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## Evaluation of IL<sub>6</sub> and TNF $\alpha$ in serum levels associated with *acinetobacter baumannii* infection in AL Diwaniya province patients

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### Summary

*Acinetobacter baumannii* is an important highly pathogenic microorganism with have a high levels of antibiotic resistance. and its extraordinary ability to develop additional resistance through the selection of chromosomal mutations and acquisition of resistance genes. In recent years, strains widely disseminated in hospitals throughout the world is being in the “critical” category of the WHO's priority pathogens list for research and development of new antibiotics.

which has caused a hike in nosocomial infection. The diminishing spectrum of treatment against these pathogens demands an alternative realm of treatment. One such nosocomial pathogen, *Acinetobacter baumannii* is known to cause pneumonia, blood stream infection, urinary tract infections, especially affecting immunocompromised individuals. Due to indiscriminate use of antibiotics, these pathogens have gained resistance to major classes of antibiotics through

mutation and horizontal gene transfer via mobile genetic elements such as plasmids, transposons and integrons.

The study conducted research on the identification and evaluation interleukin 6 and TNF  $\alpha$  of *Acinetobacter baumannii* isolates collected from patients hospitalized in government hospitals in Diwaniyah (General Diwaniyah, Women's and Children's, Al-Hussein A and Burns Hospital) in Al-Qadisiyah province, Iraq.

From Desember 2024 to January 2025, a total of 150 non-duplicate samples were collected, which included 21 (14%) wound swabs, and 36 (24%) burn swabs, 32 (21.33%) blood samples, 34 (22.66%) urine samples and 27 (18%) tracheal swabs were collected for analysis. After primary identification on MacConkey agar, Blood agar, and Chrome agar, the samples were biochemically tested and Gram stained. A VITEK2 compact system was used for diagnosis confirmation. This study demonstrated that 117 (78)% of the samples revealed bacterial growth of which 40 (34.18)% of *Acinetobacter baumannii* were confirmed to be distributed as 3 isolates (14.28%) from wound ,6 isolates (16.66%) from burn swabs , 13 isolates(40.62%) from blood, 6 isolates (17.64%) from urine samples and 12 isolates (44.44%) from trachea swabs, however, the data showed the highest percentage of *A. baumannii* were isolated from blood samples , trachea swabs followed by urin, bourn and followed by wound (44.44%),(40.62%),(17,64%),(16.66%) and (14.28%) respectively.

Blood samples were collected from all the subjects from whom clinical samples were taken, and 40 blood samples were taken from healthy subjects, where the plasma was separated from the blood, after that evaluation interleukin 6 and TNF  $\alpha$  of *Acinetobacter baumannii* isolates collected compare the interleukins with patients infected with *A. baumannii* and subjects infected with other

bacteria with normal levels.

The levels of interleukin 6 ( $20.34 \pm 1.41$ ) and tumor necrosis factor alfa ( $22.96 \pm 2.74$ ) were measured for Acetobacter-infected individuals was high level and compared with the normal levels for healthy individuals and *A. baumannii* non infected patients.

Key words: Al-Qadisyah hospital, ELASI, interlukin 6, TNF  $\alpha$ , *acinetobacter baumannii*

## Introduction

*Acinetobacter baumannii* has emerged as one of the most hostile members of the ESKAPE, is the acronym for the group of bacteria that include (Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa and Enterobacter species).<sup>[1]</sup> *Acinetobacter baumannii*, a Gram-negative bacterium, has emerged as a major threat to global public health. *A. baumannii* is one of the major human pathogens in healthcare-associated infections (both hospital- and community-acquired). The infection causes multifaceted clinical manifestations, including ventilator-associated pneumonia, catheter-associated blood and urinary tract infections, sepsis, meningitis, and skin and soft

Given the increasing difficulty of treating *A. baumannii* infections, the high mortality rates associated with the infection, and the paucity of antimicrobials in the development pipeline, there is an urgent need to develop new antimicrobials and alternative therapeutic strategies. However, progress and success have been limited to date in developing novel interventions against *A. baumannii*. Immunomodulatory therapies that stimulate the host's innate immunity have potential for use as stand-alone treatments or as immunoadjuvants for *A. baumannii* infections since many infected patients are immunocompromised prior to infection. However, the development of such therapies requires a better understanding of the host immune response to *A. baumannii*

the immune system's first line of defense, responding rapidly and nonspecifically to invading pathogens. It is activated when pathogen-associated molecular patterns (PAMPs), such as endotoxins and double-stranded viral RNA, bind to the pattern recognition receptors (PRRs) of

immune cells, causing them to secrete immune-regulating cytokines. These cytokines, such as IL-1, IL-6, IL-8, and TNF, are primarily secreted by immune cells that engulf bacteria, such as macrophages and dendritic cells. They mainly act on white blood cells, as well as on endothelial cells in blood vessels to promote an early inflammatory response.

TNF is the principal cytokine for regulating acute inflammation, though many of its functions are shared with other cytokines, especially IL-1. By binding to TNF receptors, TNF can perform functions including stimulating endothelial cells to induce coagulation, which obstructs blood flow to prevent the spread of microbes; stimulating endothelial cells and macrophages to secrete chemokines that attract white blood cells; stimulating the secretion of other cytokines such as IL-1; activating neutrophils and macrophages; stimulating the liver to produce acute phase proteins, such as C-reactive protein; inducing catabolism of muscles and fat to produce energy; and stimulating scar tissue formation, also known as fibrosis. In addition to inducing the secretion of cytokines, TNF itself can be induced by cytokines, enabling a cascade of inflammatory signals. Excessive amounts of TNF can cause septic shock<sup>[2]</sup>.

IL-6 is responsible for stimulating acute phase protein synthesis, as well as the production of neutrophils in the bone marrow. It supports the growth of B cells and is antagonistic to regulatory T cells.

## **Material and methods**

### **Patients**

A total of one hundred fifty different clinical samples were collected from visitors and hospitalized patients in governmental hospitals in Al-Qadisiyah province. (Al-Diwanyiah Teaching Hospital, Maternity and Children Teaching Hospital and Al-Diwanyiah Burns Center), during the period from September/2024 to January/2025. The clinical specimens were randomly collected from patients, and checked to recognize *Acinetobacter baumannii* isolates in this cross-sectional study. which included 21 (14%) wound swabs, and 36 (24%) burn swabs, 32 (21.33%) blood samples, 34 (22.66%) urine samples and 27 (18%) tracheal swabs were collected for analysis. These patients were males and females of different age groups,

ranging from 1 month to 80 years.

Blood samples were also taken from all patients in the focus of the current study, where the blood samples were divided into 3 groups as follows:

40 blood samples for people infected with *Acinetobacter baumannii*

40 blood samples for people infected with other bacteria

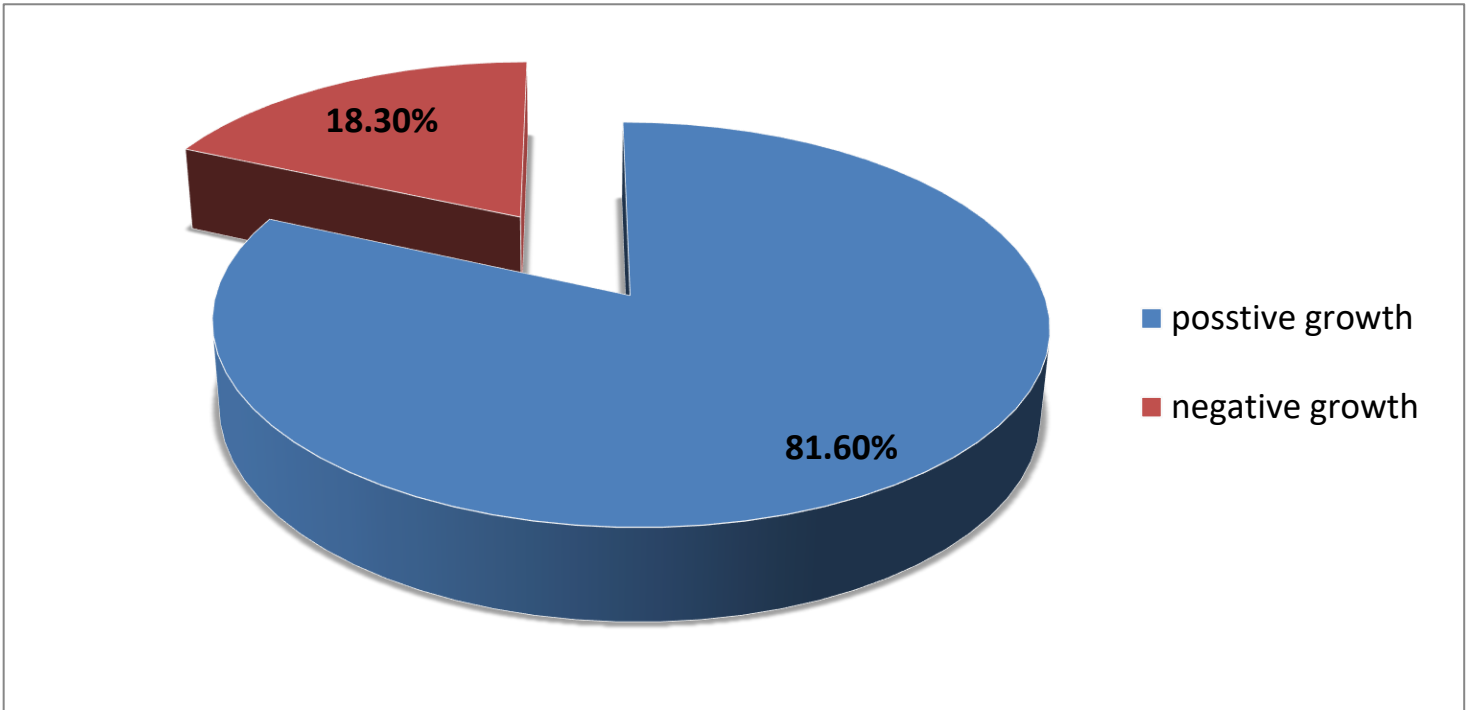
40 blood samples for healthy people

After that all blood samples are centrifuged to take serum for evaluation of IL -6 and TNF $\alpha$

### **Isolation of bacterial growth in clinical specimens**

After primary identification on MacConkey agar, Blood agar, and Chrome agar, the samples were biochemically tested and Gram stained. A VITEK2 compact system was used for diagnosis confirmation. This study demonstrated that 117 (78)% of the samples revealed bacterial growth of which 40 (34.18)% were confirmed to be *Acinetobacter baumannii* as identified by their biochemical analyses.

The results in the current study revealed that 147(81.6%) specimens had been given positive growth while 33(18.3%) specimens showed no growth as appeared in figure (1).



**Figure (1):- The percentages of clinical specimens**

Microbiological standard diagnostic criteria were used to isolate and identify the clinical isolates of *A.baumannii*, the primary identification of unknown bacterial isolates was made based on colonial morphology, appearance and pigmentation on differential and selective culture media (Blood agar, MacConkey agar, and Chromogenic agar).

The suspected colonies were initially Gram stained by preparing smear from well isolated colonies and examined under light microscope. According to Bergey's Manual of Determinative Bacteriology guidelines (Parte, 2012) and as described by Tille (2015), the isolates were further biochemically characterized and identified by several chemical tests.

All suspected isolates were confirmed by automated VITEK2 compact system using the GN

ID 222 card.

### **Ethics in Study Management**

The present study has been managed according to recommendations guide gained from the College of biology, University of Al-Qadisiyah. The task of collecting samples from hospitalized patients was facilitated according to an official administrative order (numbered as 30/4161 in 1/9/2024) issued by the College of biology , University of Al- Qadisiyah, and it was approved by the managers of visited hospitals. The study did not include forbidden biological materials or genetically modified organisms. All the *A.baumannii* isolates included in present study were obtained from hospitalized patients specimens without any additional substances.

### **Estimation of IL-6 level by ELISA**

An IL-6 ELISA kit (BT LAB, Bioassay Technology lab, China, Lot.NO: EOO75HU ) was used according to the manufacturer's instructions. The test was performed.

### **Results**

The clinical specimens were collected from patients distributed as 21(14%) wound swabs, 36(24%) bourn 32(21.33) blood,34 ( 22.66% ) urine sample, and 27(18%) trachea swab. these patients were males and females of different age groups, ranging from 1 month to 80 years including 24 males and 14 females.

of culturing on this highly selective media detected only 40 (34.18%) *A. baumannii* isolates.

The probable *A. baumannii* isolates were then put through a series of biochemical tests that performed to identify the bacteria, Table (1). These conventional biochemical tests were carried out, and the results were compared with the standard result documented by (MacFaddin's, 2000).

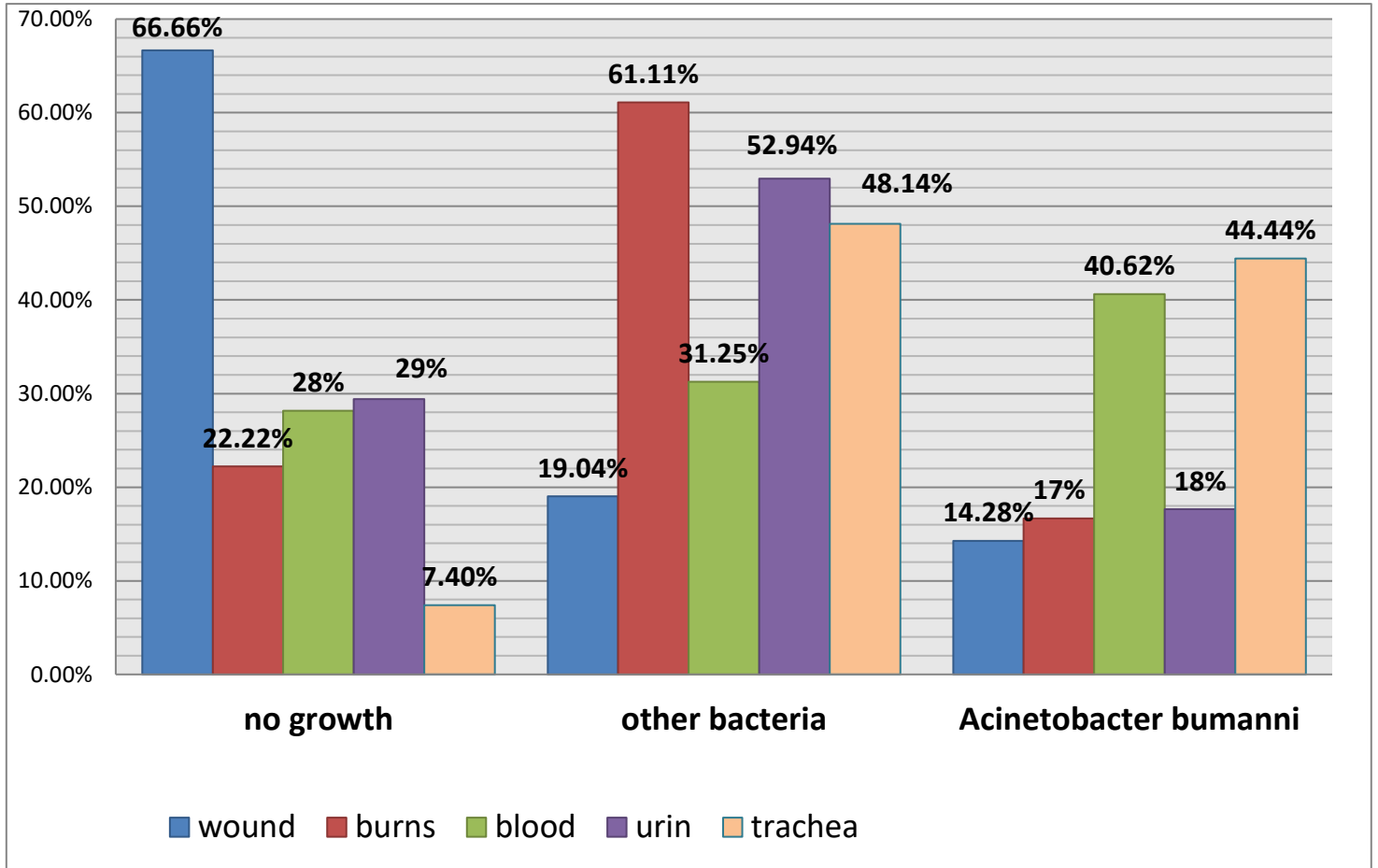
**Table (1):- Biochemical tests for *A. baumannii* isolates**

| No | Test                      | Result  |
|----|---------------------------|---|
| 1  | Catalase                  | Positive  |
| 2  | Oxidase                   | Negative  |
| 3  | Indole production         | Negative  |
| 4  | Methyl red                | Negative  |
| 5  | Kligler Iron Agar Test    | Alkaline slant / No change bottom,<br>No gas, No H <sub>2</sub> S |
| 6  | Voges-Proskauer (VP) Test | Negative  |
| 7  | Simmon Citrate            | Positive  |
| 8  | Urease production         | Negative  |

The confirmatory diagnosis of the suspected isolates was made by using the GN ID Card of the VITEK 2 compact system ,(about 99% accuracy).The results confirm that all 40 (34.18%) of the collected isolates were identified as *Acinetobacter baumannii* isolates .

According to the definite diagnosis of *A.baumannii* isolates , they distributed as showed in.

Fig. (2)



**Figure (2):- Distribution of bacterial growth according to source of samples**

The number and percentage of *A. baumannii* isolates recorded in Table (4-2) was 40 isolates (34.18%) distributed as 3 isolates (14.28%) from wound ,6 isolates (16.66%) from burn swabs , 13 isolates(40.62%) from blood, 6 isolates (17.64%) from urine samples and 12 isolates (44.44%) from trachea swabs, however, the data showed the highest percentage of *A. baumannii* were isolated from blood samples , trachea swabs followed by urin, bourn and followed by wound (44.44%),(40.62%),(17,64%),(16.66%) and (14.28%) respectively.

Human bacterial commensals and obligate pathogens have evolved features that facilitate their survival in a specific habitat. In many cases, these microbial factors are also determinants of virulence and dictate the spectrum of disease associated with the pathogen.<sup>[3]</sup> In contrast, incidental pathogens with environmental, non-human.

### Evaluation of IL-6 and TNF- $\alpha$ in patients with *A.baumannii* infection

The results show that mean serum level of IL-6 were highly increased in those who suffering from bacterial infection with *A. baumannii*  $91.43 \pm 9.69$  pg /ml , than non-infected Patient group ( $75.57 \pm 9.13$ pg/ml ) ,and serum levels of TNF-alpha in patients with *A. baumannii* are( $22.96 \pm 2.74$  pg/ml) rather than of patients without *A. baumannii* ( $18.94 \pm 3.34$  pg/ml), as compared with healthy control(  $5.64 \pm 1.07$ pg /ml ) for TLR-4 and ( $20.34 \pm 1.41$  for IL-8), with highr significant differences ( $p < 0.05$ ) . as indicated in **table (2)**

**Table (2 ):- IL-6 and TNF-alpha mean serum levels in patients with *A. baumannii* and**

**without *A. baumannii* infections**

| Subjects                                  | Cytokines ( Mean $\pm$ SE |                  |
|---|---------------------------|------------------|
|   | TNF- $\alpha$ pg./ml      | IL- 6 pg./ml     |
| <i>A. baumannii</i> infected patients     | $22.96 \pm 2.74$          | $91.43 \pm 9.69$ |
| <i>A. baumannii</i> non infected patients | $18.94 \pm 3.34$          | $75.57 \pm 9.13$ |

|                |              |            |              |
|----------------|--------------|------------|--------------|
| <b>Control</b> | <b>N(40)</b> | 5.64± 1.07 | 20.34 ± 1.41 |
|----------------|--------------|------------|--------------|

\*P ≤ 0.05, SE: Standard error.

## Discuss

When any pathogen enters the human body, this pathogen carries markers where the human immune system (innate immune system) recognizes these molecules. What identifies Gram-negative bacteria is Toll-Like Receptor 4 (TLR4) active bacteria and lipopolysaccharide (LPS). [4] Activation signals appear where immune cells are activated when LPS binds to TLR-4. As a result of this binding, immune cells secrete a number of pro-inflammatory cytokines, such as .TNF- $\alpha$ , IL-1, and IL-6

IL-6 is an important member of the cytokine family. It can stimulate the differentiation of T and B cells. It can also enhance the synthesis of acute phase response proteins by stimulating hepatocytes. In many cytokines, some are very similar in their biological activity. IL-6 functions in non-specific and specific immunity produced by mononuclear macrophages, endothelial cells, blood vessels, fibroblasts, and other cells in response to microbes and other cytokines. [5] Another study found that IL-6 levels are significantly increased in the early phase of inflammation, providing evidence for rapid diagnosis and differential diagnosis of early bacterial infections in the clinic. [6]

TNF- $\alpha$  plays a role in host defense against bacterial, viral, and parasitic infections. TNF- $\alpha$  is produced by macrophages and is activated by T lymphocytes, antigens, natural killer cells, and mast cells. [7] TNF- $\alpha$  is not normally detected in healthy individuals but is often found in serum

in conditions of inflammation and infection. TNF- $\alpha$  acts at low levels to induce acute inflammation by acting on leukocytes and endothelium, and TNF- $\alpha$  is a potent proinflammatory factor. [8]

Serum levels of cytokines IL6 and TNF- $\alpha$  were evaluated in patients infected with and associated with *Acinetobacter baumannii*, as well as 40 healthy controls

The IL-6 pathway is important in host defense against various bacterial pathogens. Most Gram-negative bacteria secrete outer membrane vesicles (OMVs), which are composed of outer membrane proteins and lipopolysaccharides. [9] These bacteria use these vesicles to secrete toxins into host cells, thereby stimulating pathogenic effects and altering host cell homeostasis. Myeloid and epithelial cells recognize pathogen-associated molecular markers (PAMPs) in the vesicles and transmit innate immune signals that secrete pro-inflammatory cytokines. [10] OMVs can also activate B and T lymphocytes, leading to adaptive immune responses. [11] The immune response to *A. baumannii* results in a very high secretion of pro-inflammatory cytokines and chemokines, and this innate immune response varies according to the nature of the individual and the nature and severity of the *A. baumannii* infection. [12] Given its importance in promoting granulocyte formation and stimulating the synthesis of antimicrobial chemokines, cytokines, and peptides such as GM-CSF, IL-6 (a neutrophil chemoattractant factor and human chemokine homologous to KC and MIP-2), and LL-37, IL-17 has emerged as a promising contender. [13] Furthermore, other chemokines, such as IL-8, promote neutrophil recruitment and activation at sites of infection; in addition, IL-17, together with IL-22, increases the expression of antimicrobial peptides, such as  $\beta$ -defensins, S100A8, and lipocalin 2, which have broad-spectrum antimicrobial activity. [9] A significant increase ( $P \leq 0.01$ ) in TNF- $\alpha$  levels was observed in the serum of patients infected with *A. baumannii*, and similar results were reported by Chen. [12] who reported that *A. baumannii* infection leads to an increase in the level of TNF- $\alpha$  in the serum of the patient due to this virulent infection. It represents a resistance mechanism for multidrug-resistant *A. baumannii*. This bacterium interacts with the host's innate pattern

recognition receptors, recruiting innate immune effector cells to the site of infection to effectively control the infection and induces a series of inflammatory cytokine and chemokine responses. TLR4 is a very important receptor for Gram-negative bacteria such as *A. baumannii* through which they recognize their hosts, by interacting with the lipid A portion of LPS, which is the main component of the *A. baumannii* cell wall. In turn, the activated TLR4 signaling pathway leads to an innate immune response in dendritic cells and macrophages, which includes activation of NF- $\kappa$ B and optimal production of interleukin (IL)-6, tumor necrosis factor (TNF) and IL-12, in addition to the death of *A. baumannii*.<sup>[14]</sup> Mast cells have been shown to promote bacterial clearance by releasing various mediators, including TNF- $\alpha$ , a chemoattractant for neutrophils.<sup>[15]</sup> *A. baumannii* adheres to mast cells via CD32 expressed on mast cells and induces TNF- $\alpha$ , leading to the development of inflammation and subsequent release of activated neutrophils.<sup>[12]</sup> Although these studies have shown that components of the *baumannii* cell membrane with mast cells influence the expression of pro-inflammatory cytokines and chemokines.<sup>[16]</sup> However, the interaction of *A. baumannii*LOS with mast cells remains unclear.

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