# Correlation between the biofilm formation and the presence of extended spectrum β - lactamase (ESBL) *bla* PER-1 gene in multiple drug resistant clinical isolates of *Acinetobacter baumannii*

*Thesis*

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# List of Abbreviations

- A.baumannii*: Acinetobacter* baumannii
- AAC: N-acetyltransferases
- ABC: ATP binding cassette
- ABEPI1: *A.baumannii* efflux pump inhibitor 1
- ADCs: *Acinetobacter*-derived cephalosporinases
- AFLP: amplified fragment length polymorphism
- AMEs: Aminoglycoside-Modifying Enzymes
- ANT: O-nucleotidyltransferases
- APH: O-phosphotransferases
- API: analytical profile index
- ARDRA: Amplified 16S rRNA gene restriction analysis
- BAP: biofilm-associated protein
- CarO: Carbepenem reasistance associated outer membrane protein
- CAUTI: Catheter-associated urinary tract infection
- CMS: Colistin sulphomethate sodium
- CRBSIs: Catheter-related bloodstream infections
- CVCs: Central venous catheters

- DDST: double disc synergy test
- EPS: Extracellular polymeric substances
- ESBLs: Extended-spectrum beta-lactamases
- ICUs: Intensive Care Units
- IS: Insertion sequence
- MATE: Multidrug and toxic compound extrusion,
- MDR: Multidrug resistant
- MFS: Major facilitator superfamily,
- NNIS: National Nosocomial Surveillance System
- OmpA: outer membrane protein A
- OMVs: Outer membrane vesicles
- PBPs: Penicillin-binding proteins
- PDR: Pan drug-resistant
- QS: Quorum sensing
- RND: Resistance–nodulation–cell division
- SEM: Scanning electron microscopy
- SMR: small multidrug resistance
- TSI: triple sugar iron
- UTI: Urinary tract infections
- VAP: Ventilator-associated pneumonia
- XDR extensively drug-resistant

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## Abstract

**Background:** *Acinetobacter baumannii* (*A. baumannii*) is responsible for the majority of hospital acquired infections. Among *A. baumannii* different virulence factors, the most important one is the ability to produce biofilm which is related to their high degree of antibiotic resistance and their survival in hospital environment .

**Objectives:** This study aimed to examine the ability of different *A. baumannii* isolates to produce biofilm and correlate the biofilm formation with multidrug resistance and the presence of *bla*PER-1gene.

**Methodology:** A total of 30 isolates of *A. baumannii* were subjected to susceptibility testing by disc diffusion method for 10 clinically relevant antibiotics followed by phenotypic detection of ESBL production by double disc synergy test (DDST) .MIC for imipenem was performed by Etest.Screening for biofilm formation was done by microtitre plate assay . The presence of *bla*PER-1 was investigated by PCR.

**Results:** *: A. baumannii* isolates showed high rate of resistance to the tested antimicrobials and 97.6% were ESBL producers . Sixteen isolates (53.3%) were biofilm producers .There was no significant relation between biofilm formation and MDR. *Bla* PER1 gene was detected in fifteen (50%) isolates but showed non significant correlation with biofilm formation and ESBL production.

**Conclusion:** This study demonstrates the propensity of *A. baumannii* isolates for biofilm formation and multiple drug resistance. There is no significant association between biofilm formation ,MDR and *bla*PER-1 gene .


**Keywords**: *A. Baumannii*; ESBL; biofilm; *bla*PER-1; MDR

# Introduction

The genus Acinetobacter includes a group of bacteria that are non-motile, Gram-negative coccobacilli, displaying strict aerobic metabolism. *Acinetobacter baumannii* (*A. baumannii*) is the main species related to outbreaks of healthcare-associated infections **(*Van Looveren and Goossens, 2004)***. They ranked the second after Pseudomonas aeruginosa among the healthcare-associated infections ***(Dheepa et al., 2011).*** They are found in diverse environments such as soil, water, food products and are often isolated from medical devices.

*A.baumannii* are now recognized as the causative agents of the most difficult healthcare-associated infections to control and to treat ***(Tabassum, 2007).***

A large number of reports describe the outbreaks of Acinetobacter-healthcare associated infections such as secondary meningitis, pneumonia, wound, burn and urinary tract infections (UTI) ***(Patwardhan et al., 2008).*** Mortality in patients suffering from *A. baumannii* infections can be as high as 75% ***(Dijkshoorn et al., 2007).***

Epidemic strains of *A. baumannii* are noted for both intrinsic resistance to antibiotics and their abilities to

acquire genes, encoding resistance determinants. Main mechanisms of resistance to β-Lactams and aminoglycosides are through the production of β-lactamases and aminoglycoside-modifying enzymes. Moreover, diminished expression of outer membrane proteins, mutations in topoisomerase, up-regulation of efflux pumps in addition to biofilm formation and regulation by quorum sensing (QS) play important roles in antibiotic resistance ***(Kazemi Pour et al., 2011).***

Biofilm formation is an important feature of most clinical isolates of Acinetobacter spp. ***(Donlan, 2002)***. Biofilms are highly structured communities of bacteria enclosed to the surfaces that are attached in an extracellular polymeric matrix, exhibiting a modified phenotype compared with corresponding planktonic cells, especially in gene transcription, as well as interaction with each other. This structure is identified as a common cause of human infection ***(Stoodley et al., 2002)***.

Bacterial biofilms have been found on the surface of different instruments such as intubation tubes, catheters and artificial heart valves in addition of water pipe lines and cleaning instruments ***(Donlan and Costerton, 2002).***

The surfaces are usual targets of complex microbial communities ***(Heydorn, 2000).***

According to the epidemiologic studies, Acinetobacter biofilms play a role in infectious diseases such as cystic fibrosis, periodontitis, bloodstream infection and urinary tracts infection because of their ability to indwell medical devices ***(Abdi Ali et al ., 2014).***

Acquisition of the ability to form biofilm could be a good strategy to enhance a microorganism's survival under stressed conditions (e.g., during host invasion or following antibiotic treatment). This is because cells growing in biofilms are highly resistant to numerous types of antimicrobial agents . In addition, the ability of horizontal gene transfer of bacterial cells is enhanced within biofilm communities, thereby facilitating the spread of antibiotic resistance ***(Nahar, 2013)***. The high colonizing capacity of *A. baumannii*, combined with its resistance to multiple drugs and transfer of resistance elements are increasing the trouble and creating additional difficulties in treating infections caused by these organisms and contributing to the organism's survival and further dissemination in the hospital setting ***(Prashanth, 2012).***

# Aim of the Work

***This study aims to:***

- Examine biofilm formation by clinical isolates of *Acinetobacter baumannii (A. baumannii)* as well as their susceptibility to antimicrobials.
- Correlate biofilm formation with the development of multiple antibiotic resistance and ESBL production.
- Verify the existence of any association between biofilm formation and the presence of extended spectrum of β - lactamase (ESBL) *bla*PER-1 gene.

# Chapter (1)

# Acinetobacter Baumannii

*Acinetobacter baumannii* has emerged as one of the most troublesome pathogens for healthcare institutions globally. Its clinical significance, especially over the last 15 years, has been propelled by its remarkable ability to upregulate or acquire resistance determinants, making it one of the organisms threatening the current antibiotic era.

## MICROBIOLOGY:

### Current Taxonomy:

The genus Acinetobacter, as currently defined, comprises Gram-negative, strictly aerobic, non-fermenting, non-fastidious, non-motile, catalase-positive, oxidase-negative bacteria with a DNA G + C content of 39% to 47%. They are short, thick, Gram-negative rods that are difficult to destain and may therefore be misidentified as either Gram-negative or Gram-positive cocci ***(Peleg et al., 2008)***.

Four species of Acinetobacters (*A. calcoaceticus*, *A. baumannii*, *Acinetobacter* genomic species 3 and