

RESEARCH ARTICLE

Identification of immunogenic proteins of the bacterium *Acinetobacter baumannii* using a proteomic approach

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Purpose: *Acinetobacter baumannii* is an important opportunistic pathogen that causes pneumoniae, urinary tract infections, and/or septicemia in immunocompromised patients. This pathogen is frequently associated with nosocomial outbreaks worldwide and has become particularly problematic because of its prevalence and resistance patterns to several antibiotics. In the present study, we used an immunoproteome-based approach to identify immunogenic proteins located on the surface of *A. baumannii* for the development of a possible immunotherapy against this devastating bacterial infection.

Experimental design: Sera from patients with *A. baumannii* infections ($n = 50$) and from a control group of healthy individuals ($n = 3$) were analyzed for reactivity against *A. baumannii* outer membrane proteins (OMPs) using Western blot analysis. To identify potential immunogenic proteins in *A. baumannii*, OMPs were separated by 2DE (2D electrophoresis), and reactive sera from infected patients were randomly selected and divided into two different pools, each containing 15 sera. Finally, MALDI-TOF/TOF mass spectrometric analysis was employed to identify the corresponding proteins.

Results: This analysis identified six immunoreactive proteins: OmpA, Omp34kDa, OprC, OprB-like, OXA-23, and ferric siderophore receptor protein. Notably, these proteins are highly abundant on the bacterial surface and involved in virulence, antibiotic resistance, and growth.

Conclusions and clinical relevance: Our results support the notion that the proteins identified in the present immunoproteome study could serve as antigen candidates for the development of vaccines and passive immunotherapies against *A. baumannii* infections.

Keywords:

Acinetobacter baumannii / Hospital infection / Immunoproteomics / MALDI-TOF/TOF



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1 Introduction

Acinetobacter baumannii is a Gram-negative, nonmotile coccobacillus that has been associated with outbreaks of nosocomial infections in hospitals all over the world. The main infection caused by this microorganism is nosocomial pneumonia, but *A. baumannii* has also been associated with bacteremia, urinary tract infections, meningitis, and skin and soft tissue infections [1]. The rapid emergence and global dissemination of *A. baumannii* as a major nosocomial pathogen is remarkable and demonstrates its successful adaptation to

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Abbreviations: ACTH, adrenocorticotrophic hormone; CBBG, CBB G-250; Fiocruz, Fundação Oswaldo Cruz; IROMP, iron-regulated outer membrane protein; MDR, multidrug-resistant; NCBI, National Center for Biotechnology Information; OMP, outer membrane protein

Colour Online: See the article online to view Fig. 1 in colour.

Clinical Relevance

Acinetobacter baumannii has been associated with outbreaks of nosocomial infections in hospitals all over the world. The main infection caused by this microorganism is nosocomial pneumonia, but *A. baumannii* has also been associated with bacteremia, urinary tract infections, meningitis, and skin and soft tissue infections. The rapid emergence and global dissemination of *A. baumannii* as a major nosocomial pathogen is remarkable and demonstrates the successful adaptation of *A. baumannii* to the hospital environment. Mortality rates associated with *A. baumannii* infection have been reported to be between 35 and 70% for nosocomial pneumonias and between 20 and 60% for bacteremic infections. Infections caused by *A. baumannii* have increased

dramatically and become more difficult to treat because of the emergence of highly antibiotic-resistant strains. These strains exhibit resistance to multiple antibiotic classes, and most problematic are recent descriptions of pan-resistant strains that are resistant to all antibiotics used in the clinic. There is particular concern regarding the development of carbapenem resistance, predominantly against imipenem and meropenem, the most important agents for the treatment of infections by MDR *A. baumannii*. For these reasons, it is necessary to identify new bacterial targets for the development of novel approaches to prevent and treat infections caused by this microorganism.

the 21st century hospital environment [2]. Mortality rates associated with *A. baumannii* infection have been reported to be between 35 and 70% for nosocomial pneumonias and between 20 and 60% for bacteremic infections [3]. Over the last three decades, the number of infections caused by *A. baumannii* has increased dramatically, and these infections have become more difficult to treat because of the emergence of highly antibiotic-resistant strains. These strains exhibit resistance to multiple antibiotic classes, and most problematic are recent descriptions of pan-resistant strains that are resistant to all clinically used antibiotics [4]. There is particular concern regarding the development of carbapenem resistance, mainly against imipenem and meropenem, which were introduced in 1985 and for years have been the most important agents for the treatment of infections by multidrug-resistant (MDR) *A. baumannii* [5]. For these reasons, it is necessary to identify new bacterial targets for the development of novel approaches to prevent and treat infections caused by this microorganism [4].

Immunization could represent a potentially effective strategy for preventing infections caused by *A. baumannii*; however, to date, there have been no vaccines developed for this pathogen [3]. The development of immunotherapy for the treatment of bacterial infections typically focuses on targets such as virulence and colonization factors located on the bacterial membrane surface. Among these factors are the outer membrane proteins (OMPs) that may act as potential targets for adhesion with other cells and binding of bactericidal compounds on the Gram-negative bacteria surface [6]. Moreover, OMPs are involved in antibiotic resistance, nutrient transport, cell–cell signaling, host cell attachment, and virulence in pathogenic strains [1]. The characterization of OMPs is necessary because these proteins are highly abundant and in direct contact with the host immune system,

making them good antigen candidates for the development of immunotherapy [7].

The immunoproteomic approach enables the identification of pathogen antigens expressed during human infection and is a simple and efficient method to identify immunogenic proteins [7]. Proteomics, the large-scale study of proteins in a cell, tissue, or entire organism, allows for the fine description and overall characterization of protein expression [8]. Soares et al. [9] identified 37 OMPs from *A. baumannii* by 2DE analysis, but immunogenic proteins were not detected. In the present study, the 2D gel technique was used for the characterization of immunogenic proteins, followed by tandem mass spectrometric analysis and data mining for protein identification. The purpose was to identify antigens by immunoblotting with sera from *A. baumannii* infected patients. This approach led to the identification of several OMPs from *A. baumannii* that might be future targets for vaccine development.

2 Materials and methods

2.1 Bacterial strain and growth conditions

A MDR clinical strain of *A. baumannii* was grown overnight in Luria–Bertani broth (LB) with 50 µg/mL ampicillin at 37°C under constant shaking. Overnight culture was diluted 1:100 v/v with fresh medium, and bacteria were grown to the exponential phase. Bacterial cells were pelleted by centrifugation at 10 000 × g for 10 min at 37°C. The supernatant was discarded, and the cell pellet was washed twice with 0.01 M PBS, pH 7.5. Washed cells were stored at –20°C.

2.2 Membrane protein extraction of *A. baumannii*

The OMPs of *A. baumannii* were extracted by a modified previously described method [10] using 4% Triton[®] X-114. Briefly, bacterial culture was pelleted by centrifugation at $10\,000 \times g$ for 10 min at 37°C. The supernatant was discarded, and the cell pellet was washed twice with 0.01 M PBS, pH 7.5. Then, 1 g of the pellet was suspended in 10 mL of ice-cold TBS (50 mM Tris-HCl, 150 mM NaCl, pH 8.0) containing 4% Triton[®] X-114. The mixture was stirred overnight at 4°C and then centrifuged at $10\,000 \times g$ for 30 min at 4°C. The supernatant was incubated at 37°C for 3 h to separate the aqueous and detergent phases. The detergent phase containing the OMPs was collected. Proteins were precipitated with cold 100% acetone for 3 h at 4°C. The samples were centrifuged at $10\,000 \times g$ for 15 min at 37°C, the supernatant was discarded, and acetone was evaporated at 37°C. The protein concentration in the extracts was determined with a Bio-Rad protein assay kit by a modified Folin–Lowry assay, as suggested by Peterson [11].

2.3 Human sera

Sera were collected from patients infected with *Acinetobacter* spp. ($n = 50$) in the period between October 2009 and December 2010 from five hospitals located in Rio de Janeiro, RJ. The identification of infected samples was performed using the automated method WalkAway Microscan (Dade Behring) or Vitek 2 (Biomérieux). All sera of patients infected with *Acinetobacter* spp. were analyzed individually by SDS-PAGE 12.5%, followed by immunoblotting. For immunoproteomic analysis, reactive sera were randomly selected and divided into two different pools, each containing 15 sera. A pool comprising three sera was obtained from healthy individuals and served as a negative control. The collected samples were properly recorded, and the data concerning the identity of patients were kept confidential. The current project was approved by the Ethics Committee in Research of the Evandro Chagas Research Institute (IPEC) at Fundação Oswaldo Cruz (Fiocruz; protocol number 0004.0.009.000–10).

2.4 1D gel electrophoresis and immunoblotting

The OMPs extracted from *A. baumannii* were analyzed by MiniProtean 3 (Bio-Rad) on 12.5% polyacrylamide gel in denaturing conditions according to Laemmli's method [12]. Sera from patients with *A. baumannii* infections ($n = 50$) and control group sera from healthy individuals ($n = 3$) were first individually analyzed for reactivity by Western blot against *A. baumannii* OMPs. Separated proteins were transferred from the 1DE gels to 0.45 mm nitrocellulose membranes (Bio-Rad), as described by Towbin et al. [13], for 90 min at 90 V. The membrane was incubated with each human serum overnight at a dilution of 1:200, followed by a secondary antibody [anti-human IgG HRP conjugate (Sigma-Aldrich[®]) at

a dilution of 1:15 000 v/v for 3 h. Proteins were detected using the color reagent Western Blue[®] Stabilized Substrate for Alkaline Phosphatase (Promega, USA).

2.5 2DE

The OMPs of *A. baumannii* were also analyzed by 2D electrophoresis. Extracts containing 60 μ g of proteins were solubilized in 7 M urea, 2 M thiourea, 4% w/v CHAPS, 1% v/v three to ten carrier ampholytes, 1% w/v bromophenol blue, and 60 mM DTT in a total volume of 125 μ L. For IEF, the IPG-phor III system was used (GE Healthcare) with 7 cm strips, pH 3–10 (GE Healthcare). IEF was performed at 30 V for 12 h, followed by 200 V for 1 h, 500 V for 1 h, 1000 V for 1 h, a gradient to 3500 V for 30 min and 18 000 Vh, all at 20°C and 50 μ A. After IEF separation, strips were equilibrated for 2×15 min in equilibration buffer containing 50 mM Tris-Cl, pH 8.8, 6 M urea, 30% v/v glycerol, 2% w/v SDS, and 0.002% w/v bromophenol blue. DTT at 1% w/v was added to the first equilibration step, and 2.5% w/v iodoacetamide was added to the second. IPG strips were then placed over a 12.5% resolving polyacrylamide gel, and electrophoresis was performed at 2.5 mA/gel for 30 min, after which the current was increased to 20 mA/gel for 1 h. To prepare the 2DE gels for analysis, CBB G-250 (CBBG) colloidal staining was performed according to Candiano et al. [14]. Protein profiles were analyzed with Image Scanner II software (Amersham Biosciences), and the gels were stored in 1% acetic acid.

2.6 Immunoblotting

Separated proteins were transferred from the 2DE gels to 0.45 mm nitrocellulose membranes (Bio-Rad), as described by Towbin et al. [13], for 90 min at 90 V. The membranes were blocked with 10% skim milk and 1% BSA in TBS (25 mM Tris-HCl pH 8.0; 0.15 mM NaCl) at room temperature for 2 h. Afterwards, the membranes were washed three times for 5 min. All washing steps were performed with TBS containing 0.05% Tween 20. Membranes were incubated overnight with two different pools, each containing 15 sera of patients infected with *A. baumannii* at a dilution of 1:200, followed by a secondary antibody [anti-human IgG HRP conjugate (Sigma-Aldrich[®]) at a dilution of 1:15 000 v/v for 3 h. Before and after the addition of the secondary antibody, the membranes were washed four times for 15 min in TBS containing 0.05% Tween-20. Proteins were detected using the color reagent Western Blue[®] Stabilized Substrate for Alkaline Phosphatase (Promega, USA). The reactions were terminated by the addition of distilled water.

2.7 Protein digestion, peptide extraction, and MS

Proteins spots were excised and treated for in-gel digestion. Gel pieces were washed once with 100% ACN and dried in

a vacuum centrifuge for 15 min. Then, the gels were incubated for 60 min at 4°C in 15 µL of 33 ng/µL trypsin (Promega, USA) diluted in 50 mM ammonium bicarbonate. Excess trypsin was removed, and the gels were incubated at 58°C for 30 min. The reactions were blocked with the addition of 1 µL of 5% v/v formic acid. Peptides were extracted with 30 µL of 5% v/v formic acid in 50% v/v ACN with sonication for 10 min. The extractions were performed twice to achieve a final volume of approximately 60 µL. Samples containing the peptides were concentrated in a speed vac to approximately 10 µL and finally desalted using a C₁₈RP ZipTip (Millipore, Bedford, USA) microcolumn.

The sample solutions (0.3 µL) were mixed with an equal amount of a saturated matrix solution (CHCA (Aldrich, Milwaukee, WI) 10 mg/mL in 50% ACN/0.1% TFA) on the target plate and allowed to dry at room temperature (dried-droplet method). Raw data for protein identification were acquired on an AB Sciex 5800 (AB Sciex, Foster City, CA) MALDI mass spectrometer (see Supporting Information for raw data). Up to 12 of the most intense ion signals with S/N above 2 were selected as precursors for MS/MS acquisition, and common trypsin autolysis and keratine masses were excluded. External calibration in MS mode was performed using a mixture of five standard peptides: des-Arg1-Bradykinin ($m/z = 904.468$), angiotensin I ($m/z = 1296.685$), Glu1-fibrinopeptide B ($m/z = 1570.677$), adrenocorticotrophic hormone (ACTH) (18–39 clip) ($m/z = 2465.199$), and ACTH (7–38 clip) ($m/z = 3657.929$). MS/MS spectra were externally calibrated using known y-ion masses (175.119, 684.346, 813.389, 1056.475, 1441.634) observed in the MS/MS spectrum of Glu1-fibrinopeptide B.

2.8 Database search

The tandem mass spectra were searched against all entries of NCBIInr (www.ncbi.nlm.nih.gov/index.html) using the Mascot software (www.matrixscience.com). The following search parameters were used: no restrictions on species of origin or protein molecular mass, tryptic cleavage products, two tryptic missed cleavages allowed, variable modifications of cysteine (carbamidomethylation), asparagine and glutamine (deamidation), methionine (oxidation), and pyroglutamate formation at the N-terminal glutamine of peptides.

3 Results and discussion

In the present study, OMPs of *A. baumannii* were extracted by a modified method using 4% Triton X-114 and resolved on a 1D gel. Individual sera of patients diagnosed with *A. baumannii* infection were used to identify OMPs that were recognized by the human immune system. Fifty sera of patients infected with *A. baumannii* were analyzed by immunoblotting with proteins resolved on 1D gels to determine the frequency of occurrence of the major immunogenic proteins in an individual serum. Examples could be seen in Fig. 1 and 1D

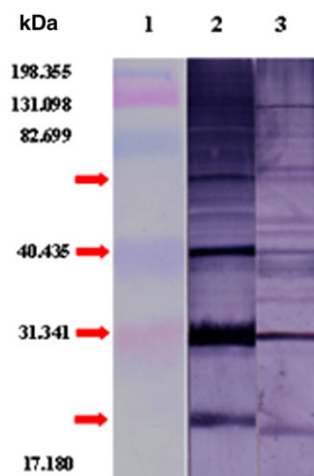


Figure 1. Representative Western blot of a 1D gel of *A. baumannii* OMPs probed with sera from patients infected with *A. baumannii*. Lane 1: standard molecular weight ladder; lanes 2 and 3: OMPs identified with serum from patients infected with *Acinetobacter* spp.

Table 1. Immunoreactive electrophoretic bands identified in 50 individual sera from *A. baumannii* infected patients

Molecular weight range of bands	Frequency (%)
38–40	88
26–27	64
62–64	62
32–33	54
71–73	52
42–44	50
28–29	48
30–31	42
45–47	42
22–23	42

Sera were collected from 50 individuals infected with *A. baumannii*, and immunogenic bands present in each serum were identified by immunoblotting with proteins resolved on 1D gels.

immunoblottings of all 50 patients are represented in Supporting Information Fig. 1. Healthy human sera were used as negative controls. We identified a protein with molecular weight in the range 38–40 kDa in 88% of the sera from infected patients with *Acinetobacter* spp., and other proteins at lower frequency were also detected (Table 1).

To better identify *A. baumannii* OMPs, reactive sera from infected patients were randomly selected and divided into two different pools, each containing 15 sera for immunoproteomic analyses. First, the OMPs of *A. baumannii* were resolved on a 2D CBBG colloidal stained gel to yield approximately 35 spots within the pI 3–10 range (Fig. 2), indicating that the 2DE analysis provided a representative view of the *A. baumannii* proteome (Supporting Information Table 1).

A total of 15 immunoreactive protein spots were observed from immunoblotting (Fig. 3A and B), and these spots matched with the protein spots observed in the preparative 2D

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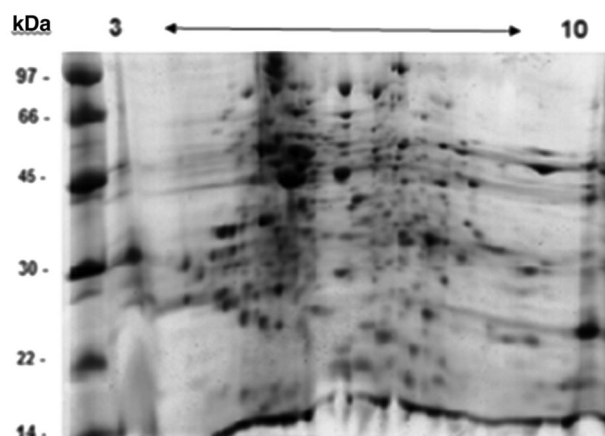


Figure 2. A 2D gel displaying *A. baumannii* OMPs proteins. Proteins identified by MALDI-TOF/TOF are indicated by spot number in Supporting Information Table 1. Preparative 2D gel of *A. baumannii* OMPs stained with CBB G-250 colloidal. Molecular size markers are indicated on the left in kDa.

gel (Fig. 2). When the blot was probed with sera obtained from healthy individuals, no immunoreactive protein spots were observed. Protein identification was conducted using MALDI-MS/MS mass spectrometric analysis of the digested proteins followed by a search against the nonredundant National Center for Biotechnology Information (NCBI) database.

Despite the large amount of proteins present in the outer membrane fraction, few were immunogenic. Some reasons can be noted:

- (i) These proteins are generally embedded in the outer membrane, and therefore, only the outermost portion is in

contact with the external environment. Thus, if only a few epitopes are present in the outermost region, it is normal to expect a lower immune response to these proteins in bacteria. In the 2DE, all proteins in the extracted membrane protein were represented, including proteins not exposed to the external environment.

- (ii) Some OMPs may be differentially expressed according to the environmental conditions [15], particularly during the infectious process, which is different from in vitro culture, for example.
- (iii) In the 1D IB, we observed a low number of bands in the sera of some patients. This result may be because of the immune status of the patient, who is potentially immunocompromised [16], therefore reflecting a lower response to the surface proteins of the bacterium.
- (iv) The low number of the identified immunogenic proteins may be because of the limitations of the methodology used. However, immunoproteome analyses have been employed in previous studies to identify immunogenic proteins from the sera of infected patients [7, 16].
- (v) Kurupati et al. [7], employing immunoproteome analysis from *Klebsiella pneumoniae*, detected 60 proteins spots by 2DE and identified nine highly immunogenic proteins; only 15% of the spots were identified as immunogenic proteins. In our study, we detected 35 spots, and eight immunogenic proteins were identified; therefore, we conclude that our results are similar.

The immunogenic proteins identified in this work are summarized in Table 2. Among the identified proteins, some have been previously characterized and are involved in pathogenesis, metal transport (iron, copper), and antibiotic resistance.

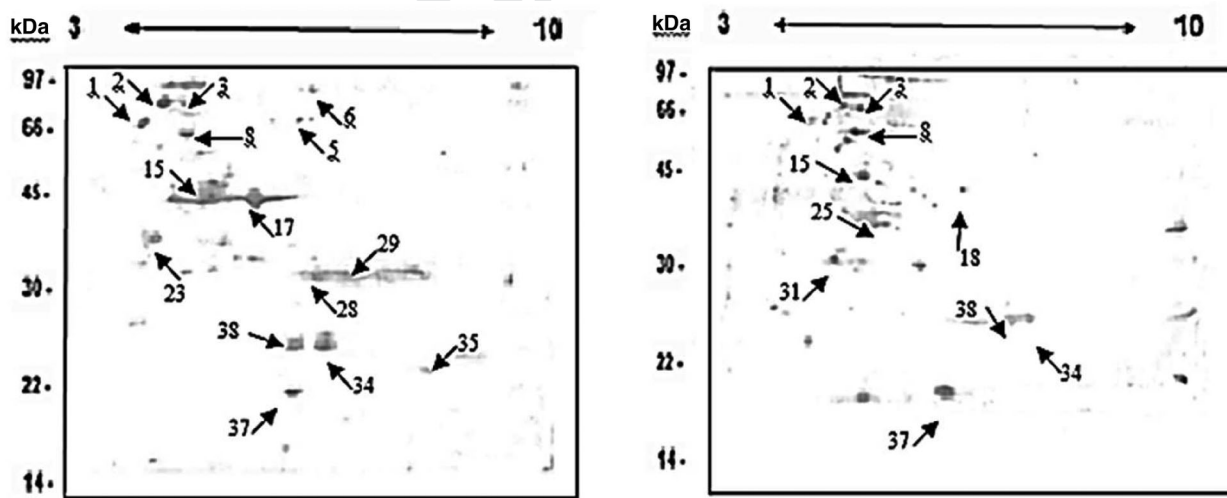


Figure 3. *Acinetobacter baumannii* 2D map (pI 3–10) with identified immunoreactive proteins (listed in Table 2). Identified proteins are indicated by spot number in Fig. 3 and Table 2. Molecular size markers are indicated on the left in kDa. (A) Western blot of a 2D gel of *A. baumannii* OMPs probed with sera pooled from 15 patients infected with *A. baumannii*. (B) Western blot of a 2D gel of OMPs probed with sera pooled from a second group of 15 patients infected with *A. baumannii*. All sera were diluted to 1:200.

Table 2. Immunoreactive proteins from *A. baumannii*

Spot	Protein
2	Outer membrane receptor (<i>A. baumannii</i> ACICU) gi 184159397 or TonB-dependent receptor (<i>A. baumannii</i> 6014059) gi 332876237
2	Outer membrane protein (<i>A. baumannii</i> ATCC 17978) gi 126642014
5	Outer membrane copper receptor (OprC) (<i>A. baumannii</i> SDF) gi 169632179 and other proteins of <i>Acinetobacter</i>
6	Ferric siderophore receptor protein (<i>A. baumannii</i> ATCC 17978) gi 126640547, ou TonB-dependent siderophore receptor (<i>A. baumannii</i> OIFC137) gi 395522750
8	TonB-dependent receptor (<i>A. calcoaceticus</i> PHEA-2) gi 375135955
15	Outer membrane protein A (<i>A. baumannii</i> ATCC 17978) gi 126642864 and other “outer membrane proteins” de <i>Acinetobacter</i>
17	Glucose-sensitive porin (OprB-like) (<i>A. baumannii</i> ATCC 17978) gi 126642873 ou porin B precursor (outer membrane protein D1) (<i>A. baumannii</i> AB900) gi 239501737
19	Hypothetical protein A1S_1462 (<i>A. baumannii</i> ATCC 17978) gi 126641508
23	34 kDa outer membrane protein (<i>A. baumannii</i> ACICU) gi 184159810
25	34 kDa outer membrane protein (<i>A. baumannii</i> ACICU) gi 184159810
28	β -Lactamase OXA-23 (<i>A. baumannii</i> AB0057) gi 213155910 ou OXA23-like protein (<i>A. baumannii</i>) gi 371927247
29	β -Lactamase OXA-23 (<i>A. baumannii</i> AB0057) gi 213155910 ou OXA23-like protein (<i>A. baumannii</i>) gi 371927247
31	Hypothetical protein ABAYE0137 (<i>A. baumannii</i> AYE) gi 169794337
34	Outer membrane protein (<i>A. baumannii</i> ATCC 17978) gi 126640934 and other outer membrane proteins
34	Hypothetical protein A1S_1296 (<i>A. baumannii</i> ATCC 17978) gi 126641342 ou type VI secretion system effector (<i>A. calcoaceticus</i> RUH2202) gi 262278650
37	β -Lactamase OXA-23 (<i>A. baumannii</i> AB0057) gi 213155910 ou OXA23-like protein (<i>A. baumannii</i>) gi 371927247
38	Hypothetical protein A1S_1296 (<i>A. baumannii</i> ATCC 17978) gi 126641342 ou type VI secretion system effector (<i>A. calcoaceticus</i> RUH2202) gi 262278650
38	β -Lactamase OXA-23 (<i>A. baumannii</i> AB0057) gi 213155910 ou OXA23-like protein (<i>A. baumannii</i>) gi 371927247

3.1 OmpA

OMPs of Gram-negative bacteria are known to be key players in bacterial adaptation and pathogenesis in host cells [18]. OMP A (OmpA), identified in this study, has previously been well characterized in *A. baumannii*. This protein belongs to the OmpA superfamily of proteins, which are highly conserved across multiple species of Gram-negative bacteria, and OmpA of *A. baumannii* is also involved in many interactions with the host during infection, including adherence to epithelial cells, induction of apoptosis in host cells, and differentiation of host immune cells [4]. According to Soares [9] and co-workers, this protein is a precursor of Omp38, of the OmpA family, which is involved in the transport of β -lactams and saccharides with molecular masses of up to 800 Da. Furthermore, it has been suggested by the same authors that this protein can act as a potential virulence factor inducing apoptosis of epithelial cells in the early stages of infection by *A. baumannii*. For these reasons, OmpA might be an attractive target for the development of novel antibacterial therapies.

3.2 Omp34kDa

Omp34kDa, identified in this work, has been previously observed to be located on the outer membrane of *A. baumannii*. Importantly, this protein was recognized by antibodies of patients with an *A. baumannii* infection, but not by antibodies of patients without infection or infected by other pathogens. The protein has been demonstrated to be a specific antigenic protein in OMPs of *A. baumannii*, but further studies are re-

quired to decipher its role in the virulence or survival of the bacteria under adverse conditions [19].

3.3 IROMP

When colonizing a host, bacterial pathogens, including *A. baumannii*, must compete with the host for essential nutrients. One of the most coveted nutrients in biological systems is iron because of its essentiality to almost all living organisms and limited availability under physiological conditions [20]. To survive in a human host, bacteria form siderophores called iron-regulated OMPs (IROMPs) [21]. In this study, we identified the ferric siderophore receptor protein. In host tissues, the availability of free Fe is minimal because most Fe is sequestered by high-affinity iron-binding proteins, such as transferrin and lactoferrin. Notably, OmpA is potentially involved in iron metabolism. Taken together, these findings open new avenues to explore and better understand the role of iron in the pathophysiology of *A. baumannii* [20].

3.4 OprC

In the present study, OMPs associated with transport across the membrane of several compounds were also identified, including proteins such as outer membrane copper receptor (OprC), which allows the penetration of small cations and putative glucose-sensitive porin (OprB-like). This porin is often referred to as carbohydrate-selective porin because it acts as a central component of glucose, mannitol, glycerol, and fructose transport across the outer membrane [9].

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Porins can play a variety of roles depending on the bacterial species, including the maintenance of cellular structural integrity, bacterial conjugation and bacteriophage binding, antimicrobial resistance, and pore formation to permit the penetration of small molecules [22–25].

3.5 OXA-23

Acinetobacter baumannii is an important opportunistic Gram-negative pathogen frequently associated with nosocomial outbreaks worldwide. Carbapenems generally represent the last resort in treating life-threatening infections caused by *Acinetobacter* spp. because of their resistance against most β -lactamases, including extended-spectrum β -lactamases; however, their efficacy is increasingly compromised by the emergence of carbapenem-hydrolyzing β -lactamase enzymes of Ambler molecular class B (metallo- β -lactamases) and D (oxacillinases) [26]. In this study, we identified OXA-23-like protein or β -lactamase OXA-23. The OXA-type carbapenemases have emerged globally as the main mechanism responsible for this resistance. Outbreaks of OXA-23-producing *Acinetobacter* have been reported in various regions of the world. Although MDR *Acinetobacter* spp. are a leading cause of nosocomial infections in Brazilian hospitals, there are few studies regarding the mechanisms of resistance to carbapenems. Therefore, the presence of MDR OXA-23-producing *A. baumannii* genotypes emphasizes the need to control the use of carbapenems to prevent the spread of these organisms [26].

3.6 Hypothetical proteins of *A. baumannii*

Three immunogenic hypothetical proteins were identified in this study. Hypothetical proteins A1S_1462 (spot 19) and A1S_1296 (spot 34), both from the ATCC 17978 strain of *A. baumannii*, are 242 and 167 amino acid long proteins, respectively, of unknown function. However, these proteins have sequence similarity with other protein sequences of other species of *Acinetobacter*, such as *A. calcoaceticus*, *A. junii*, *A. johnsonii*, and *A. haemolyticus*. Notably, hypothetical protein A1S_1296 has sequence homology with the type VI secretion system effector of *A. calcoaceticus* (RUH2202). A1S_1462 and A1S_1296 belong to the tetratricopeptide repeat domain and DUF superfamily.

The hypothetical protein ABAYE0137 of *A. baumannii* is a protein with 259 amino acids and exhibits sequence homology with other strains of *A. baumannii*, such as ATCC19606. ABAYE0137 belongs to the Phenol_Meta_Superfamily and is most likely involved in phenol degradation pathways.

3.7 Immunotherapy strategies

An important characteristic of a protein considered to be a good target for use in immunotherapy strategies is its degree

of conservation in the same species (*A. baumannii*), in different species of *Acinetobacter*, and with other bacteria. This conservation ensures specific immune response against this pathogen, avoiding cross-reactivity with other microorganisms. Most of the proteins identified in this study are present in the genome of *Acinetobacter* deposited in Gene Bank and in another species of *Acinetobacter*, particularly *A. calcoaceticus*, *A. lwoffii*, *A. nosocomialis*, *A. radioresistens*, and *A. johnsonii*.

Infectious diseases are the second leading cause of death worldwide, and the acquisition of antibiotic resistance by many pathogenic bacteria has recently spurred interest in generating vaccines to cure or prevent diseases. OMPs are highly abundant proteins in direct contact with the host immune system, making them good targets for the development of immunotherapy for the treatment of bacterial infections [7].

The feasibility of a vaccine against *A. baumannii* has been investigated in previous studies. McConnell et al. employed a killed whole-cell *A. baumannii* vaccine that protected immunized mice from infection [27]. These results confirm the effective potential of a vaccine against *A. baumannii* and are complementary to our study, which identified candidate antigens for the development of a recombinant protein-based vaccine.

In this work, the characterization of OMPs based on an immunoproteomic approach identified antigen candidates by immunoblotting with sera from *A. baumannii* infected patients. The presence of antibodies against a particular antigen in many patients is indicative of the expression of the respective protein in vivo and demonstrates that most individuals can mount an immune response against this protein; both of these results are requirements for a successful vaccine candidate [7]. It is particularly surprising that we identified an immunogenic protein related to antimicrobial resistance; however, previous reports have identified antibodies against chromosomal β -lactamase from *Pseudomonas aeruginosa* isolated from infected patients [28].

4 Conclusions

The specific antigens OmpA, Omp34kDa, OprC, OprB-like, OXA-23, and ferric siderophore receptor protein of *A. baumannii* were major OMPs identified in this study as immunogenic proteins. Most of these antigens were conserved among *A. baumannii* and other *Acinetobacter* species.

These results suggest that these proteins could serve as antigen candidates for the development of immunotherapies against *A. baumannii* infections. Further studies must be performed with these immunogenic proteins to analyze their specific immunogenicities and protection abilities in animal models.

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The authors have declared no conflict of interest.

5 References

- [1] Marti, S., Sanchez-Cespedes, J., Oliveira, E., Bellido, D. et al., Proteomic analysis of a fraction enriched in cell envelope proteins of *Acinetobacter baumannii*. *Proteomics* 2006, 6, 82–87.
- [2] Fattahian, Y., Rasooli, I., Mousavi Gargari, S. L., Rahbar, M. R. et al., Protection against *Acinetobacter baumannii* infection via its functional deprivation of biofilm associated protein (Bap). *Microb. Pathog.* 2011, 51, 402–406.
- [3] McConnell, M. J., Domínguez-Herrera, J., Smani, Y., López-Rojas, R. et al., Vaccination with outer membrane complexes elicits rapid protective immunity to multidrug-resistant *Acinetobacter baumannii*. *Infect. Immun.* 2011, 79, 518–526.
- [4] McConnell, M. J., Pachon, J., Expression, purification, and refolding of biologically active *Acinetobacter baumannii* OmpA from *Escherichia coli* inclusion bodies. *Protein Expr. Purif.* 2011, 77, 98–103.
- [5] Dijkshoorn, L., Nemec, A., Seifert, H., An increasing threat in hospitals: multidrug-resistant *Acinetobacter baumannii*. *Nat. Rev. Microbiol.* 2007, 5, 939–951.
- [6] Vila, J., Marti, S., Sanchez-Cespedes, J., Porins, efflux pumps and multidrug resistance in *Acinetobacter baumannii*. *J. Antimicrob. Chemother.* 2007, 59, 1210–1215.
- [7] Kurupati, P., Teh, B. K., Kumarasinghe, G., Poh, C. L., Identification of vaccine candidate antigens of an ESBL producing *Klebsiella pneumoniae* clinical strain by immunoproteome analysis. *Proteomics* 2006, 6, 836–844.
- [8] Soares, A. J., Santos, M. F., Trugilho, M. R., Neves-Ferreira, A. G. et al., Differential proteomics of the plasma of individuals with sepsis caused by *Acinetobacter baumannii*. *J. Proteomics* 2009, 73, 267–278.
- [9] Soares, N. C., Cabral, M. P., Parreira, J. R., Gayoso, C. et al., 2-DE analysis indicates that *Acinetobacter baumannii* displays a robust and versatile metabolism. *Proteome Sci.* 2009, 7, 1–10.
- [10] Kittelberger, R., Hansen, M. F., Hilbink, F., de Lisle, G. W. et al., Selective extraction of bacterial macromolecules by temperature-induced phase separation in Triton X-114 solution. *J. Microbiol. Methods* 1995, 24, 81–92.
- [11] Peterson, G. L., Determination of total protein. *Methods Enzymol.* 1983, 91, 95–119.
- [12] Laemmli, U. K., Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970, 227, 680–685.
- [13] Towbin, H., Staehelin, T., Gordon, J., Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA* 1979, 76, 4350–4354.
- [14] Candiano, G., Bruschi, M., Musante, L., Santucci, L. et al., Blue silver: a very sensitive colloidal Coomassie G-250 staining for proteome analysis. *Electrophoresis* 2004, 25, 1327–1333.
- [15] Renzone, G., D'Ambrosio, C., Arena, S., Rullo, R. et al., Differential proteomic analysis in the study of prokaryotes stress resistance. *Ann. Ist. Super. Sanita* 2005, 41, 459–468.
- [16] García-Garmendia, J. L., Ortiz-Leyba, C., Garnacho-Montero, J., Jiménez-Jiménez, F. J. et al., Risk factors for *Acinetobacter baumannii* nosocomial bacteremia in critically ill patients: a cohort study. *Clin. Infect. Dis.* 2001, 33, 939–946.
- [17] Dennehy R., McClean, S., Immunoproteomics: the key to discovery of new vaccine antigens against bacterial respiratory infections. *Curr. Protein Pept. Sci.* 2012, 13, 807–815.
- [18] Lin, J., Huang, S., Zhang, Q., Outer membrane proteins: key players for bacterial adaptation in host niches. *Microbes Infect.* 2002, 4, 325–331.
- [19] Islam, A. H., Singh, K. K., Ismail, A., Demonstration of an outer membrane protein that is antigenically specific for *Acinetobacter baumannii*. *Diagn. Microbiol. Infect. Dis.* 2011, 69, 38–44.
- [20] Nwugo, C. C., Gaddy, J. A., Zimpler, D. L., Actis, L. A., Deciphering the iron response in *Acinetobacter baumannii*: a proteomics approach. *J. Proteomics* 2011, 74, 44–58.
- [21] Hwa, W. E., Subramaniam, G., Mansor, M. B., Yan, O. S. et al., Iron regulated outer membrane proteins (IROMPs) as potential targets against carbapenem-resistant *Acinetobacter* spp. isolated from a Medical Centre in Malaysia. *Indian J. Med. Res.* 2010, 131, 578–583.
- [22] Vordermeier, H. M., Hoffmann, P., Gombert, F. O., Jung, G. et al., Synthetic peptide segments from the *Escherichia coli* porin OmpF constitute leukocyte activators. *Infect. Immun.* 1990, 58, 19–24.
- [23] Weiser, J. N., Gotschlich, E. C., Outer membrane protein A (OmpA) contributes to serum resistance and pathogenicity of *Escherichia coli* K-1. *Infect. Immun.* 1991, 59, 2252–2258.
- [24] Hauschildt, S., Kleine, B., Bacterial stimulators of macrophages. *Int. Rev. Cytol.* 1995, 161, 263–331.
- [25] Saint, N., El Hamel, C., Dé, E., Molle, G., Ion channel formation by N-terminal domain: a common feature of OprFs of *Pseudomonas* and OmpA of *Escherichia coli*. *FEMS Microbiol. Lett.* 2000, 190, 261–265.
- [26] Carvalho, K. R., Carvalho-Assef, A. P., Peirano, G., Santos, L. C. et al., Dissemination of multidrug-resistant *Acinetobacter baumannii* genotypes carrying bla(OXA-23) collected from hospitals in Rio de Janeiro, Brazil. *Int. J. Antimicrob. Agents* 2009, 34, 25–28.
- [27] McConnell, M. J., Pachón, J., Active and passive immunization against *Acinetobacter baumannii* using an inactivated whole cell vaccine. *Vaccine* 2010, 29, 1–5.
- [28] Giwercman, B., Rasmussen, J. W., Cioufu, O., Clemmetsen, I. et al., Antibodies against chromosomal beta-lactamase. *Antimicrob. Agents Chemother.* 1994, 38, 2306–2323.

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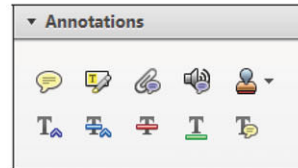
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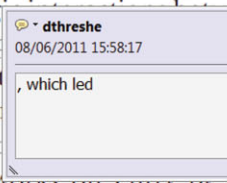


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there is no room for extra profits as mark-ups are zero and the number of firms (net) values are not determined by the number of firms. Blanchard and Kiyotaki (1987), perfect competition in general equilibrium. The effects of aggregate demand and supply shocks in a classical framework assuming monopolistic competition and an exogenous number of firms

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dynamic responses of mark-ups consistent with the VAR evidence

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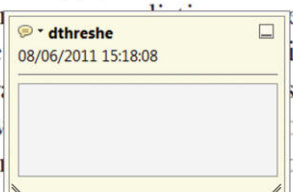


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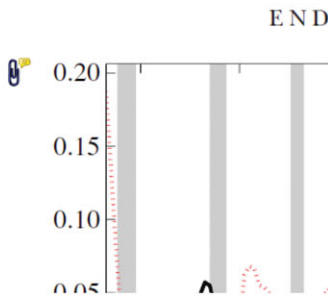
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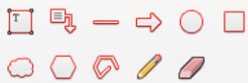
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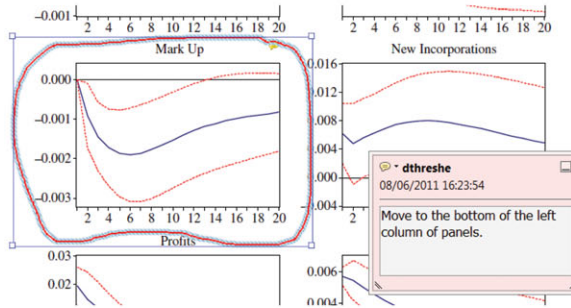


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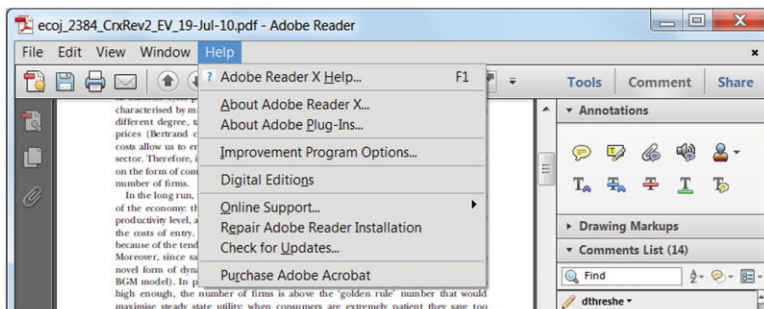
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Identification of immunogenic proteins of the bacterium *Acinetobacter baumannii* using a proteomic approach