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Exploration of the Potential of the ATP-Binding Cassette (ABC) Transporter Antigen as a Vaccine Candidate Against *Acinetobacter baumannii* Infections

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ABSTRACT

Objectives: The emerging multidrug-resistant strains of *Acinetobacter baumannii* and the limited available treatment options, have led the World Health Organization to enlist this pathogen among the microorganisms with critical priority demanding the development of novel treatment alternatives. This study aimed at exploring, for the first time, the potential of an efflux-related protein, the ATP-binding cassette (ABC) transporter substrate-binding protein, as a vaccine candidate against *A. baumannii* infections. The ABC transporter substrate-binding protein is known to play a role in the iron acquisition pathway of *A. baumannii*. It utilizes the energy released from the hydrolysis of ATP in the transportation of the iron-bound siderophore across the plasma membrane into the cell. **Methods:** In this work, the ABC transporter substrate-binding protein was expressed in *Escherichia coli* after cloning its respective gene into pQE31 plasmid vector. The protein was purified using metal affinity chromatography. The purified antigen was administered to mice in combination with Bacillus Calmette–Guérin (BCG) and alum nanoparticles as adjuvants. Immunological parameters were assessed, and protection was tested following bacterial challenge in a murine infection model. **Results:** Two weeks after the last immunization dose, serum samples were tested for antigen-specific IgG antibody response which was extremely significant in immunized mice when compared with negative control mice. Following challenge of mice with *A. baumannii*, only a short-lived protection lasting for 24 hours was obtained. **Conclusion:** The diversity of the virulence factors exhibited by *A. baumannii* including several iron acquisition mechanisms might necessitate the design of a multi-component vaccine to elicit effective protection. Furthermore, the immunization regimen, the used adjuvant, and the route of administration are critical factors which are worthy of further investigation to fulfill complete long-lasting protection.

Keywords: *Acinetobacter baumannii; ABC transporter substrate binding protein; Vaccine; BCG; Iron acquisition mechanisms.*

INTRODUCTION

Arising as important etiological agent of many hospital-acquired infections, *Acinetobacter baumannii* represents a serious threat to public health globally [1]. For the last decades, *A. baumannii* has demonstrated an exceptional ability to persist in the environment of health care facilities, where it causes severe infections among the critically ill patients in intensive care units, including meningitis, pneumonia, urinary tract, skin, wound, and bloodstream infections [2]. Following the extensive use of antimicrobial agents in clinical practice to treat

infections caused by *A. baumannii*, multidrug-resistant (MDR) strains of this pathogen started to emerge and to disseminate worldwide [1, 2]. This accelerated dispersion has been fueled by the capacity of *A. baumannii* to form biofilms on abiotic surfaces and its genomic plasticity which facilitates the acquisition of a variety of resistant determinants and virulence genes [3]. The high mortality rates caused by these emerging MDR strains of *A. baumannii*, and the limited available treatment options had led the World Health Organization (WHO) in 2017, to enlist this pathogen among the microorganisms with critical priority demanding the development of novel treatment alternatives [4]. The implementation of prophylactic vaccination might be considered as an effective strategy to guard against *A. baumannii* infections. A key aspect in designing a successful vaccine candidate is the recognition of the antigens with potential ability to elicit immune protection [5]. So far, several promising antigens have been explored as vaccine candidates that could contribute to the control and prevention of *A. baumannii* infections [3]. They include the outer membrane vesicles (OMVs), outer membrane proteins (OmpA, Omp22, OmpK), Acinetobacter trimeric auto-transporter (Ata), biofilm-associated protein (Bap), K1 capsular polysaccharide and poly-N-acetyl-β-(1-6)-glucosamine (PNAG) among others [6]. Unfortunately, these vaccines are still in the preclinical research phase. An ultimate antigen would be the one located on the surface of the bacterial cell, present in the majority of *A. baumannii* strains, highly conserved at the amino acid level, and well-expressed during infections [7].

It is widely recognized that iron plays a crucial role in the pathogenicity and virulence of *A. baumannii* [8]. Iron is an essential micronutrient for all bacteria. It acts as a cofactor for different enzymes, such as those involved in electron transport, and in DNA and amino acid synthesis. The colonization and the ability of *A. baumannii* to survive within an infected host are directly related to its ability to uptake iron from this host. During infection, the host tends to withhold this essential metal from invading microorganisms, by increasing its iron storage, thus creating an iron-deficient environment [8]. To counteract the challenge of iron starvation, *A. baumannii* has evolved numerous iron acquisition systems, primarily based on the production of siderophores which sequester iron from the host, forming an iron-siderophore complex [9]. The established complex binds to the siderophore receptors located on the outer membrane of the bacterium and then is transferred into the periplasm. Next, the periplasmic binding proteins direct the ligation of the complex to an inner membrane, efflux-related, ATP-binding cassette (ABC) transporter. This transporter utilizes the energy released from the hydrolysis of ATP in the transportation of the iron-bound siderophore across the plasma membrane into the cell [10]. This pathway highlights the pivotal role mediated by the siderophore receptors on the outer membrane, as well as by ABC transporter, in the translocation of iron chelators and hence, in the pathogenesis of *A. baumannii* infections. Recent studies have identified the iron-regulated outer membrane proteins, such as BauA and BfnH, as effective vaccine candidates providing protection against *A. baumannii* infections [10, 11]. In the current study, for the first time we explored the potential of the efflux-related protein belonging to ABC superfamily transporters, ABC transporter substrate-binding protein, as a vaccine candidate against *A. baumannii* infections.

MATERIAL AND METHODS

Bacterial strains and plasmids

A. baumannii ATCC 19606 and *Escherichia coli* DH5α were obtained from the laboratory stock of the department of Microbiology and Immunology, Faculty of Pharmacy, Alexandria University. *E. coli* M15 (pREP4) (Qiagen, Germany) and pQE31 plasmid (Qiagen, Germany) were used as an expression host and a cloning vector for the selected gene, respectively. A clinical isolate of *A. baumannii*, obtained from Mabaret Al Asafra Laboratories, was used in the *in vivo* challenge experiment.

Culture media, antibiotics, and incubation conditions

The bacterial strains used in this study were inoculated into Luria Bertani (LB) broth (HiMedia Laboratories, India) or subcultured on LB agar, which was prepared by adding agar (2% w/v) to LB broth before autoclaving. Ampicillin (AMP, Epico, Egypt) and kanamycin sulfate (KM, Sigma Aldrich, USA) were added to the culture media at a final concentration of 100 µg/mL and 25 µg/mL, respectively, whenever required. *A. baumannii* clinical isolate that was used in mice challenge experiments was cultured in Roswell Park Memorial Institute medium (RPMI) 1640 (Gibco, UK) containing 10% v/v fetal bovine serum (FBS) (Biowest, France). All liquid cultures used in this study were incubated for 18 h at 37°C under shaking conditions (200 rpm) except for cultures used for the induction of protein expression which were incubated for 3 h at 37°C and 200 rpm.

Design of primers and amplification of the gene encoding ABC transporter substrate-binding protein by polymerase chain reaction (PCR)

The ABC transporter substrate-binding protein (GenBank accession no. AP022836, Region: 2035184 to 2037004) was checked for the presence of signal peptide (SP) sequences using SignalP Server v.4.1 (http://www.cbs.dtu.dk/services/SignalP/).

Consequently, when designing the forward (TATAGGATCCGAAAAGCCCAGCCGATCCAAAT) and reverse (TATAAAGCTTTTAATGATTAGATTTCGTATTTGTA) primers (Eurofins Genomics, USA), the coding sequence of SP was excluded. These forward and reverse primers were supplemented with *BamHI* and *HindIII* restriction sites (underlined) at their respective 5' ends to enable directional cloning in pQE31 plasmid. The OligoEvaluator™ online tool (www.oligoevaluator.com) was used for primer design. A single colony of *A. baumannii* ATCC 19606, isolated on LB agar plate, was heated in sterile water at 95°C for 10 min and 5 µL of the supernatant served as a template to amplify the target gene by PCR using the designed primers. COSMO PCR RED Master Mix (Willowfort®, Birmingham, UK) was used to amplify the gene of interest according to the following PCR cycling conditions: an initial denaturation step at 95°C for 3 min, then 35 cycles of denaturation at 95°C for 15 sec, annealing at 55°C for 15 sec and extension at 72°C for 45 sec, then a final extension at 72°C for 5 min.

Cloning of the gene encoding the ABC transporter substrate-binding protein

The resulting purified PCR amplicon was double digested with *BamHI* and *HindIII* (ThermoScientific, USA), then ligated to a similarly digested pQE31 plasmid using T4 DNA ligase (NEB, England). Chemically competent cells of *E. coli* DH5-α were prepared [12], then transformed with the ligation reaction mixture, and positive clones were selected by inoculation on LB plates containing AMP and by colony PCR [13]. A single transformed colony was selected and subcultured overnight for subsequent plasmid extraction. Next, chemically competent cells of *E. coli* M15 (pREP4) were transformed with the construct of the gene encoding ABC transporter substrate-binding protein in pQE31 plasmid. The transformants were plated onto LB supplemented with AMP and KM and positive clones were confirmed by colony PCR [13]. The cloning results were verified by sequencing using specific primers at Colors Medical Labs facility, Cairo, Egypt.

Induction of protein expression and purification

A fresh overnight culture of *E. coli* M15 (pREP4), harboring pQE31-gene encoding ABC transporter substrate-binding protein construct, was inoculated in 200 mL of LB broth containing 100 µg/mL AMP and 25 µg/mL KM at 37°C with vigorous shaking till reaching an optical density (OD_{600}) of 0.6-0.8. For overexpression of the target protein, isopropyl β-D-1-thiogalactopyranoside (IPTG, Melford, UK) [12] was used for induction at a final concentration of 1mM for 3 h at 37°C. This was followed by purification of the ABC transporter substrate-binding protein under denaturing conditions using nickel-nitrilotriacetic acid (Ni-NTA, Qiagen, USA) metal affinity chromatography according to the protocol recommended by Qiagen (The QIAexpressionist™). Purified ABC transporter substrate-binding protein was analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) using acrylamide/bis-acrylamide 40% (37.5:1) (Noragen Biotek corp., Canada) in the preparation of separating gel (12%) and stacking gel (4%). SDS-PAGE gel was stained using Coomassie brilliant blue R-250 dye (MP Biomedicals, USA) [14].

Refolding of ABC transporter substrate-binding protein

ABC transporter substrate-binding protein was subjected to buffer exchange, to eliminate any urea traces resulting from the denaturing buffers [15], using PD-10 desalting column containing Sephadex™ G-25 medium (GE Healthcare, USA) and 1X phosphate buffered saline (PBS) (0.137 M NaCl, 0.0027 M KCl, 0.01 M Na_2HPO_4 and 0.0018 M KH_2PO_4, pH to 7.4).

Mice immunization with ABC transporter substrate-binding antigen

A murine immunization/challenge model was employed to validate the potential vaccine efficacy of ABC transporter substrate-binding protein as previously described with slight modifications [16]. Eight-week-old female Swiss albino mice (20–25 g) were supplied by the animal house of Pharos University in Alexandria, Egypt. The mice were divided into groups (6 mice/group), where food and water were provided *ad libitum*. All animal experimental procedures were carried out according to the international and institutional ethical guidelines and had been ethically approved by the Animal Care & Use Committee (ACUC) of Faculty of Pharmacy, Alexandria University (Approval reference no. 062020112168). Two adjuvants were used for the immunization in this study: BCG (Immune BCG-T®), purchased from VACSERA co., Giza, Egypt, as well as aluminum phosphate (alum). Immune BCG-T® is a suspension of live attenuated *Mycobacterium bovis* BCG at a concentration of 3×10^8 CFU/mL which was 10-fold diluted in 0.9% saline to reach a concentration of 3×10^7 CFU/mL before administration. Alum nanoparticles were prepared according to the method of Vrieling *et al.* and their particle size was confirmed to be within the size range of 49 – 342 nm using Zetasizer (Malvern Panalytical Ltd, UK) [17]. Four groups of mice were used to assess the protective effect of ABC transporter substrate-binding protein against *A. baumannii* infection. Group 1 represented the negative control group and were injected with 0.9% saline. Group 2 received the adjuvant while group 3 mice were immunized with ABC transporter substrate-binding protein (20 µg for the first administrated dose and 15 µg for subsequent booster

doses) without adjuvants. Group 4 mice were immunized with both the antigen and the adjuvant according to the administration regimen shown in Table 1. Each group was injected by a total of four doses subcutaneously (S.C.) (each dose 200 µL), at two-week intervals. Before the administration of each immunization dose, blood samples were collected from mice by the submandibular bleeding method, and sera were kept at -20°C for subsequent analysis [18].

Assessment of anti-ABC transporter substrate-binding protein IgG response using indirect enzyme-linked immunosorbent assay (ELISA)

Anti-ABC transporter substrate-binding protein IgG response was assessed using ELISA as previously described with slight modifications [19]. Briefly, refolded ABC transporter substrate-binding antigen was quantified using NanoDrop and diluted with 1X PBS to a final concentration of 10 µg/mL. Diluted antigen was used for coating 96-well high binding ELISA plate (Greiner Bio One, Germany) (100 µl/well). Coated plate was incubated statically overnight at 4°C. After coating, the plate was washed 3 times with 1X PBS followed by blocking with 5% skimmed milk 3 h. After blocking, the plate was washed 3 times with PBS and 100 µl of diluted mouse serum (1:300 dilution in blocking buffer) were added and incubated for 1 h. After thorough washing, horseradish peroxidase-labeled goat anti-mouse IgG antibody (KPL, USA) was added for 1 h. After washing the plate, 100 µL of 3,3',5,5'-Tetramethylbenzidine (TMB) ELISA Substrate System (KPL, USA) were added to each well and left for color development in darkness for 20min at room temperature. This was followed by adding 50 µL of 1 M sulfuric acid (stopping solution) and measuring the absorbance at 450 nm using microplate reader (BIOTEK, USA).

Mice challenge with *A. baumannii*

Three weeks after the administration of the last immunization dose, mice were challenged by an intraperitoneal injection (200 µL) of a clinical isolate of *A. baumannii* equivalent to 10^9 CFU/mouse. Inoculum of *A. baumannii* was prepared from an overnight culture in RPMI 1640 medium containing 10% FBS. Challenged mice were observed for survival during a period of one week at different time intervals.

Statistical Analysis

ANOVA and Tukey's test were employed for statistical analysis using GraphPad Prism software version 9.3.1 (GraphPad Software, San Diego, CA, United States). For the analysis of survival curves, Log-rank (Mantel-Cox) test was used. A p-value < 0.05 was considered statistically significant.

RESULTS

PCR amplification and successful antigen expression in *E. coli* M15 (pREP4) host

Following the bioinformatic analysis of the gene encoding ABC transporter substrate-binding protein, the first 31 amino acids were recognized as signal peptide (SP) and their corresponding coding sequences were omitted during the forward primer design. The target gene was successfully PCR-amplified where agarose gel electrophoresis displayed the gene under investigation as a single band at its expected size (1728 bp) as shown in Figure 1 (lane 1). The gene was then cloned in pQE31 plasmid vector, and subsequently transformed into *E. coli* M15 (pREP4) expression host (**Figure 1**, lane 2).

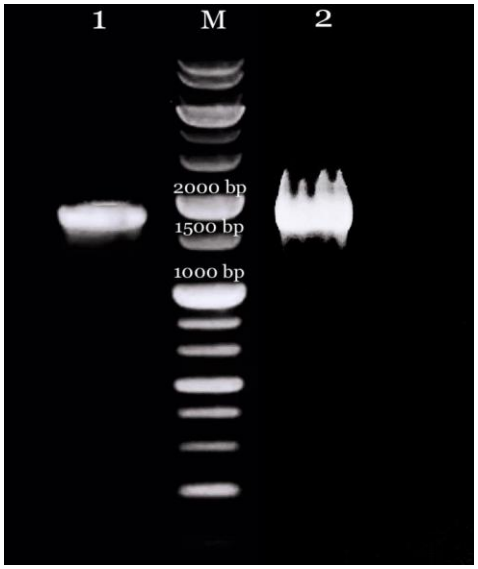

Figure 1. Agarose gel electrophoresis of gene amplicon of ABC transporter substrate-binding protein. Lane 1 shows the PCR-amplified gene (1728 bp) using gene-specific primers in a colony PCR using *A. baumannii* ATCC 19606 DNA template. Lane 2 shows the gene recovered from *E. coli* M15 (pREP4) transformants (1728 bp). Lane M: DNA molecular weight marker.

Sequencing analysis (**Supplementary file S1**) confirmed the sequence and identity of the gene encoding ABC transporter substrate-binding protein of *A. baumannii*. Following protein induction with IPTG, SDS-PAGE demonstrated successful protein expression in *E. coli* M15 (pREP4) host where the protein appeared as a pure band at its expected size (65 kDa) following Ni-NTA metal affinity chromatography (**Figure 2**).

Table 1. The immunization regimen used in the present study.

Dose	Week of experiment	Antigen (100 µL)	Adjuvant (100 µL)
First	First	20 µg per mouse	BCG
Second	Third	15 µg per mouse	alum
Third	Fifth	15 µg per mouse	alum
Fourth	Seventh	15 µg per mouse	alum

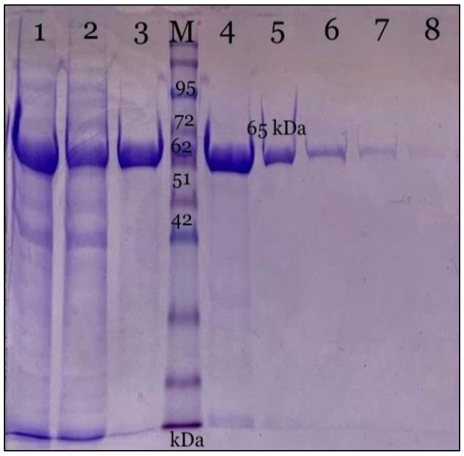

Figure 2. SDS-PAGE showing ABC transporter substrate-binding protein purified using metal affinity chromatography. Cells were induced with 1 mM IPTG at 37°C for 3 h. Lanes 1, 2, and M show lysate, flow through, and pre-stained protein ladder, respectively. Lanes 3 and 4 - 8 represent successive eluates of purified ABC transporter substrate-binding protein (65 kDa), where lanes 3 and 4 show high protein concentration in the eluates, while lanes 5 to 8 demonstrate gradually decreasing concentrations.

Active immunization and anti-ABC transporter substrate-binding protein IgG titer

Indirect ELISA was performed before initiating the immunization procedure and after the administration of each immunization dose to monitor and compare the antigen-specific antibody response among the different groups. When compared to the negative control groups, an extremely significant anti-ABC transporter substrate-binding protein IgG antibody response was observed in the group receiving the antigen and the group receiving the antigen/adjuvant mixture (**Figure 3**).

Moreover, a highly significant response was observed when comparing the total IgG titer of the group receiving the antigen/adjuvant mixture when compared with group receiving the antigen alone (**Figure 4**).

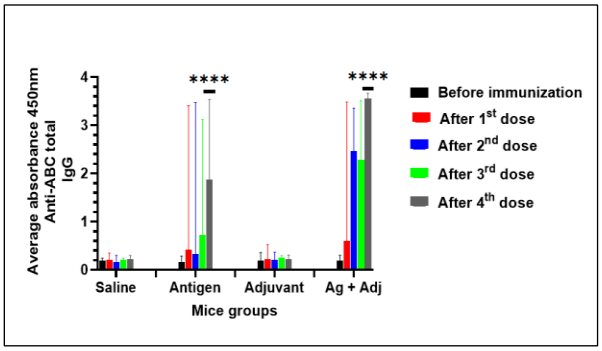

Figure 3. Mouse anti-ABC transporter substrate-binding protein IgG response at different time intervals during the vaccination regimen. Indirect ELISA technique was used and the serum was added at a dilution of 1/300. The error bars represent mean reading +/- SEM (standard error of the mean). The *p*-values indicate significance where ****$p < 0.0001$.

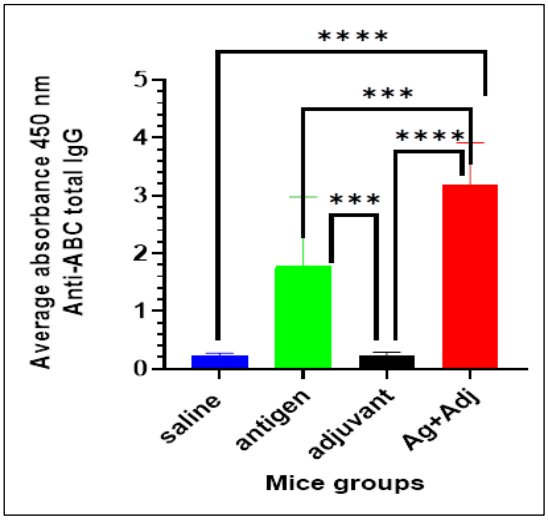

Figure 4. Antibody immune response elicited by ABC transporter substrate-binding antigen two weeks after the last booster. Antigen-specific IgG response was measured using indirect ELISA technique. The serum was at a dilution of 1/300. The error bars represent mean reading +/- SEM (standard error of the mean). The *p*-values indicate significance, where ***$p < 0.001$ and ****$p < 0.0001$.

Survival experiment

Mice groups were challenged intraperitoneally with a lethal dose of a clinical isolate of *A. baumannii* three weeks after the last vaccine booster. The mice were observed at different time intervals for one week. By the end of the first day of observation, the group receiving the antigen and that receiving the antigen/adjuvant mixture showed a survival rate of 83.3%. In addition, the saline group showed 50% survival and the adjuvant group showed 83.3% survival on day 1 (**Figure 5**). Further monitoring for one week revealed a final survival rate of 33.3% for the adjuvant, the antigen and the antigen/adjuvant mixture groups and 50% for the saline group. Overall, there was no significant difference in survival between all groups as assessed by Log-rank (Mantel-Cox) test (*p*-value > 0.05).

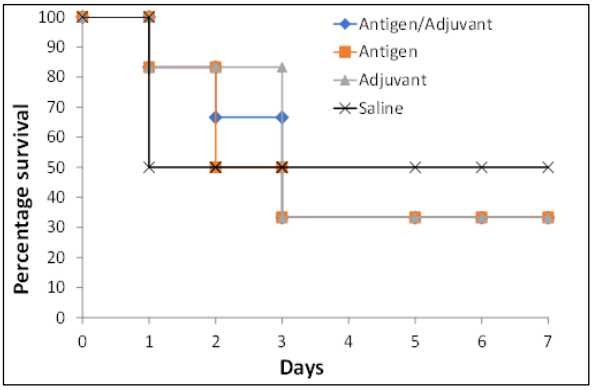

Figure 5. Mice survival in the four tested groups at different time intervals monitored for one week.

DISCUSSION

Despite the tremendous efforts dedicated towards vaccine development for infections caused by one of the most problematic bacteria, *A. baumannii*, only partial protection has been reported with several earlier experimented protein antigens [6]. In the current study, the immunogenicity and the protective effect of ABC transporter substrate-binding protein was investigated in a murine *A. baumannii* infection model. The selection of an efflux-related protein belonging to ABC superfamily transporters was based on its periplasmic location, its high conservancy among *A. baumannii* strains, and on its importance in the translocation of iron chelators, thus playing role in the pathogenicity of *A. baumannii* [11].

According to Di Pasquale *et al*., adjuvants enhance the immunogenicity of the used antigen and ameliorate vaccine efficacy [20]. Furthermore, adjuvants were reported to improve the vaccine stability and to increase the duration of the immune response enabling the antigen dose-sparing strategy [21]. Two types of adjuvants were experimented in this study: alum, and BCG. Despite being discovered a century ago, alum is still considered as the most widely used adjuvant, and recently, it has been included in the preparation of COVID-19 vaccines [21, 22]. Antigen particles tend to adsorb on alum forming an alum-antigen complex that induces local inflammation, increases antibody production, and stimulates Th2 immune response [20, 22]. On the other hand, BCG enhances a mixed Th1/Th2 immune response associated with IgG1, IgG2a and IgG2b antibodies [23]. Besides, the selection of BCG as a vaccine adjuvant was based upon its prior use in the clinical practice and its ability to activate and recruit NK cells and neutrophil granulocytes [24]. The use of alum in form of nanoparticles is reported to be advantageous as the surface/volume ratio is increased, resulting in higher antigen absorption capacity which exceeds 10 to 20 times that of the conventional alum [25]. Moreover, the immunogenicity of alum, when used as nanoparticles, is documented to be more potent as compared to that of the microparticles [25]. Therefore, alum nanoparticles were prepared within a size range of 49 – 342 nm and were used in the current study as vaccine adjuvant.

Indirect ELISA results revealed an extremely significant ABC transporter substrate-binding protein-specific IgG antibody response in the immunized group receiving the antigen combined with adjuvant when compared to the unimmunized mice group (*p*-value < 0.0001). Confirming the importance of the immuno-adjuvant effect, a highly significant difference was noticed between the group receiving the antigen and that immunized with the antigen combined with adjuvant (*p*-value = 0.0003). In accordance with our results, a recent study indicated that the addition of BCG to alum in the preparation of COVID-19 vaccine showed to be effective and resulted in a remarkable increase in the antibody titer when compared to the results of using BCG or alum alone [26]. Upon challenge of immunized mice, a short-lived protection, lasting for 24 h, was observed in the mice group receiving antigen combined with adjuvant with a survival rate reaching 80.3%. Unfortunately, this rate decreased eventually reaching 33.3% on day 7.

Several aspects might explain the inability of the ABC transporter substrate-binding protein to induce protection in mice against infection with *A. baumannii* observed in the present study. This might be attributed to the diversity of the virulence factors exhibited by *A. baumannii*. These involve several other iron acquisition mechanisms including the production of siderophores such as fimsbactins A-F, acinetobactin (acinetobactin and preacinetobactin), and baumannoferrins A and B; the outer membrane TonB-dependent receptor responsible for the translocation of the iron-siderophore complex to the periplasmic; and the haem uptake systems [27]. Therefore, a single component vaccine might not be enough to produce effective protection.

Moreover, the S.C. route of administration of the vaccine constitutes a suspected drawback standing behind the low survival rates obtained. In a study conducted by Ranjbar *et al*., the percentage of protection detected in the immunized mice group receiving the antigen S.C. reached 34% as compared to 100% protection against the same challenging dose of *A. baumannii* in the mice group receiving the antigen intranasally [28]. Furthermore, Poland *et al*. detected in their study, a slow-going mobilization and processing of the target antigen after being injected into the S.C. fat layer as a consequence of the poor vascularization [29]. A study comparing between two different routes for administration of hepatitis B vaccine, intramuscular and S.C., described a significant decrease in the seroconversion rates and a rapid decline of the antibody response upon using the S.C. administration route [30].

The incorporation of alum as an adjuvant in the vaccine used in the current study might be another reason behind the unsatisfactory immunization outcome. Despite their ability to induce strong antibody-mediated immune responses, aluminum-based adjuvants had been reported to produce an inadequate induction of the cell-mediated immune responses [31]. Cell-mediated immunity is known to play a major role in the activation of phagocyte responses, antigen-specific T-lymphocytes, and the production of cytokines after the antigen recognition [32]. In a study conducted by Oleszycka *et al*., the authors explained that alum promotes the activation of interlukin-10, thus inhibiting the expected Th1 immune responses mediated by the BCG immuno-adjuvant effect [33]. Th1 cells are responsible for the activation of macrophages and the induction of B cells to produce IgG antibodies that are important for opsonizing foreign pathogens to be eliminated by phagocytes [34, 35].

CONCLUSION

In conclusion, ABC transporter substrate-binding protein has been explored, for the first time, as a potential vaccine candidate against *A. baumannii*. This antigen fulfilled the *in silico* requirements of a successful vaccine candidate with its periplasmic location, its high conservancy among *A. baumannii* strains, its known expression during infections due to the its crucial role in iron acquisition, and its ability to elicit high antigen-specific IgG titers following active immunization. Nevertheless, the S.C. immunization with this candidate did not provide the expected vaccine efficacy when compared to the controls, where only a short-lived unsustainable protection, lasting for 24 h, was achieved in the mice infection model. Further evaluation of the ABC transporter substrate-binding protein as a vaccine candidate is necessitated. The modification of the immunization regimens, the type of the used adjuvant and/or the route of administration are potential approaches to evaluate the vaccine efficacy of this antigen. Furthermore, the use of the ABC transporter substrate-binding protein in the context of a multi-antigen vaccine which can elicit a multi-faceted immune response against *A. baumannii*, could be an effective vaccine strategy that is worthy of further investigation.

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Contributions

All authors read and approved the final version of the manuscript.

Supplementary material

Sequencing analysis (S1) confirming the sequence and identity of the gene encoding ABC transporter substrate-binding protein of *A. baumannii* can be accessed through the journal website at https://aprh.journals.ekb.eg/article_296898.html.

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