Genotype detection of *fimH* gene of *Acinetobacter baumannii* isolated from different clinical cases

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Abstract

Background: Forty isolates were obtained of *Acinetobacter baumannii* from (200) samples, the samples were collected from different cases including: - wounds, burns, Stool, Urinary tract infection urine.

Aim of study: The aim of study is to isolate and diagnose *Acinetobacter baumanniii* from different clinical cases and to investigate the *fimH* gene.

Objective: Respiratory tract infection and blood sample.For the period between 1/9/2016 to 30/11/2016.

Materials and methods: Identification of 40 isolates confirmed to be *Acinetobacter baumannii* included 9 isolates from blood, 1 isolate from urinary tract infections, 4 isolates from each of wound infections and Respiratory tract infection, 8 isolates from burns and 14 isolates from stool sample.

Results: The result revealed that the *fimH* gene was present in (20) isolates (50%) of *Acinetobacter baumannii.* The result Showed (7) isolate (17.5%) of Stool and blood has *fimH* gene to each of them and Burns (3) isolates (7.5%) has *fimH* gene and (2) isolate (5%) of respiratory tract infection has *fimH* gene and finally only (1) isolate (2.5 %) of Wound infection. While all isolated for each of the urinary tract infections doesn't the *fimH* gene. The gel electrophoresis showed that the molecular weight of *fimH* gene was 508 bp.

Conclusion: Sequential analysis detection of *fimH* showed three silent mutations that did not affect the amino acid translation.

Key Words: *Acinetobacter baumannii, fimH gene* , PCR polymorphism.

Introduction

Acinetobacter species are Gram negative bacteria , Strict aerobic, catalase positive, oxidase negative, non-motile, but some species bacteria have a kind of movement Twitching Motility because of polarity fimbriae (1, 2).

These bacterial opportunistic pathogens are responsible for many opportunistic infections in the hospital including pneumonia, burns infection wound infection, septicemia, endocarditis and urinary tract infection (3, 4).

Many virulence factors have an effect on the risk and morbidity of these bacteria, including the age of the patient, length time of stay in the hospital, resistance to antibiotics, long treatment of broad-spectrum antibiotics and immunosuppressive drugs, as well as chronic diseases such as heart disease, diabetes and hypertension (5, 6).

Acinetobacter species include several types of *A. baumannii* species. These bacteria have many of the virulence factors that help them cause disease like capsule formation, production of biofilm and production of enzymes such as lipase, protease, Gelatinase activity, Pellicle assay, Siderphores, production of colic in, formation of polysaccharides, adhesion to living cells and non-living surfaces (7, 8).

Several studies have indicated that the *fimA* gene encodes the large secondary unit while the *fimF* and *fimG* genes encode the small subunits and the *fimH* gene encodes the top of the cilia that are sensitive to the manus and the *fimC* gene encodes the attached protein that helps the fim protein pass through the Periplasms and *fimD* encodes the outer membrane proteins and *fimI* encodes For the structural of the grass and molecular weight of *fimH* gene was 508 bp (9, 10). The *fimH* gene is an important virulence agent for bacteria. Which encodes the Type 1 fimbraie, that helps bacteria bind to the surface of host cells and then cause injury.

The aim of study is to isolate and diagnose *A. baumanniii* from different clinical cases and to investigate the *fimH* gene.

Materials and methods

1. Sample collection: 200 samples of different diseases were collected from (wounds, burns, stools, urinary tract infection, respiratory tract infections, bacteremia) from several hospitals in Baghdad, from several hospitals in the of Baghdad city (Central Children's Hospital, Al Karama Hospital, Karkh General Hospital, Al-Ameen Medical City Hospital, Educational Labs, Baghdad Teaching Hospital, Child Protection Hospital, Burns and Wounds Hospital). For the period from 1/9/2016 to 30/11/2016. Samples collection included 50 isolates from blood, 20 isolates from urinary tract

infections, 30. isolates from wound infections, 40 isolates from burn infections , 35 isolate from Respiratory tract infection and 25 isolates from stool samples

2. Bacterial isolation and diagnosis: Samples were diagnosed using blood agar and MacConkey agar, and biochemical tests such as Catalase and Oxidase were used. For the final diagnosis of isolates, API 20E and Vitek 2 were used (11).
3. DNA extraction: A special kit (Promega, USA) was used to extract the Genomic DNA Purification Kit from bacterial isolates according to the instructions of the manufacturer Promega (USA).
4. Detection of the *fimH* gene: Prepare the primer solution for *FimH* F Forward and *FimH* R Reveries accordance the instructions of the manufacturer Alpha (DNA) using sterilized ionic distilled water to obtain a concentration of 100 picommol / microliter and present each solution of the initiates separately at 10 picomol / microliter by taking 10 microliters of the Stock Solution and add 90 microliters of distilled ionic distilled water and store the stock solutions under - 20 ° C, mixing of the solution after removing it from the ice using vortex before use. GO Taq Green Master Mix PCR Promega (USA).
5. PCR reaction: Using PCR amplification conditions shown in Table (1).

Table (1): The initiator used in this study and the interactive conditions used in PCR reaction

Source	Product size (bp)	Primer sequence ´ 5 ⟶ ´3		primer
Johnson and Stell (2000)	508	TGCAGAACGGATAAGCCGTGG	F	*fimH*
		GCAGTCACCTGCCCTCCGGTA	R	

6. Detection of the *fimH* gene: The steps of replication to investigate the gene *fimH* (12). The program was modified as follows initial denaturation (94 º for 4 minutes), 34 cycles (94°C for 60 second), (45 seconds at 56°C) and (60 seconds at 72°C). The final step included (10 minutes and at 72°C for final elongation)
7. The reaction products were extracted using Bio-Basic INC (Canada) (5 µl) of products with a concentration of (agarose gel) 2% containing 5 microliters of Eithidium bromide Bio Basic INC (Canada) and using (5µl) of DNA ladder (100-1500) (Promega, USA) base pairs and 100 volts for 80 min. UV light Optima (Japan) (13).
8. Sequencing analysis of DNA sequencing: DNA samples fim H gene with F and R primer were sent to the Macrogen Inc. South Korea Geumchen, Seoul) The results of the BioEdit Sequence Alignment Editor Software (DNASTER, Madison, WI, USA) are available on the National Center for Biotechnology Information (NCBI) and protein translation by Clustar Omega to determine the number of nucleotides Mutations and their effect on protein translation.

Result and discussion

Acinetobacter baumannii is one of the most common types of bacteria that cause septicemia, followed by respiratory tract infections (NSA) and isolated from hospital-acquired pneumonia (pneumoconial infection) and persistent ambulatory peritoneal dialysis (CAPD) (14).

After identification for bacterial diagnosis and relying on (11) was obtained 40 isolates confirmed to be *Acinetobacter baumannii* included 14 isolates (35%) from stool sample , 9 isolates (22.5%) from blood, 8 isolates (20%) from burns, 4 isolate (10%) from each of wound infections and Respiratory tract infection and 1 isolate (2.5%) from urinary tract infections Table (2).

Table (2): Source, number and percentages of *Acinetobacter baumannii* isolates

No.	Source *of A. baumannii*	number	%
1	Bacteremia (blood)	9	22.5
2	Respiratory tract Infection	4	10
3	Wounds	4	10
4	Burns	8	20
5	Stool	14	35
6	UTI	1	2.5
	Total	40	100

A.baumanni, *fimH* genes were detected using a PCR. The results showed that *A. baumannii* isolates possessed these genes in different proportions. As shown in Table (3). The results showed that 19 isolates (47.5%) of *A. baumannii* possessed the *fimH* gene. The resulting packets with a molecular weight were 508 bp as shown in Fig. (1).The result was closely correlated with the findings of the researchers (15).Who found 60% of *A. baumannii* isolates possessed the *fimH* gene with molecular weight of was 508 bp and the results were agreed with researchers Momtaz *et al.* (2). Who found that 90 isolates (47.38%) of *A. baumannii* had this gene that isolate from hospital infection.

Table (3): percentage of virulence genes *(fim H)* possessed by *A. baumannii* in different type of sampling

Source	Virulence genes *fimH (%)*
Bacteremia	**(17.5) 7**
Respiratory tract Infection	**(5)2**
Burns	**(7.5) 3**
Wounds	**(2.5) 1**
Stool	**(17.5) 7**
UTI	**0**
Total	**(50) 20**

***(*fimH*= fimbriae)**

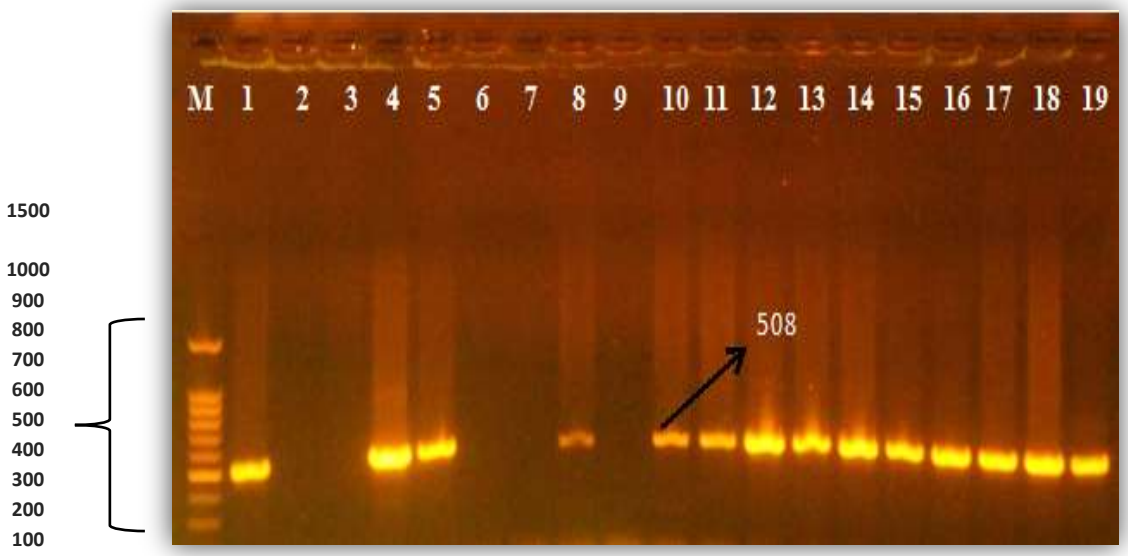

Figure (1- A): Agarose gel electrophoresis of the PCR product of the *fimH* gene (508 bp) of *A. baumannii* isolates on 2% Agarose gel and 100 volts for 80 min. Line M: DNA marker (100-1500bp Ladder, Promega, USA); Lanes (1, 4, 5, 8, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19) *A. baumannii* PCR positive isolate

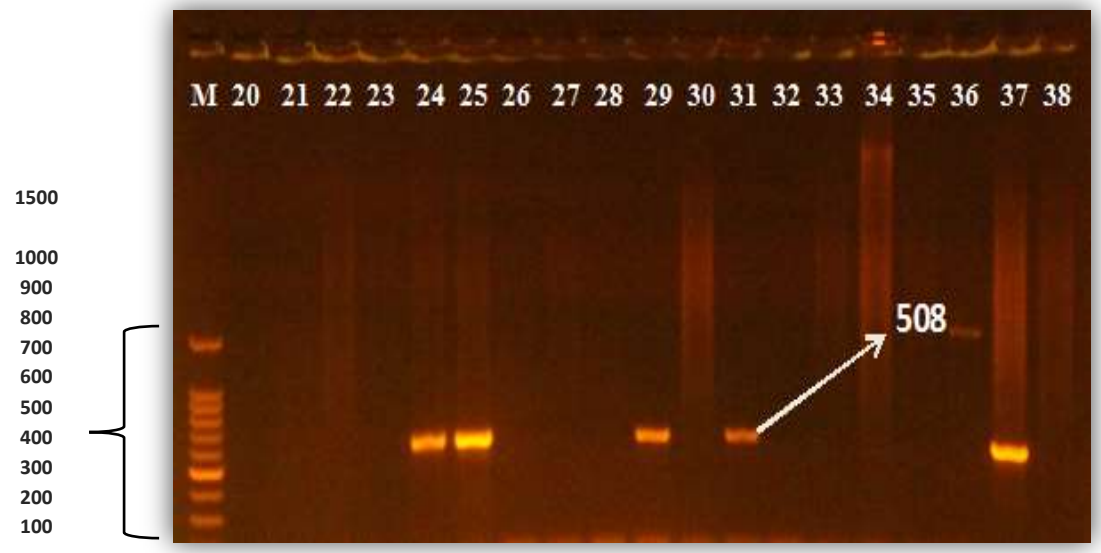

Figure (1-B): Agarose gel electrophoresis of the PCR product of the *fimH* gene (508 bp) of *A. baumannii* isolates on 2% Agarose gel and 100 volts for 80 min. Line M: DNA marker (100-1500bp Ladder, Promega, USA); Lanes (24,25,29,31,37) *A. baumannii* PCR positive isolate

Analyzed the DNA sequence of the *fimH* gene. The results showed that there were mutations in the *fimH* gene of isolation A23 and A23 from respiratory tract infection from *A. baumannii* . The substitution of the thymine with the adenine was obtained at 88 Subject- 4549236. Thymine were substituted with cytosine at 205 (4549353) and 237 (459384) respectively, as shown in Table (4) and Figure (2). When analyzing the results of the amino acid translation of the *fimH* gene with the original amino acid found that this mutation did not affect the amino acid change as described in the Figure (2).The results of the mutations analysis showed that all mutations were silent mutations which produced the same amino acid and did not affect protein translation. Lamhef and Schlotterer s Studies showed that the types of insertions and Deletion mutations result from adding or removing one or more nucleotides to a DNA tape that may not affect In the translation of protein function (16).

Table (4): Changes in nitrogen bases and their effect on the translation of *fimH* gene for the isolates A20 and A23 of *A. baumannii*

Nitrogenous base	Changes in nitrogen bases	Position	Subject	Amino acid	Changes in amino acid
Thymine	Adenine	88	4549236	Valine	Valine
Thymine	Cytosine	205	4549353	Serine	Serine
Thymine	Cytosine	237	4549384	Threonine	Threonine

The *fim H* gene has been registered at National Center for Biotechnology Information (NCBI) at the following link:- https://www.ncbi.nlm.nih.gov/nuccore/LC338015

Conclusions

The results of the investigation of *fimH* in *Acinetobacter baumannii* showed that *A. baumannii* had a *fimH* gene 47.5% of isolates were studied. Sequencing analysis of *fimH* gene showed mutant mutations that did not affect protein translation and other mutations that led to a change in amino acid order and protein translation.

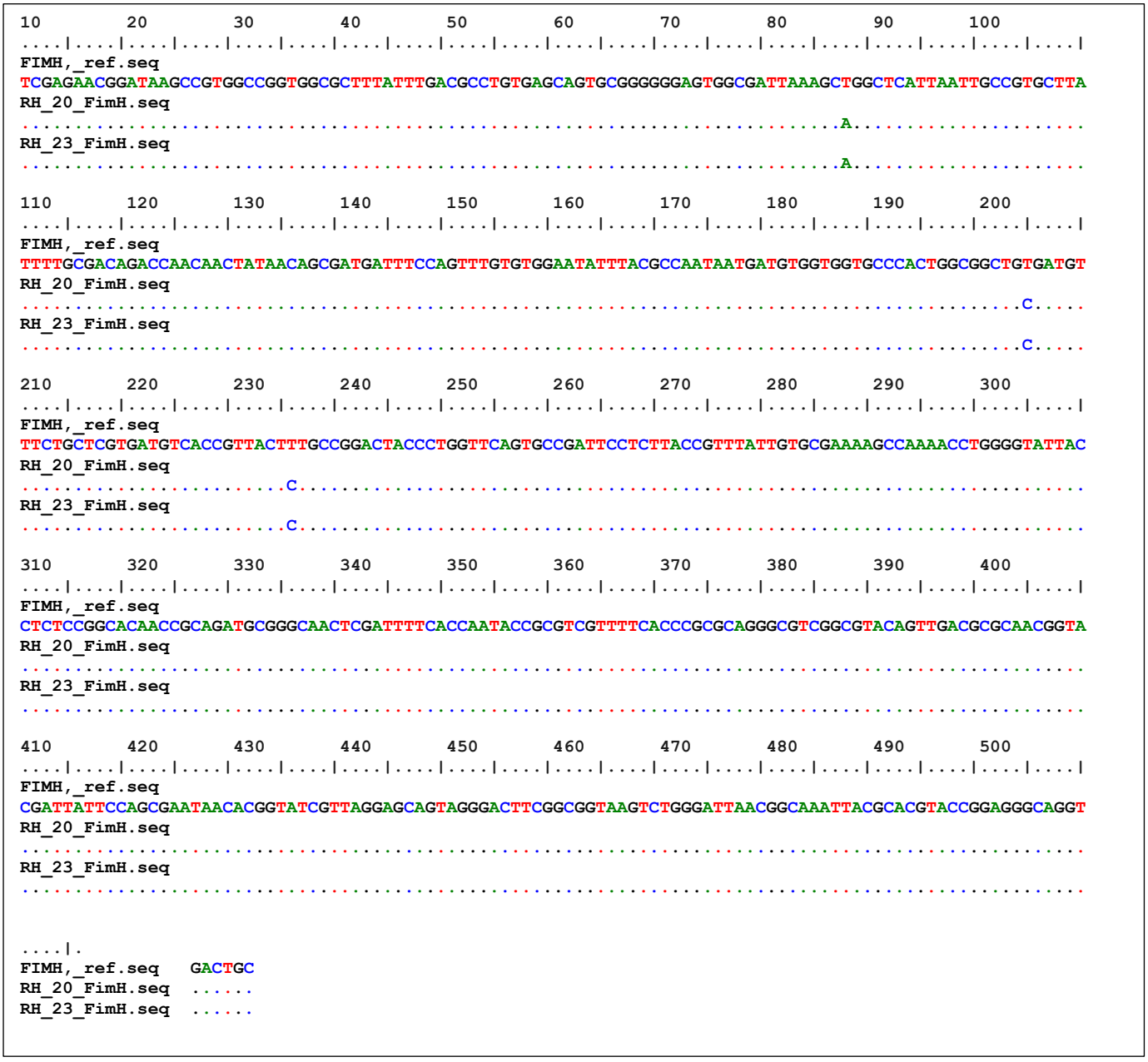

Figure (2): Analysis of multiple gene sequences of *fimH* reference with two changes to A20 and A23 isolates of *A. baumannii* using the BioEdit Sequence Alignment Editor Software

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