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MOLECULAR DETECTION OF *RPOB* GENE FROM *ACINETOBACTER BAUMANNII* ISOLATED FROM DIFFERENT CLINICAL SOURCES

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ABSTRACT

This study carried out to detection of *rpoB* gene from *Acinetobacter baumannii* isolated from different clinical sources in Baghdad governorate. A total (200) clinical samples were collected from different clinical sources were (50) samples from urine, (50) samples from wound, (50) samples from sputum and (50) sample from otitis infections. After identification by routine methods and confirmation by using VITEK- 2 Compact system**,** the results showed found (20) isolates of *Acinetobacter baumannii* from (200) clinical samples in percentage (10%). Antibiogram of these isolates were carried out against five antibiotics and the result showed high multidrug resistant. DNA extraction of all isolates extracted by specific kit, then amplification of rpoB gene by specific primers take place and the results showed about 80% of these isolates contain *rpoB* gene.

KEYWORDS: *rpoB* gene, Molecular detection, clinical sources.

INTRODUCTION

Acinetobacter baumannii consider a non-fermentative Gram negative bacillus very important as an opportunistic nosocomial pathogenic bacteria. In recent studies, this bacteria has high clinical importance because its recurrent association with healthcare infections (healthcare - associated infections), most of which with capacity of it to the gene expression of different antimicrobial resistance mechanisms to the antibiotics.[1,2,3]

Classification of the genus *Acinetobacter* carry out by Bouvet and Grimont in 1986, who recorded 12 genospecies (DNA groups) according to techniques of DNA-DNA hybridization.

Recent application of molecular methods carry out to diagnosis about (33) named and unnamed species of *Acinetobacter* bacteria.[4] *Acinetobacter baumannii* caused different infections which reached a point of concern (critical infection) and has formed dangerous to populations in the world. It is cauased a wide spectrum of nosocomial infections like pneumonia, meningitis, urinary tract infections, bacterimia and wound infections.[5] This studies recorded infection with *Acinetobacter baumannii* to account for about 2 to 10% of infections in intensive care units.[6]

rpoB gene encoding the highly conserved (β- subunit) of the bacterial RNA polymerase, which consider as a suitable target to the identification of enteric bacteria. This gene is more distinguishable than the 16S ribosomal DNA (rDNA) gene. In recent studies, species diagnosis by using the developed molecular techniques are 16S rRNA and RNA polymerase β-subunit (rpoB) genes sequences for the description and characterization of *Acinetobacter* species.[7,8] So the aim of this study is molecular detection of *rpoB* gene from *Acinetobacter baumannii* isolated from different clinical sources in Baghdad governorate.

MATERIALS AND METHODS

Samples collection

This study included collecting (200) clinical samples from different clinical sources were (50) samples were collected from urine, (50) samples were collected from wound, (50) samples were collected from sputum and (50) sample were collected from otitis in some hospitals in Baghdad during the period from September into December 2016.

Isolation and identification

The samples were cultured onto MacConkey agar and incubated for 18-24 hrs at 37^{o}C. The isolates were non lactose fermenting were cultured onto CHROMagarTM medium and incubated for 18-24 at 37^{o}C, *Acinetobacter* appears as a red colonies after the incubation period. The isolates were tested by morphologic characteristics and standard biochemical tests according to MacFaddin, (2000).[9] Then confirmation of *Acinetobacter* spp. isolates was carried out by VITEK- 2 Compact system to identification *Acinetobacter* isolates to species level according to manufactures' instructions (Biomerieux/ France).

Antibiogram testing

The isolates were tested against 15 antibacterial agents and the results were compared with National Committee for Clinical Laboratory Standard (CLSI, 2014).[10]

DNA extraction and PCR assay

DNA of all isolates was extracted by wizard® genomic DNA purification kit (Promega, USA) according to manufactures' instructions. Amplification of the *rpoB* gene was performed with specific primer table (1).

Table (1): Sequence of oligonucleotides primers used for amplification of *rpoB* gene

Primer type	Sequence 5'→3'	Reference
Forward	GGCGAAATGGCDGARAACCAC	Laure *et al*[11]
Reverse	GARTCYTCGAAGTTGTAACC	

The cycling conditions were: Initial denaturation at 94ºC for 2 minutes and 30 cycles of denaturation at 94ºC for 30 second, annealing at 50ºC for 30 second, extension at 72ºC for 30 second and a final extension at72ºC for 5 minutes. A molecular marker (promega/ USA effective size range: 100 to 1500 bp) was used to assess PCR product size.

RESULTS AND DISCUSSION

Isolation and identification

collected (200) clinical samples from different clinical sources were (50) samples were collected from urine, (50) samples were collected from wound, (50) samples were collected from sputum and (50) sample were collected from otitis infections. After identification by routine methods and confirmation by using VITEK- 2 Compact system**,** the results showed found (20) isolates of *Acinetobacter baumannii* from (200) clinical samples in percentage (10%) as shown in table (2).

Table (2): Distribution of *Acinetobacter baumannii* isolates in clinical samples

Clinical samples	No. of samples	No. of isolates	Percentage %
Urine	50	11	22%
Wound	50	4	8%
Sputum	50	3	6%
otitis	50	2	4%

In locally study carried out by Adnan *et al*[12] the percentage of infection with this bacteria was (10.3%) in different clinical samples. Another locally study by Mosafer[13] isolated *Acinetobacter baumannii* from different clinical sources and the percentage of infection was (7%). The infection with *Acinetobacter* increased significantly and continuous in different region in worldwide because this bacterium an important nosocomial pathogens and has different virulence factors.[14]

Antibiogram test

The results showed that the isolates had very high rates of resistance against 5 antibiotics were Ceftriaxone 30 µg (85%), Meropenem 10µg (60%), Amikacin 30 µg (30%), Tetracycline 30 µg (85%), Levofloxacin 5 µg (90%), as shown in table (3).

Table (3): Antibiogram results of *Acinetobacter baumannii* isolated from clinical sources

LEV	MEM	TE	AK	CRO	No. of isolate
R	R	R	S	R	**1**
R	R	R	S	R	**2**
R	R	R	S	R	**3**
R	R	R	R	R	**4**
R	R	R	R	R	**5**
R	S	R	S	R	**6**
R	R	R	S	R	**7**
R	R	R	R	S	**8**
R	R	R	R	R	**9**
R	R	S	S	R	**10**
R	S	R	S	R	**11**
R	R	R	S	S	**12**
R	R	R	S	S	**13**
R	S	R	R	R	**14**
R	S	R	R	R	**15**
R	S	S	S	R	**16**
R	S	R	S	R	**17**
R	R	R	S	R	**18**
S	S	S	S	R	**19**
S	S	R	S	R	**20**

R: Resistant

S: Sensetive

(**CRO**)=Ceftriaxone 30 µg, (**MEM**)=Meropenem 10 µg, (**AK**)=Amikacin 30 µg, (**TE**)=Tetracycline 30 µg, (**LEV**)=Levofloxacin 5 µg,

Acinetobacter baumannii showed high resistance rates against cephalosporin, antibiotic combinations, aminoglycosides, nitrofurantoin and quinolones. There are different mechanisms of resistance this bacteria to antibacterial agents included it ability to develop multiple resistance mechanisms against several major antibiotic classes that mediated by mobile genetic elements like conjugative plasmids, integrons, insertion sequences and transposons.[15,16] Also production of enzymes like β-lactamases, alterations in cell-wall channels (porins) and efflux pumps. Extended spectrum cephalosporinase AmpC are predominantly in this bacteria, which is act typically hydrolyze penicillins and narrow- and

extended-spectrum cephalosporins but not carbapenems[17,18] In study by Abd AL-Kareem[19] in Baghdad and Chaiwarith *et al* in India[20] the concluded high rate of resistance this bacteria to antibacterial agents.

These multidrug resistance may be achieved by horizontal transfer of genetic information and mutation of endogenous genes lead to increase resistance.[21] *Acinetobacter baumannii* contain an 86-kb resistance island, AbaR1, contains genes encoded to resistance as many as 25 antibiotic and 20 antiseptic and heavy metal.[22] This island variants are integrated at the same chromosomal locus in a significantly high proportion of multidrug resistance.[23] There are endogenous functions in this bacteria increased resistance like overexpression process of chromosomally encoded β-lactamases ADC and OXA-51-like; loss of porins CarO and Omp33–36 contributing to carbapenem resistance; mutation in the GyrA and ParC fluoroquinolone targets and overexpression of efflux systems.[24]

Mechanisms of efflux pump in this bacteria play a role in homeostasis of the cell and extrusion of toxic compounds and there are two RND efflux systems are AdeABC and AdeIJK.[25] It composed of an efflux protein (AdeB or AdeJ) that interacts with a membrane fusion protein (AdeA or AdeI) and an outer membrane factor (AdeC or AdeK) to facilitate drug export across both the inner and the outer membranes. *Acinetobacter baumannii* contain *adeABC* operon and its overexpression leads to increase resistance to aminoglycosides, cefepime, fluoroquinolones, chloramphenicol and tetracycline-tigecycline. So, the AdeABC efflux pump is a major mechanism very important of multiple drug resistance in this bacteria and its clinical significance has been established.[26,27]

Detection of *rpoB* gene

Detection of *rpoB* gene in all isolates carried out to investigate to the presence of *rpoB* gene. 16 isolates from 20 isolates (in percentage 80%) were positive to presence of this gene as shown in figure (1).

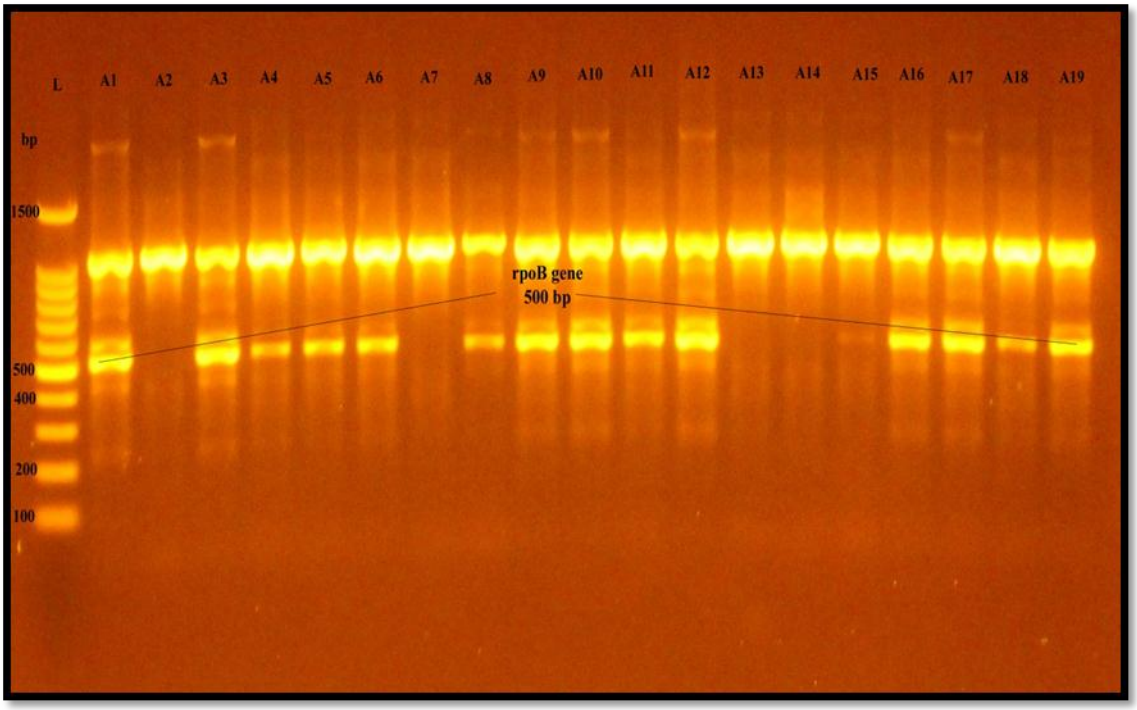

Figure (1): Gel electrophoresis for amplified *rpoB* gene on agarose gel (1%), 50V for 1 hour.

L: DNA ladder (1500 bp)

A1-A19: isolates of *Acinetobacter baumannii*

CONCLUSION

Acinetobacter baumannii isolated from clinical sources in Baghdad is highly resistance to the antibiotics in this study and about (80%) of this bacteria contain *rpoB* gene in product PCR size about 500 bp.

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