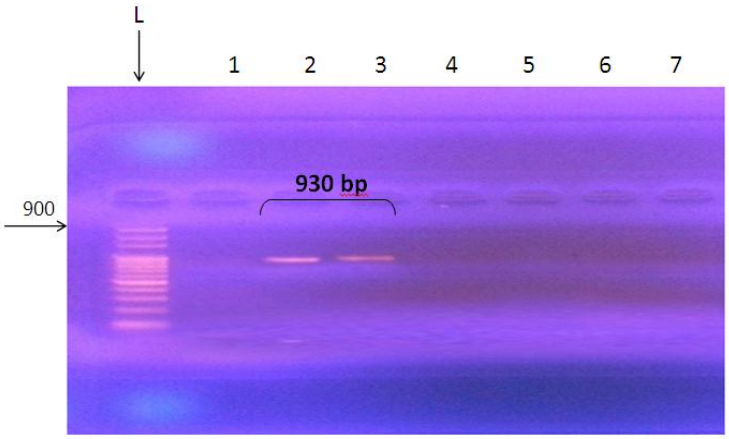

Figure. (3-4) Gel electrophoresis of PCR products of *PAI* gene.

3.2.1.5. Invasion protein A *(ibe A)* gene

Atotal of (7) isolates of *A.baumannii*,it was found that five(71.4%) isolates contain this gene with base pair 170 when compart with allelic leader as shown in figure (3-12).

In this study, this gene found with highest percentage (71.4%), comparable with another studies where, Asadi *et al*., 2014(29), have percentage 20% from 116 samples, also Chmielarczyk *et al*., 2015(30), was detect *ibeA* gene in 37.5% of total isolates, the cause of this different results in these studies, back to types of clinical samples (blood, wounds, urine, sputum, etc.) so, presence this gene according location of isolation.

Giray *et al*., 2012(31), showed that *ibeA* was indeed involved in adhesion of strain to eukaryotic cells but only indirectly via the modulation of the synthesis of type 1 fimbriae.

The presence of *ibeA*, a gene encoding a known virulence factor of *Escherichia coli* strain responsible for neonatal meningitis in humans, was investigated in the genome of 213 avian pathogenic *E. coli* (APEC) strains and 55 non-pathogenic *E. coli* strains of avian origin.Fifty-three strains were found to be *ibeA*, all of which belonged to the APEC group The *ibeA* gene is therefore positively linked to the pathogenicity of strains(32).

Schippa, *et al*., 2014(33). From 20 isolates of *E.coli* found *ibeA*gene approximately 5 from origin 20 samples 25%. Isolated from soft tissue.

ibeA are present at different locations of the genetic island GimA as a pair of homologous proteins that are encoded by two different operons, *cgl*(GimA2) relating presumably to glycerol metabolism and *ibeA* (GimA4) contributing to invasion and regulation(34).

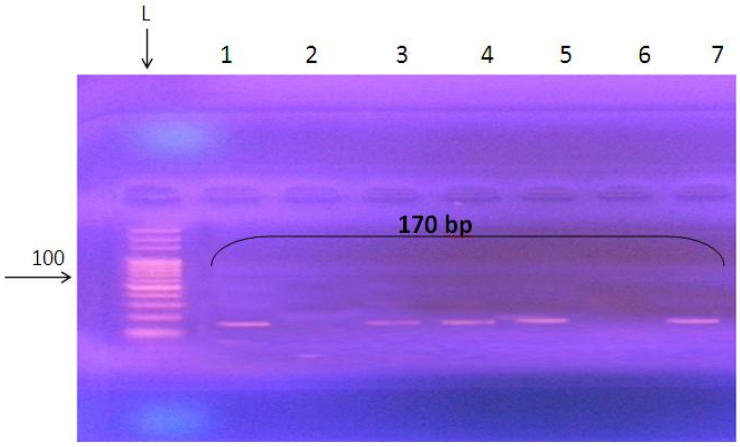

Figure. (3-5) Gel electrophoresis of PCR products of *ibeA* gene.

3.2.1.6. S-fambrial adhesion (*sfaS*) gene

Atotal of (7) isolates of *A.baumannii* ,it was found that one(14.2%) isolates contain this gene with base pair 240 when compared with allelic leader as shown in figure (3-13).

The S adhesion molecule, a protein of 12 kilodaltons (kDa), was isolated and characterized, and the sequence ofthe gene, coding for the S fimbrial protein subunit, was determine. The *sfa* determinants from differentstrains have high sequence homology. Surprisingly, itwas found that the *sfa* determinant is also related to anothergene cluster (foc) coding for FlC fimbriae (6).

The result in this study will agree with farshad*et al*., 2011(35), refers the prevalent of *sfa* (18.75%) in *E.coli* isolated from urine in pateints with UTIS.

The result in this study disagree with Momtaz *et al*., 2015(10), who detect this gene in high percentage 51% in *A. baumannii* isolated from different clinical samples.

Also, mohajeri *et al*., 2013(23), found that no isolates of *A.baumannii* isolated from different clinical samples contain this genes and explain that may be this isolates contain other adhesive virulences genes like *csg*A and *fim*H genes.

Although this study disagree with Asadi *et al*., 2014 (29), founds that this gene with percentage 32(53.3)% from isolates 116 urine samples of *E. coli*

Shetty *et al*., 2014 (36), also detect this gene with percent 39.1% in *E.coli* isolates urinary tract infection.

The strains that lack S fimbriae and afimbrial adhesion perhaps use a different adhesion such as P fimbriae for attachment.

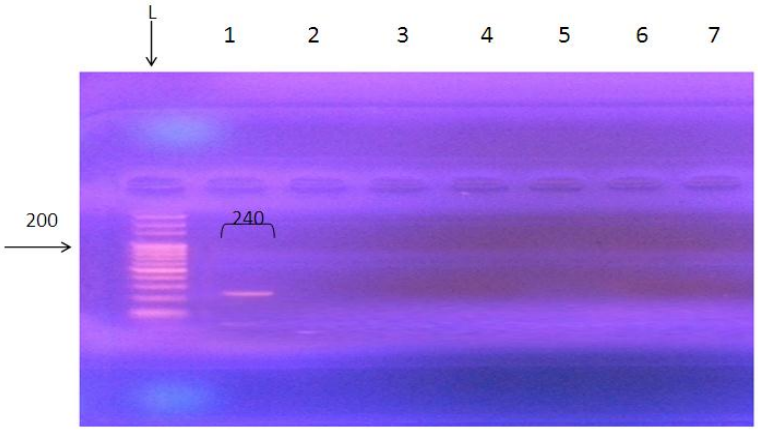

Figure. (3-6) Gel electrophoresis of PCR products of *sfaS* gene.

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