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Molecular study of *Acinetobacter baumannii* Isolated from skin infection

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Abstract :

Eight isolates of *Acinetobacter baumannii* were identified among one hundred fifty of Gram-negative bacteria grown on MacConkey agar that was isolated from different clinical skin specimens in Al-Najaf Al-Ashraf province hospitals. *A.baumannii* identification depends on morphological ,microscopic examination and biochemical tests and confirmed by Api20E .The results showed that 60% of all *A.baumannii* isolates produce biofilm and carry *csuE* gene while 40% of all *A.baumannii* isolates carry *ompA* gene .

The outcomes showed 70 % of *A.baumannii* isolates carry *bla*TEM gene and 40% of *A.baumannii* isolates carry *bla*SHV gene while none carry of *bla*NDM-1gene..The results of *bla*TEM, *bla*SHV genes become 77.7% and 44.4% respectively.

Introduction

Acinetobacter baumannii is an emerging opportunistic human pathogen responsible for a growing number of nosocomial infections mainly affecting patients who are immunosuppressed, who suffer other underlying diseases or who have been treated using certain invasive procedures and in Intensive Care Units (ICUs) patients (1,2). *A baumannii* represent the most clinically important and frequently detected *Acinetobacter* species (3).

During the last two decades, increase clinical importance of *A. baumannii* isolates due to its remarkable ability to cause outbreaks of infections and to acquire resistance to almost all currently used antibiotics, including the carbapenems (4). The ability of *A. baumannii* to adhere to and persist on surfaces as biofilms could be central to its pathogenicity. The production of pili and a biofilm-associated protein and the expression of antibiotic resistance are needed for robust biofilm formation on abiotic and biotic surfaces. This multistep process also depends on the expression of transcriptional regulatory functions, some of which could sense nutrients available to cells. (5) .Outer membrane proteins have been shown

to contribute to the virulence of *A. baumannii*. OmpA was implicated to facilitate adhesion (6).

A. baumannii was produced ESBLs(extended spectrum β-lactamases) that mediate resistance to extended spectrum cephalosporins (ESCs) (7). NDM , IMP and VIM genes responsible for MBLs production are horizontally transferable via plasmids and can rapidly spread to other bacteria. it may be chromosomally or plasmid mediated (8,10).

Methodology :

Samples Collection and Processing

Clinical samples including ; burn, wounds swab and blood were collected from patients attending to four hospitals in AL- Najaf Government include:, Al-Hakeem General Hospital and Al-Sadder Medical City ,.All samples were cultured on the selective and differential media and incubated at 37°C under aerobic condition for 18 - 24 hours. All media presented were prepared according to the manufacturing company instructions.

Bacterial Diagnostics

Primary diagnosis based on morphological characteristic of the colonies including colony shape ,texture ,color and edges were studied depending on bacterial growth on MacCokey agar and blood agar(incubated for 24 hr. at 37 C°)(18).

One isolated colony was transported to a microscopic slide,fixed well and stained with Gram stain .Gram reaction ,cell shape and arrangement were observed (11). All the biochemical tests were carried out according to (12)then Acinetobacter baumannii were detected by dex (API) 20E system (13**).**

Detection for Extended spectrum- β -lactamase(ESBLs) by CDT

Each isolate that showed resistance to third generation of cephalosporins was subjected to disc potentiating test to investigate the production of ESBLs, the results interpreted according to (14).

PCR Assay

In this study PCR assay was performed to detect the(*csuE,ompA)* to detection some of virulence factors in *A. baumannii* and to detect some of β-Lactam resistance gene*(bla*TEM,*bla*SHV,*bla*NDM*)* By using monoplex PCR technique . primers used in this study as in table 1.

Table (1): primers used in this study

Primer Type	Target Gene	Primer Sequence (5'-3')	Amplicon size (bp)	Reference
OmpA	*OmpA*	**F:** CAATTGTTATCTCTGGAG **R:** ACCTTGAGTAGACAAACGA	966	*15*
CsuE	*CsuE*	**F:** ATGCATGTTCTCTGGACTGATGTTGAC **R:** CGACTTGTACCGTGACCGTATCTTGATAAG	976	*16*
NDM	*bla*NDM-1	**F:** ACC GCC TGG ACC GAT GAC CA **R:** GCC AAA GTT GGG CGC GGT TG	264	17
SHV	*bla*SHV	**F:** GGTTATGCGTTATATTCGCC **R:** GGTTAGCGTTGCCAGTGCTC	867	18
TEM	*bla*TEM	**F:** TGCAACAGTGCCTCTCGATA **R:** CTCGTGCACCCAACTGATCT	717	19

Results:

Identification of bacterial isolates

The bacterial isolates obtained from clinical specimens were identified initially according to cultural morphology, microscopic characteristics and biochemical tests. From those isolates, the cultural identification of *A. baumannii* . On blood agar *Acinetobacter baumannii* colonies have appeared white/cream colored, smooth, circular with entire edges ,while on MacConky agar, a pinkish tint due to non-lactose fermentation, no pigmentation, small in size, with regular edges and nutrient agar appears white/ cream (Figure 1). Microscopically, *A. baumannii* shows as Gram negative coccobacilli arranged in single or aggregates.

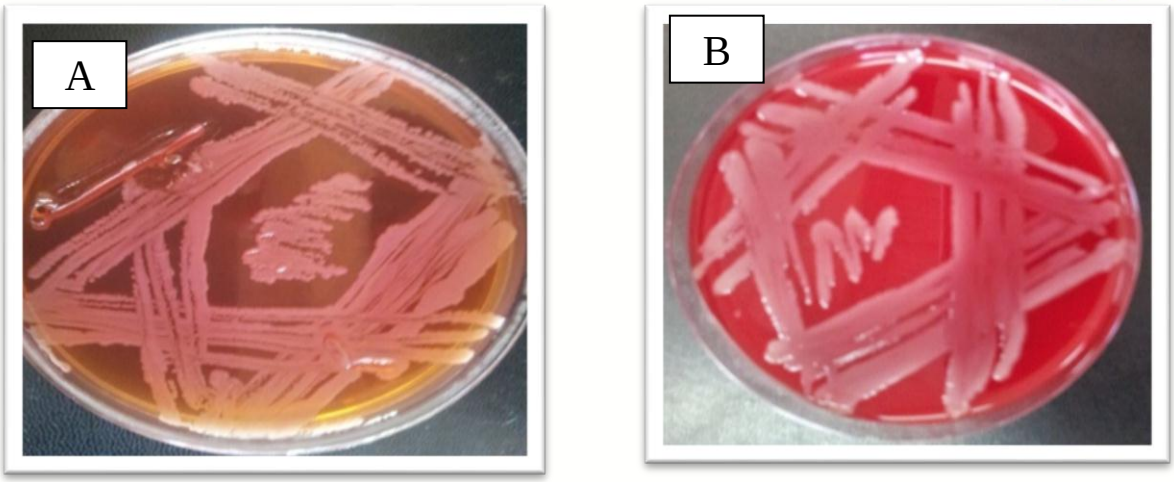


Figure (1): The morphology and microscopic form of *Acinetobacter baumannii* on(A)blood agar (B) MacConky agar.

Eight isolates of *A. baumannii* were obtained from 150 isolated from various clinical samples and distribution as in table (2).

Table (2): Sources & distribution of *A. baumannii* according to Various clinical specimen.

specimen Type	specimens No.	No. *A. baumannii*	Percentage of *A baumannii.*
Wound	68	4	50%
Burn	51	3	40%
Blood	31	1	10
Total	150	8	100%

The results of biochemical tests were considered as a complementary of the initial identification of *A. baumannii* isolates. All isolates showed general characteristics, that were oxidase, indole ,methyl red and Vogus-Proskuar and urease and gelatin production give negative result while catalase ,citrate are positive They are non-motile and lactose non-ferment . On triple sugar iron alkaline/alkaline red color without gas and not producing of H2S,none- Hemolysis. All isolates were (8) gave positive when confirmed by Api-20E system.

A. baumannii Isolates also identified by discoloration of blood agar containing D-glucose, in which all isolates of *A. baumannii* gave a positive result to this test by produced a unique light-brown discoloration of the surrounding blood agar (browning effect), while *Pseudomonas aeruginosa* did not cause similar discoloration (the browning effect was not observed) as it depicted in figure (2).

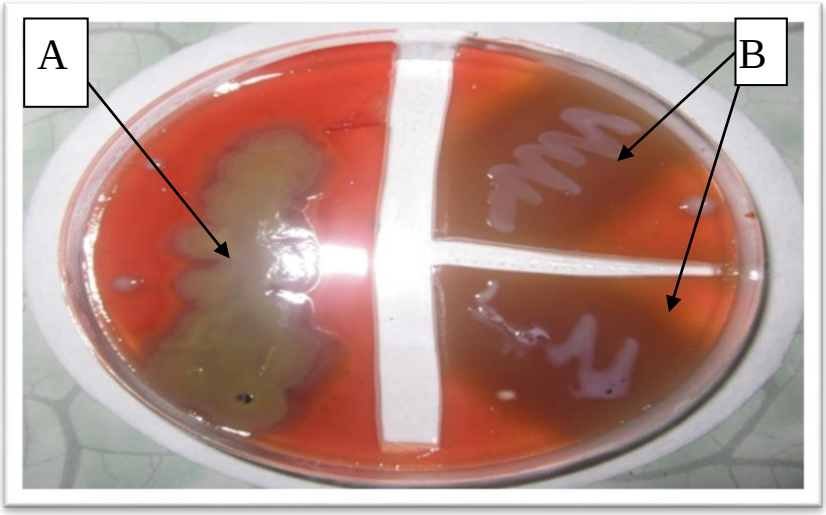


Figure (2): Blood agar containing D-glucose after 24 hours of incubation at 37ºC, in which shown A- *Pseudomonas aeruginosa* B- *Acinetobacter baumannii*.

Detection of Extended Spectrum β - lactamases production

The result showed 9 isolates of *A. baumannii* (90%) give the positive ESBLs enzymes by CDT method. Figure (3) shows increase in inhibition zone which surround the disc (5mm or more) contain clavulanic acid comparable to without it. The percentage of ESBLs were 90% among all *A. baumannii* isolates

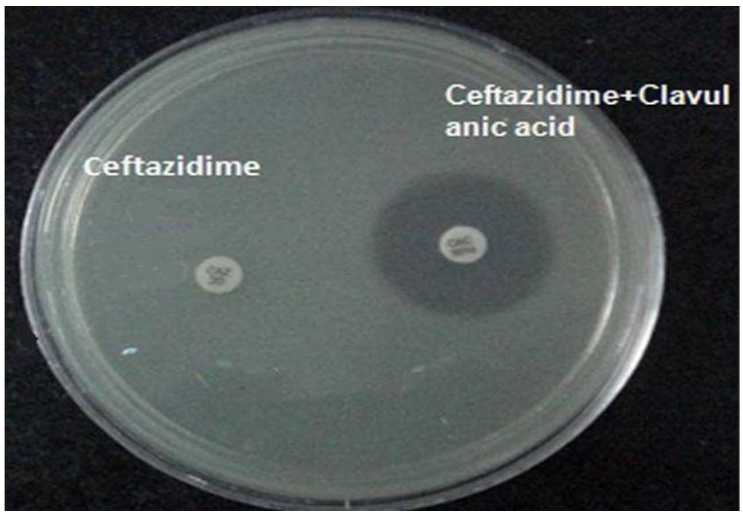
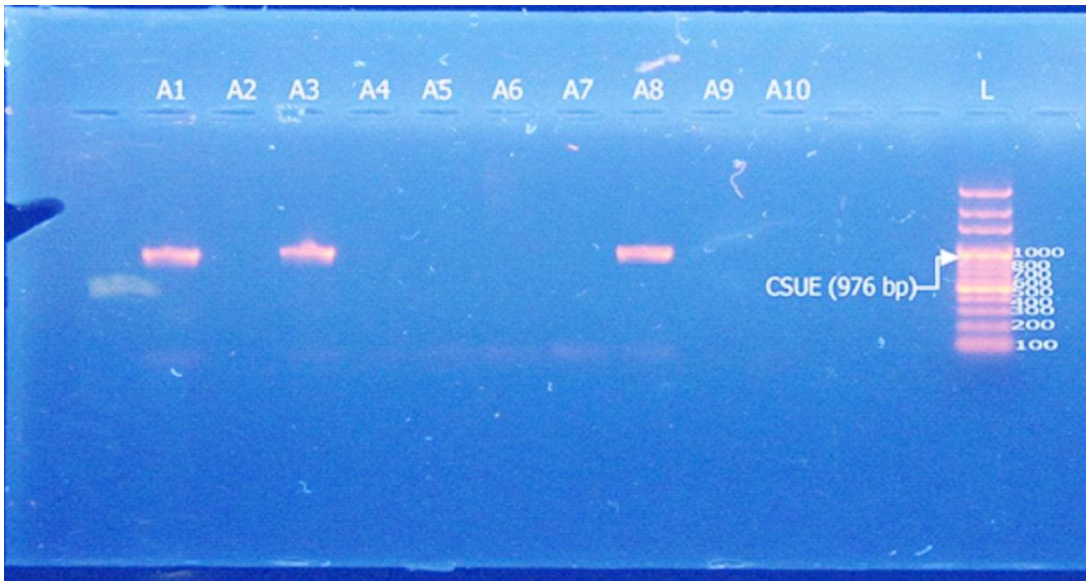


Figure 3:Positive Phenotypic confirmatory test (CDT) for detection of ESBLs , Muller – Hinton agar plate showing an isolate resistant to ceftazidime (CAZ) (30µg) and along with an increase zone of inhibition around ceftazidime -clavulanic acid(A1)

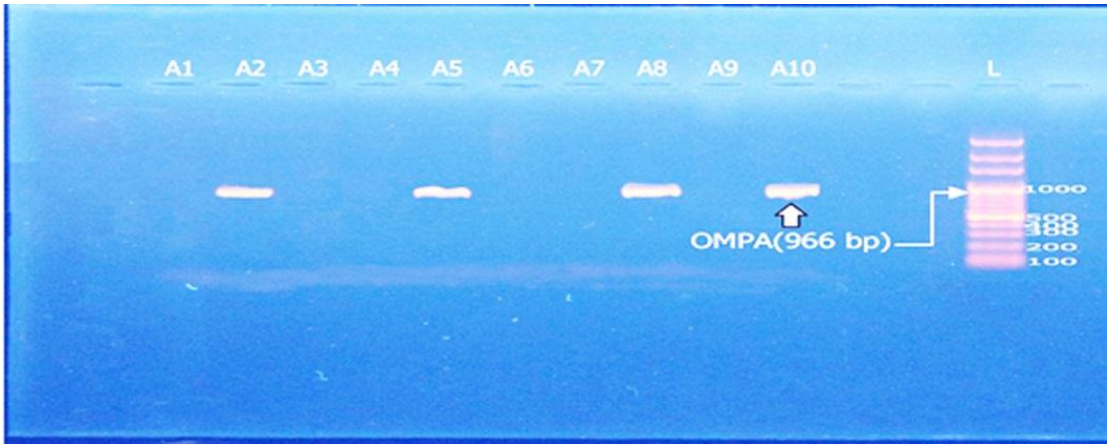
Molecular detection of *csuE* and *ompA* genes producing isolates.

All *A. baumannii* isolates were investigated to determine the occurrence of *csuE* **and *ompA* genes** that responsible on biofilm

formation and adhesion ability respectively. The result manifested is that out of the 8 of *A. baumannii* isolates ,only 3 isolates showed presence of this gene as in figure (4) that represent 30% of total number of isolates . The result manifested from 8 of *A. baumannii* isolates, only 4 isolates showed the positive result for this gene as in figure(5) that represent 40% of total number of isolates .



figure(4): PCR amplification products of *Acinetobacter baumannii* isolates that amplified with *csuE* primers with product 976 bp . Lane (L), DNA molecular size marker (2000-bp ladder). Lanes (1-10) isolates number. Lanes 1,3,8,give positive result to *csuE* gene while lanes 2,4,5,6,7,9,10,give negative result.



figure(5): PCR amplification products of *Acinetobacter baumannii* isolates that amplified with *ompA* primers with product 966 bp . Lane (L), DNA molecular size marker (2000-bp ladder). Lanes (1-10) isolates number.lanes 2,5,8,10, give positive result to *ompA* gene while lanes 1,3,4,6,7,9, give negative result.

Molecular detection of both (*bla*SHV,*bla*TEM ,*bla*NDM-1) producing isolates.

A. baumannii isolates were furthers exanimated to determine the occurrence of (*bla*SHV, *bla*TEM, blaNDM-1) genes responsible on some antibiotic resistance for B-lactam antibiotic. The result of *bla*SHV manifested from 8 *A. baumannii* isolates, only 4 isolates showed the positive result for *bla*SHV gene as in figure(6) that represent 40% of total number of isolates while represent 44.4% of 9 isolates that have ESBLs and the result of *bla*TEM manifested from 8 of *A. baumannii* isolates, only 7 isolates showed the positive result for *blaTEM* gene as in figure(7) that represent 70% of total number of isolates while represent 77.7% of 9 isolates that have ESBLs While *blaNDM* not show any result and are negative in all isolates. The results showed that the genetic detection of antibiotic resistance genes in *A. baumannii* were the A1,A3 and A5 contain both *bla*TEM and *bla*SHV genes while other isolates contain either *bla*TEM or *bla*SHV. Regarded to *bla*NDM-1 the results showed all isolates give negative results.

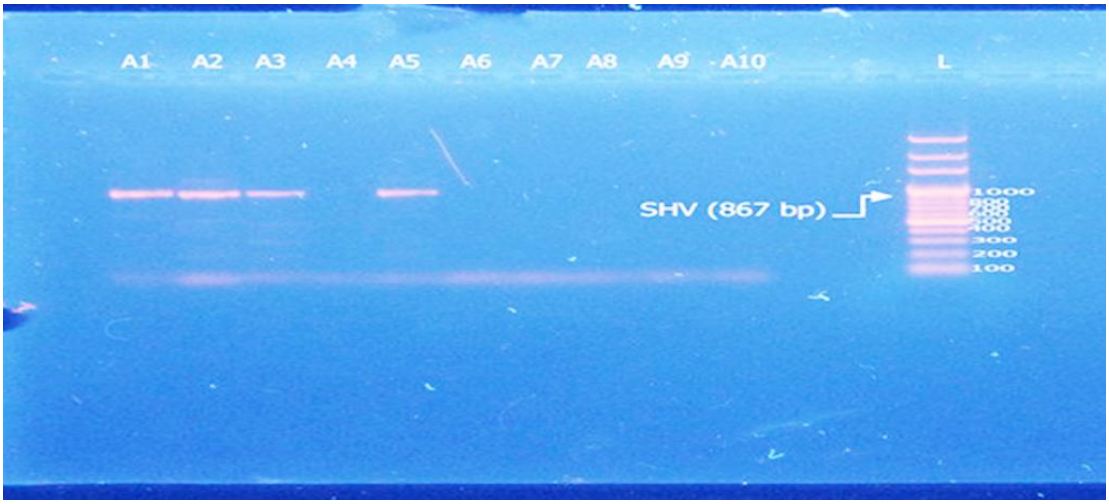


Figure (6): PCR amplification products of *Acinetobacter baumannii* isolates that amplified with *blaSHV*primers with product 867 bp . Lane (L), DNA molecular size marker (2000-bp ladder). Lanes (1-10) isolates number. Positive result showed in isolates A1,A2,A3,A5 while negative result in A4,A6,A7,A8,A9,A10.

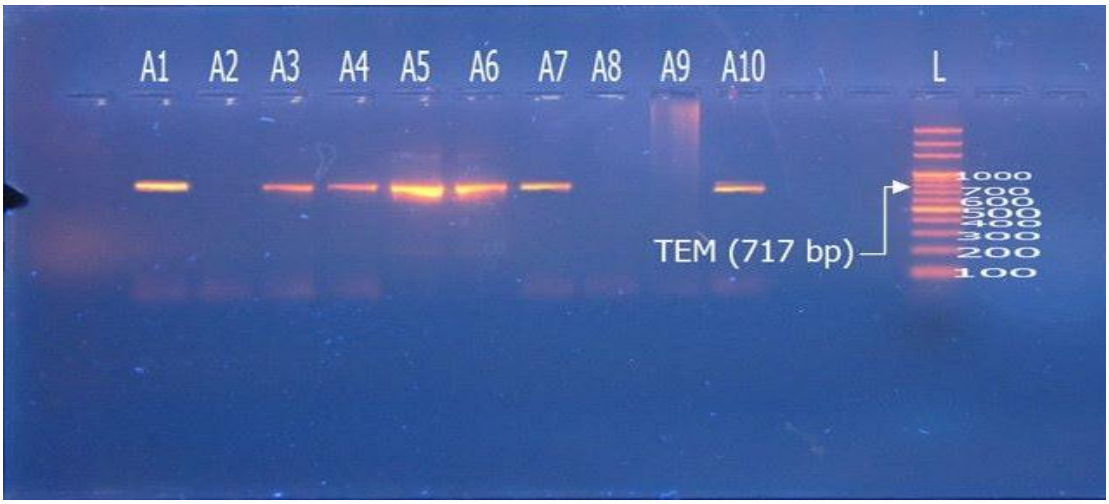


Figure (7): PCR amplification products of *Acinetobacter baumannii* isolates that amplified with *blaTEM* primers. Lanes (1-10) isolates number. Positive result in A1,A3,A4,A5,A6,A7,A10 while negative result in A2,A8,A9.

Discussion:

Isolation and identification

Acinetobacter baumannii has emerged as a medically important pathogen because of the increasing number of infections produced by this organism over the preceding three decades and the global spread of strains with resistance to multiple antibiotic classes (20). The morphological result of *A. baumannii* is similar (21,22)

Distribution of *Acinetobacter baumannii*

The results of distribution of *A. baumannii* among various clinical specimens in this study showed the higher percentage of *A. baumannii* obtained from wound specimen (40%) and lower percentage obtained from blood specimens (10%) . this may be due to *A.baumannii* which is a nosocomial pathogen, make it more spread in wound because of disregarding cleaning the hands staff and beds and surgical tools in hospital and also opportunistic pathogen , that mean it utilizes any impair in natural defenses or immunity of the body , wounds cause by surgical operation more account due to disregard of hygiene and sterilization(23) .

Encoding genes of some virulence factors in *A. baumannii*

The detection both (*csuE,ompA*) in *A. baumannii* that responsible on biofilm formation and adhesion respectively .*csuE.* which is one part of usher-chaperone pili assembly system that consist of six genes are *csuA/BABCDE* this system is required for the initial steps of bacterial

attachment on abiotic surfaces, resulting in microcolony formation followed by the full development of biofilm(24).

In present study showed that 3 isolates of 8 have the gene *csuE* that represent 30% of total number of *A. baumannii* and this gene responsible on biofilm formation . Goh,(25) showed that (97.7) of *A.baumannii* have the gene (*csu*) that is one of three chaperone-usher (CU) fimbrial gene clusters.

. Distribution of gene among the various clinical specimens where higher in wound and burn isolates . The results of present study showed (40%) of 10 *A. baumannii* isolates have *ompA* gene that responsible on adhesion activity and represent (44.4%) of 9 isolates that have adherence activity .

The distribution of *ompA* gene among various clinical sample in wounds reached to (35) while the highest percentage one isolates obtained of blood (100%).

The highest percentage of ompA gene in blood may be due to low number *A. baumannii* in blood that is one isolates only.

detection of some antibiotic resistance genes in *Acinetobacter baumannii*

By using polymerase chain reaction (PCR) technique to detection of some of extended spectrum Beta_lactamases gene include (blaSHV,blaTEM) and also to detect one of MBLs (blaNDM-1).

The present study showed (70%) of Acinetobacter baumanni had blaTEM gene when compare with the total number of isolates while (77.7%) when compare with 9 isolates that have phenotypically ESBLs. The result of blaSHV gene(40%) gene when compare with the total number of isolates while (44.4%) when compare with 9 isolates that have phenotypically ESBLs. not presence of blaNDM-1 gene on the genetic level .

Hujer *et al*. (26) found 40% of isolates of A.baumannii have blaTEM, while 1% of isolates have blaSHV. Study by Adams-Haduch *et al* . (27) were demonstrated that percentage of detecting about, TEM and SHV in studied A.baumannii isolates were 73.5% and 0% ,respectively.

This results of not presence of blaNDM-1 gene although they have 20% of MBLs in phenotypic detection these may be return to presence another type of MBLs such as IMP.

The difference in results of this study with another studies may be return to several factor such as number of isolates and the geographic site and time of collection and state of isolates.

In conclusions

The study revealed the prevalence of of each *csuE,ompA* among all *A. baumannii* isolates which can play role in biofilm formation and adhesion and high rate of ESBLs among all *A. baumannii* isolates and the high predominant of gene *bla*TEM and follow by *bla*SHV while low prevalence of MBLs and not presence of *bla*NDM-1gene.

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