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(54) Title: VACCINE AGAINST ACINETOBACTER BAUMANNII BASED ON CELLULAR COMPONENTS DEFICIENT IN LIPOPOLYSACCHARIDE

(57) Abstract: The invention refers to a composition comprising inactivated cells deficient in LPS from the genus Acinetobacter and/or outer membrane vesicles from the same and their use for the manufacture of a medicament, preferably a vaccine, for the prevention of diseases produced by organisms of the genus Acinetobacter.
Vaccine against *Acinetobacter baumannii* based on cellular components deficient in lipopolysaccharide

**Field of the invention**

The present invention refers, in general, to the field of pharmacology and immunology, and, in particular, to a vaccine composition suitable for the prophylactic treatment of an infection caused by an *Acinetobacter baumannii* strain in a mammal.

**Background of the invention**

The increasing clinical importance of infections caused by multidrug resistant *A. baumannii* warrants the development of novel approaches for the prevention and treatment of infections caused by this pathogen. In this context, immunization of certain patient populations could contribute to reducing the morbidity and mortality caused by this pathogen. Vaccines against Gram-negative bacteria based on inactivated whole bacterial cells are highly immunogenic and have been shown to produce protective immunity against a number of bacterial species. However, the high levels of endotoxin, due to the presence of lipopolysaccharide, in these vaccines complicate their use in humans.

*Acinetobacter baumannii* is a Gram-negative coccabacillus with clinical importance in the hospital environment. This organism is highly distributed in soil and environmental water sources (Baumann, P. 1968. *J. Bacteriol.* 96, 39-42), and can cause different types of infections as a nosocomial pathogen such as pneumonia, bacteremia, meningitis and skin and soft tissue infection, among others (García-Quintanilla et al., 2013. *Curr. Pharm. Biotechnol.* In press). This pathogen typically infects patients receiving mechanical ventilation and patients sustaining burn injury (Muñoz-Price y Weinstein. 2008. *N. Engl. J. Med.* 358, 1271-1281). However, it has also been isolated in cases of community-acquired pneumonia (Ho et al., 2009. *Chest.* 136, 1119-1127; Ong et al., 2009. *Respirology* 14, 1200-1205) and in military personnel sustaining war-related trauma in Vietnam, Iraq, Kuwait and Afghanistan (Jones et al., 2006. *Lancet Infect. Dis.* 6, 317-318; Tong, 1972. *JAMA* 219, 1044-1047). The crude
mortality rates associated with infection by A. baumannii are between 35% and 70% for nosocomial infections (Vila y Pachón, 2008. Expert Opin. Pharmacother. 9, 587-599). Due to the ability of A. baumannii to acquire resistance to antibiotics, the number of multidrug resistant strains has increased dramatically over the preceding years (Tasbakan et al., 2009. Mikrobiyol Bul. 43, 61-70; Valencia et al., 2009. Infect. Control Hosp. Epidemiol. 30: 257-263). The appearance of these highly resistant strains has complicated the clinical Management of infections caused by A. baumannii. In this context, the development of a vaccine against A. baumannii could reduce the morbidity and mortality caused by this pathogen (Pachón y McConnell, 2014. Vaccine, In press).

The experimental vaccines that have been described for A. baumannii can be classified into two broad groups, vaccines consisting of a single purified antigen, and multicomponent vaccines. Within the first group, the outer membrane protein OmpA (Luo et al., 2012. PLoS One 7, e29446), the biofilm associated protein Bap (Fattahian et al., 2011. Microb. Pathog. 51, 402-406), the membrane transporter Ata (Bentancor et al., 2012. Infect. Immun. 80, 3381-3388), and the surface polysaccharide poly-N-acetyl-β-(1-6)-glucosamine (Bentancor et al., 2012. Infect Immun 80, 651-656) have been described as good candidates due to their capacity to elicit a specific immune response. However, the experiments testing survival after active immunization have only demonstrated that OmpA provides partial protection, and that the expression of Bap has not been clearly demonstrated in strains that do no form biofilm. The approaches that employ multicomponent vaccines include outer membrane complexes (McConnell et al., 2011. Infect. Immun. 79, 518-526), outer membrane vesicles (outer membrane vesicles; McConnell et al., 2011. Vaccine 29,5705-5710) and inactivated whole cells (McConnell y Pachón, 2010. Vaccine 29: 1-5). Each of these vaccines induces a strong immune response and is able to elicit high levels of protection against infection in a murine model using the ATCC 19606 strain and clinical isolates. However, in spite of these promising results, the use of these vaccines in humans is complicated in view of the high levels of endotoxin in these vaccines due to the high amounts of lipopolysaccharide (LPS) present in these preparations.
LPS is formed by the O antigen, a core polysaccharide and lipid A, which is responsible for the endotoxin activity of LPS. The first studies that employed *Escherichia coli* demonstrated that the production of LPS was essential for bacterial viability (Raetz, 1990. *Annu. Rev Biochem.* 59, 129-170). However, subsequent work demonstrated that certain bacterial species, such as *Neisseria meningitidis* y *Moraxella catarrhalis*, were viable after mutation of the genes encoding enzymes involved in LPS biosynthesis, resulting in strains completely lacking LPS (Peng *et al*., 2005. *Infect. Immun.* 73, 7569-7577; Steeghs *et al*., 1999. *Infect. Immun.* 67, 4988-4993). A recent study demonstrated that *A. baumannii* can acquire resistance to the antibiotic colistin through mutation of the genes involved in LPS biosynthesis *lpxA*, *lpxC* and *lpxD* (Moffatt *et al*., 2010. *Antimicrob. Agents Chemother.* 54, 4971-4977), living rise to strains completely deficient in LPS. This results indicate that *A. baumannii* is also viable in the absence of LPS, raising the possibility of developing vaccines based on these strains.

Outer membrane vesicles (OMVs) are vesicles derived from the bacterial outer membrane that are secreted from numerous Gram-negative bacteria (Kulp *et al*., 2010. *Annu. Rev. Microbiol.* 64, 163-184). OMVs are spherical vesicles of approximately 20-200 nm that are composed of outer membrane proteins, periplasmic proteins and LPS (Kuehn *et al*., 2005. *Genes Dev.* 19, 2645-2655; Mashburn *et al*., 2005. *Nature.* 437, 422-425). Secreted OMVs have been shown to participate in the detection of quorum sensing, the transport of virulence factors and the transfer of genes, indicating that they play a role in bacterial patogenesis. It has also been demonstrated that OMVs can deliver proteins to the interior of host cells through fusion with lipid rafts, suggesting that OMVs can be used to transport bacterial products over large distances (Kesty *et al*., 2004. *Embo J.* 23, 4538-4549). A recent study by Kwon et al demonstrated that a clinical isolate of *A. baumannii* secreted OMVs during growth in vitro (Kwon *et al*., 2009. *FEMS Microbiol. Lett.* 297, 150-156). A proteomic analysis of the OMVs demonstrated that they contain multiple virulence factors and immunomodulating proteins, suggesting that OMVs play an important role in the pathogenesis of *A. baumannii*. 
Vaccines based on OMVs have been developed for various Gram-negative bacteria including *Neisseria meningitidis*, *Helicobacter pylori*, and *Vibrio cholerae* (Bjune *et al.*, 1991. *NIPH Ann.* 14, 125-130; Keenan *et al.*, 2003. *FEMS Immunol. Med. Microbiol.* 36, 199-205; Bishop *et al.*, 2012. *J. Infect. Dis.* 205, 412-21). Immunization with OMVs has been shown to induce antibodies against multiple bacterial antigens, and the capacity to provide protective immunity in animal models of infection. In addition, the OMVs isolated form *N. meningitidis* serogroup B have been shown to be safe and immunogenic in humans, and have been used to control an outbreak of meningococcus meningitis in New Zealand (Nokleby *et al.*, 2007. *Vaccine.* 25, 3080-84).

The infections caused by *A. baumannii* often occur in outbreaks caused by a single clone. For this reason, a vaccine based on OMVs could be more effective if the OMVs are isolated form the causative clone. The purification of OMVs from bacterial cultures is rapid and simple, requiring only filtering of the culture supernatant and concentration of the OMVs.

**Brief description of the invention**

**Aspect A:** This aspect of the invention refers to a pharmaceutical composition, preferably a vaccine composition, suitable for the prophylactic treatment (before infection) of an infection caused by an *Acinetobacter baumannii* strain in a mammal, which comprises:

a. an *Acinetobacter baumannii* strain whole cell deficient in lipopolysaccharide (LPS) characterized by the partial or complete inactivation of one or various cellular nucleic acid molecules that encode endogenous LPS; and/or

b. an outer membrane vesicle (OMV) derived from an *Acinetobacter baumannii* strain deficient in lipopolysaccharide (LPS) as defined in paragraph a) above.

In a prefer embodiment of this aspect of the invention, the *Acinetobacter baumannii* strain deficient in lipopolysaccharide (LPS) is characterized by the partial or complete inactivation of the genes selected from the list consisting of lpxA, lpxB and/or lpxC.
In another prefer embodiment of this aspect of the invention or of any of its preferred embodiments, the pharmaceutical composition, preferably the vaccine composition, further comprises a, preferably recombinant, polypeptide selected from the list consisting of:

a. Amino acid sequence SEQ ID No 27 (putative ferric siderophore receptor (A. baumannii ATCC 17978; accession number YP_001084684)) or a fragment thereof, wherein the term fragment is understood herein as biologically active fragments selected from the list consisting of SEQ ID No 1 to SEQ ID NO 11 or any combination thereof, or an amino acid sequence having at least 85% identity with any of sequences SEQ ID NO 1 to SEQ ID No 11; and/or

b. Amino acid sequence SEQ ID No 28 (putative ferric hydroximate siderophore receptor (A. baumannii ATCC 17978; accession number YP_001084696)) or a fragment thereof, wherein the term fragment is understood herein as biologically active fragments selected from the list consisting of SEQ ID NO 12 to SEQ ID NO 23 or any combination thereof, or sequences that have at least 85% identity with the amino acids SEQ ID NO 12 to SEC ID NO 23.

In another prefer embodiment of this aspect of the invention or of any of its preferred embodiments, the pharmaceutical composition, preferably the vaccine composition, further comprises a purified outer membrane protein sequence of A. baumannii selected from the list consisting of: SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49; SEQ ID NO: 50; SEQ ID NO: 51; SEQ ID NO: 52; SEQ ID NO: 53; SEQ ID NO: 54; SEQ ID NO: 55; SEQ ID NO: 56; SEQ ID NO: 57; SEQ ID NO: 58; SEQ ID NO: 59; SEQ ID NO: 60; SEQ ID NO: 61; SEQ ID NO: 62; SEQ ID NO: 63; SEQ ID NO: 64; SEQ ID NO: 65; SEQ ID NO: 66; SEQ ID NO: 67; SEQ ID NO:
In yet another prefer embodiment of this aspect of the invention or of any of its preferred embodiments, the pharmaceutical composition, preferably the vaccine composition, further comprises fusion recombinant polypeptides sequences SEQ ID NO: 24 and/or 25 and/or the amino acid sequence coded by nucleotide sequence SEQ ID NO: 26.

In yet another prefer embodiment of this aspect of the invention or of any of its preferred embodiments, the *Acinetobacter baumannii* strain deficient in lipopolysaccharide (LPS) comprises or is transformed, transduced or transfected with a nucleotide sequence capable of coding for any of the amino acid sequences as defined in any of the precedent paragraphs so that such strain is capable of producing the exogenous expression any of these amino acid sequences.

In yet another prefer embodiment of this aspect of the invention or of any of its preferred embodiments, said pharmaceutical composition, preferably the vaccine composition, further comprises a vector, such as viral vector, a plasmid or an expression cassette comprising a nucleotide sequence capable of coding for any of the amino acid sequences as defined in any of the precedent paragraphs and expressing said amino acid sequences.

In yet another prefer embodiment of this aspect of the invention or of any of its preferred embodiments, the *Acinetobacter baumannii* strain deficient in lipopolysaccharide (LPS) is inactivated. Preferably, said strain or cell is derived from ATCC strain 19606.

**Aspect B:** This aspect of the invention refers to the pharmaceutical composition, preferably the vaccine composition, of the aspect A of the invention or of any of its preferred embodiments, for use in the prophylactic
treatment or for the active immunization of an infection caused by A. baumannii in a mammal, preferably in a human.

**Aspect C:** This aspect of the invention refers to a vaccine composition comprising an antibody (monoclonal or polyclonal) or a fragment thereof, preferably selected from the list consisting of Fab, Fab', Fab'-SH, Fv, scFv, F(ab')₂, Vhh, nanobody and diabody, having affinity or binding affinity against the *Acinetobacter baumannii* strain deficient in lipopolysaccharide (LPS) and/or against an outer membrane vesicle (OMV) derived therefrom. In a preferred embodiment, said antibody or fragment thereof specifically binds the *Acinetobacter baumannii* strain deficient in lipopolysaccharide (LPS) and/or to the outer membrane vesicle (OMV) derived therefrom.

"Affinity" or "binding affinity" KD are often determined by measurement of the equilibrium association constant (ka) and equilibrium dissociation constant (kd) and calculating the quotient of kd to ka (KD = kd/ka).

The term "specifically binding" means that the antibody binds to the LPS deficient strain or to the OMV derived therefrom with an affinity KD of lower than or equal to 10⁻⁹M (monovalent affinity). The antibody may have substantially greater affinity for the target antigen compared to other unrelated molecules. The antibody may also have substantially greater affinity for the target antigen compared to homologs, e.g. at least 1.5-fold, 2-fold, 5-fold 10-fold, 100-fold, 10²-fold, 10⁴-fold, 10⁵-fold, 10⁶-fold or greater relative affinity for the target antigen. Such affinities may be readily determined using conventional techniques, such as by equilibrium dialysis; by radioimmunoassay using radiolabeled target antigen; or by another method known to the skilled artisan.

The affinity data may be analyzed, for example, by the method described in [Kaufman RJ, Sharp PA. (1982) Amplification and expression of sequences cotransfected with a modular dihydrofolate reductase complementary dna gene. J Mol Biol.159:601 -621 ].

The term "antibody" is used in the broadest sense and includes fully assembled antibodies, monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), antibody fragments that can bind the antigen ( e.g., Fab', F'(ab)₂, Fv, single chain antibodies, diabodies),
camelbodies and recombinant peptides comprising the forgoing as long as they exhibit the desired biological activity.

A "functional fragment" or "antigen-binding antibody fragment" of an antibody/immunoglobulin hereby is defined as a fragment of an antibody/immunoglobulin (e.g., a variable region of an IgG) that retains the antigen-binding region. An "antigen- binding region" of an antibody typically is found in one or more hypervariable region(s) of an antibody, i.e., the CDR-1 , -2, and/or -3 regions; however, the variable "framework" regions can also play an important role in antigen binding, such as by providing a scaffold for the CDRs.

The term "hypervariable" region refers to the amino acid residues of the variable domains VH and VL of an antibody or functional fragment which are responsible for antigen- binding.

Nonlimiting examples of antibody fragments include Fab, Fab', F(ab')2, Fv, domain antibody (dAb), complementarity determining region (CDR) fragments, single-chain antibodies (scFv), single chain antibody fragments, diabodies, triabodies, tetrabodies, minibodies, linear antibodies [Johnson G , Wu TT. (2000) Kabat database and its applications: 30 years after the first variability plot. Nucleic Acids Res. 28:214-218]; chelating recombinant antibodies, tribodies or bibodies, intrabodies, nanobodies, small modular immunopharmaceuticals (SMI Ps), an antigen-binding-domain immunoglobulin fusion protein, a camelized antibody, a VHH containing antibody, or muteins or derivatives thereof, and polypeptides that contain at least a portion of an immunoglobulin that is sufficient to confer specific antigen binding to the polypeptide, such as a CDR sequence, as long as the antibody retains the desired biological activity; and multispecific antibodies formed from antibody fragments [Chothia C, Lesk AM. (1987) Canonical structures for the hypervariable regions of immunoglobulins. J Mol Biol. 196:901 -917; Zapata G, Ridgway JB, Mordenti J, Osaka G, Wong WL, Bennett GL, Carter P. (1995) Engineering linear F(ab')2 fragments for efficient production in Escherichia coli and enhanced antiproliferative activity. Protein Eng. 8:1057-1062].
In a preferred embodiment of aspect C of the invention, the pharmaceutical or vaccine composition is obtained or obtainable after immunizing a mammal with the vaccine composition as defined in the first aspect of the invention.

**Aspect D:** This aspect of the invention refers to the vaccine composition as defined in aspect C of the invention, for use in the therapeutic treatment (after the infection), or in the passive immunization, of an infection caused by A. baumannii in a mammal, preferably in a human.

**Aspect E:** This aspect of the invention refers to an *Acinetobacter baumannii* strain deficient in lipopolysaccharide (LPS), transformed, transduced or transfected with a nucleotide sequence capable of coding for any of the amino acid sequences as defined in aspect A of the invention so that such strain is capable of producing the exogenous expression any of said amino acid sequences. Preferably, said *A. baumannii* deficient strain is use as a medicament.

**Aspect F:** This aspect of the invention refers to a method for the production of antibodies or fragments thereof, preferably selected from the list consisting of Fab, Fab', Fab'-SH, Fv, scFv, F(ab')2, Vhh, nanobody and diabody, which comprises:

a. Selecting an antibody or a fragment thereof, preferably from an antibody library;

b. Using an *Acinetobacter baumannii* strain deficient in lipopolysaccharide (LPS) and/or an outer membrane vesicle (OMV) derived therefrom, as an antigenic target;

c. Selecting those antibodies or fragments thereof having affinity or binding affinity or capable of specifically binding such strain and/or OMV; and

d. Producing those antibodies or fragments thereof identified in step c) above

It is noted, that Serotype specific variability in *A. baumannii* relies on variations in epitopes of the LPS component. By eliminating the LPS component
according to the method as detailed in aspect F above, we focus the antibody raising to antibodies against less variable epitopes of the bacterial surface, not related to the LPS component. These epitopes are conserved among A. baumannii strains or individual isolates belonging to all A. baumannii international clones used in A. baumannii classification. This method thus provides the tools for a universal treatment against A. baumannii infections independently of the origin of the strain(s) responsible for the host infection.

**Aspect G:** This aspect of the invention refers to an antibody or fragment thereof produced, obtained or obtainable by the method of aspect F of the invention.

**Aspect H:** This aspect of the invention refers to the use of an Acinetobacter baumannii strain deficient in lipopolysaccharide (LPS) and/or an outer membrane vesicle (OMV) derived therefrom, for the production of antibodies or fragments thereof having affinity or binding affinity or capable of specifically binding such strain and/or OMV.

**Detailed description of the invention**

The present invention refers to compositions and vaccines that consist of whole cells deficient in LPS and/or outer membrane vesicles of Acientobacter baumannii (A. baumannii) capable of conferring protection against infection caused by infectious pathogens.

The authors of the present invention demonstrate that inactivated cells of A. baumannii deficient in LPS and/or outer membrane vesicles from A. baumannii, upon being inoculated produce immunization, which provides protection against posterior infection by said bacteria, which demonstrates the utility of these cells or strains as prophylactic vaccines against infections caused by A. baumannii.

**A first aspect** of the present invention refers to an Acinetobacter cell or strain that is deficient in LPS, hereinafter cell or strain of the invention.

The species of Acinetobacter are strictly aerobic non-fermenting and non-motile bacilli that are oxidase negative and appear in pairs by microscopy. They are distributed widely in nature, and are important in soil and contribute to its mineralization.
It is understood that "inactivated cell" in the present invention is a cell that does not have the ability to replicate but that conserves its immunogenic capacity. The cells of the present invention are inactivated prior to their inoculation to prevent their replication in the host, and therefore prevent invention produced by their administration. The inactivation of the cells of the invention can be performed using diverse methods known in the state of the art for example, although not limited to, adsorption, heat, ultraviolet light, ionizing radiation, ultrasound, phenol, formol, formaldehyde, crystal violet, glyceraldehyde, ethylene oxide, propiolactone, ethylenamina, bromoethylenamina or formalin.

In a preferred embodiment, the cells of the invention are inactivating with formalin. In another preferred embodiment the cells of the invention are from the species *Acinetobacter baumannii* and they are inactivated with formalin.

In a preferred embodiment of this aspect of the invention, the deficiency in LPS can be achieved by partial or complete inactivation of one or various cellular molecules of nucleic acids that encode the endogenous genes for the LPS subunits, particularly *lpxA, lpxB* and/or *lpxC* of LPS.

In another preferred embodiment of the invention, the cell or strain of *Acinetobacter* deficient in LPS is obtained by deletions and/or insertions of one or various nucleotides in nucleic acid sequences encoding the gene involved in the biosynthesis of LPS and/or the sequences that control their expression. The deletions and/or insertions can be generation by homologous recombination, insertion of transposons, or other adequate methods known in the state of the art.

In preferred embodiment of the invention, the sequence is inactivated e.g by construction of a suicide vector that contains the gene *lpxA, lpxB, lpxC, lpxD, lpxK, lpxL* and/or *lpxM* or any of their combination, or interrupting with a marker gene for selection, transforming the target cells with the vector and screening for positive cells that are negative for LPS expression.

In another preferred embodiment of the invention, the cell or strain of the invention is preferably an *A. baumannii* cell, particularly an attenuated *A. baumannii* cell or other *Acinetobacters; Acinetobacter bayyi, A. beijerinckii, A. bereziniae, A. boissieri, A. bouvetii, A. brisouii, A. calcoaceticus, A.gernerii,* A.

In this report, it is understood that Acinetobacter refers to the kingdom Bacteria, phylum Proteobacteria, class Gammaproteobacteria, order Pseudomonadales, family Moraxellaceae.

It is understood that "cells or strains of the Acinetobacter baumannii" in the present invention are those cells pertaining to the domain Bacteria, phylum Proteobacteria, class Gammaproteobacteria, order Pseudomonadales, family Moraxellaceae, genus Acinetobacter, species Acinetobacter baumannii.

It is understood that "lipopolysaccharide (LPS) or lipooligosaccharide" is a component that is found on the external membrane of various Gram-negative bacteria. The term LPS is used often and interchangeably with "endotoxin", due to its history of discovery. LPS consists of a polysaccharide chain and the rest is lipid, known as lipid A, which is responsible for the endotoxin activity. The polysaccharide chain is variable between different bacteria and determines the serotype. Endotoxin is of approximately 10 kDa in size, but can form large aggregates of up to 1000 kDa. Humans are able to produce antibodies against LPS, but in general these antibodies can only protect against bacteria of a specific serotype. Endotoxin is responsible for many of the clinical manifestations of infections caused by Gram-negative bacteria such as Neisseria meningitidis and Acinetobacter baumannii.

COMPOSITION OF THE INVENTION

A second aspect of the invention refers to a composition, hereinafter composition of the invention comprising:

a) a cell or strain of the invention, and

b) optionally a sequence of nucleotides and/or amino acids or polypeptides.

In a more preferred embodiment of this aspect of the invention, the nucleic acid and/or the amino acid sequence or polypeptide is recombinant.
In a still more preferred embodiment, the polypeptide is selected from

I) the peptide sequence SEQ ID NO: 27 (putative ferric siderophore receptor (A. baumannii ATCC 17978; accession number YP_001084684)) or a fragment thereof, wherein the fragments are biologically active fragments, preferably selected from the list consisting of SEQ ID NO: 1 to SEQ ID NO: 11, or any of its combinations, or sequences having at least 85% sequence identity with the peptide sequences SEQ ID NO: 1 to SEQ ID NO: 11, and/or

II) the peptide sequence SEQ ID No 28 (putative ferric hydroximate siderophore receptor (A. baumannii ATCC 17978; accesoión number YP_001084696)) or a fragment thereof, wherein the fragments are biologically active fragments, preferably selected from the list consisting of SEQ ID NO: 12 to SEQ ID NO: 23, or any of its combinations, or sequences having at least 85% sequence identity with the peptide sequences SEQ ID NO: 12 to SEQ ID NO: 23.

In a still more preferred embodiment, the composition of the invention comprises the amino acid sequence SEQ ID NO: 28 and the amino acid sequence SEQ ID NO: 27.

In another preferred embodiment, the composition of the invention comprises a fusion protein that consists of at least 2, preferably 3, more preferably 4, amino acid sequences from the following list consisting of: SEQ ID NO: 1 to SEQ ID NO: 23 or a variant of these sequences having at least 85% identity with the sequences SEQ ID NO: 1 to SEQ ID NO: 23.

In another preferred embodiment, the fusion protein comprises the amino acid sequence SEQ ID NO: 24 or the amino acid sequence SEQ ID NO: 25.

In another preferred embodiment, the composition of the invention comprises a nucleotide sequence, hereinafter nucleotide sequence of the invention, capable of transcribing an amino acid sequence described in the invention. More preferably, the nucleotide sequence is SEQ ID NO: 26.

The fragments described previously differ in amino acid sequence by at least one amino acid. The most preferred variations are those having at least 85%,
or more, including 90%, 93% or more, and preferably 95% or more, 96% or more, 97% or more, 98% or more, 99% or more of sequence identity with any of the polypeptides shown in SEQ ID NO: 1 to SEQ ID NO: 25. More preferably, the invention refers to a sequence variant characterized by at least one (at least two, at least three, at least four) mutation(s) in relation to any of the polypeptides SEQ ID NO: 1 to SEQ ID NO: 25. In agreement with the invention as described in the descriptive section, mutation can refer to any mutation selected by insertion(s), deletion(s), and substitution(s). Preferably substitution(s).

In another preferred embodiment, the composition of the invention comprises an expression vector (hereinafter expression vector of the invention), comprising a nucleotide sequence of the invention.

In another preferred embodiment, the composition of the invention also comprises outer membrane vesicles, hereinafter outer membrane vesicles of the invention, deficient in LPS.

In another preferred embodiment, the composition of the invention also comprises at least one or the proteins purified from the membrane of A. baumannii with amino acid sequence SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46; SEQ ID NO: 47; SEQ ID NO: 48; SEQ ID NO: 49; SEQ ID NO: 50; SEQ ID NO: 51; SEQ ID NO: 52; SEQ ID NO: 53; SEQ ID NO: 54; SEQ ID NO: 55; SEQ ID NO: 56; SEQ ID NO: 57; SEQ ID NO: 58; SEQ ID NO: 59; SEQ ID NO: 60; SEQ ID NO: 61; SEQ ID NO: 62; SEQ ID NO: 63; SEQ ID NO: 64; SEQ ID NO: 65; SEQ ID NO: 66; SEQ ID NO: 67; SEQ ID NO: 68; SEQ ID NO: 69; SEQ ID NO: 70; SEQ ID NO: 71; SEQ ID NO: 72; SEQ ID NO: 73; SEQ ID NO: 74; SEQ ID NO: 75; SEQ ID NO: 76; SEQ ID NO: 77; SEQ ID NO: 78; SEQ ID NO: 79; SEQ ID NO: 80; SEQ ID NO: 81; SEQ ID NO: 82; SEQ ID NO: 83; SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, or SEQ ID NO: 89, or any of their combinations.

More preferably, at least one of the proteins of the outer membrane of A. baumannii is obtained by a process comprising:
a. Inoculating one liter of Mueller-Hinton broth with a colony of A. baumannii ATCC 19606;

b. incubating the culture until an optical density of 0.6 at 600 nm;

c. Washing bacterial cells with 30 ml of 10 mM phosphate buffer pH, 7.2;

d. Centrifuge at 6000 x g for 10 min;

e. Resuspend the bacterial pellet sin 10 ml of 10 mM phosphate buffer pH 7.2 and lysing by sonication 5 times for 1 minute;

f. Eliminate unlysed cells by centrifugation at 6000 x g for 5 minutes;

g. Centrifuge the supernatant at 4 °C at 20000 x g for one hour;

h. Eliminate proteins of the external membrane by solubilising with 5 ml of 2% N-lauraylsarcosinante in 10 mM phosphate buffer pH 7.2 for 30 minutes at 37 °C;

i. Precipitate the insolube fraction (that contains the outer membrane proteins) by centrifuging at 4 °C at 20000 x g for one hour;

j. Wash the pellet once with 2 ml 62.5 mM Tric-HCl ph, 6.8 and centrifuge at 4 °C for one hour;

k. Resuspend the pellet in a solution of 5% SDS and precipitate with methanol/chloroform; and

l. Resuspend the pellet in sterile PBS.

In another preferred embodiment of the invention, the composition of the invention is a pharmaceutical composition, more preferably also comprises an acceptable pharmaceutical vehicle, and still more preferably, also comprises another active ingredient.

In another preferred embodiment, the composition of the invention also comprises an adjuvant. In another preferred embodiment, the composition of the invention is a vaccine.
In the context of the present invention, the term “vaccine” refers to an antigenic preparation employed for inducing an immune response to a disease. They are prepared from antigens that, once inside the host, provoke an immune response through the production of antibodies, and generate immunologic memory producing transient or permanent immunity. It is noted that as used herein the term “vaccine” can also be understood as a preparation from antibodies or fragments thereof suitable for the therapeutic treatment or for passive immunization of an infection caused by an Acinetobacter strains, in particular from an A. baumannii strain.

NUCLEOTIDE SEQUENCE AND EXPRESSION VECTOR

A third aspect of the invention refers to a nucleotide sequence, hereinafter second nucleotide sequence of the invention, which encodes any of SEQ ID NO 1 to 25, 27 or 28 or any combination thereof. Said second nucleotide sequence of the invention also includes nucleic acid sequence SEQ ID NO: 26.

A fourth aspect of the invention refers to an expression vector, hereinafter expression vector of the invention, comprising the second nucleotide sequence of the invention.

In a preferred embodiment of this aspect, the cell of the invention comprises the expression vector of the invention.

The nucleic acid can be localized in a recombinant vector. Preferably, the recombinant vector is a prokaryotic vector, that is a vector that contains elements for replication and/or integration into the genome of prokaryotic cells. Preferably, the recombinant vector contains the nucleic acid molecule of the present invention operatively linked to an expression control sequence. The control sequence is preferably a sequence controlling the active expression in Acinetobacter, particularly in A. baumannii. The vector can be an extrachromosomal vector adequate for integration in the chromosome. Examples of such as vectors are known by experts in the field, for example in Sambrook et al. supra.

OUTER MEMBRANE VESICLES OF THE INVENTION
A fifth aspect of the invention refers to an outer membrane vesicle that is deficient in LPS. In a preferred embodiment of this aspect, the outer membrane vesicle is obtained from the cell or strain of the invention.

USE OF THE CELLS, VESICLES AND THE COMPOSITIONS OF THE INVENTION

A sixth aspect refers to the composition of the invention for use as a medicament, or alternatively, to the use of the composition of the invention for the manufacture of a medicament.

The term “medicament” or “pharmaceutical composition” as used in this report, makes reference to any substance used for the prevention, alleviation, treatment or cure of a disease in man or animals. The context of the present invention refers to a composition comprising the composition of the invention. This composition of the invention comprises inactivated cells of the genus Acinetobacter and/or outer membrane vesicles of the same in a quantity therapeutically effective, that is able to inducer an immune response in the organism in which they are administered against an organism of the genus Acinetobacter. The term “medicament” or “pharmaceutical composition” therefore is known as a vaccine.

The dosing for obtaining the effective therapeutic quantity depends on a variety of factors such as for example, age, weight, sex, tolerante,... of the mammal. As used in this description, the “effective therapeutic quantity” refers to the quantity of inactive cells of the genus Acinetobacter and/or outer membrane vesicles that produce the desired effect, and in general are determined by the therapeutic effect that is desired.

A seventh aspect of the invention refers to the composition of the invention for the prevention, the improvement or the treatment of an infection caused by A. baumannii, en a mammal, or alternatively, to the use of the composition of the invention for the elaboration of a medicament for the prevention, improvement or treatment of an infection caused by A. baumannii in a mammal.

It is understood by “disease produced by organisms of the genus Acientobacter” those diseases in which the causal agent of the pathology is from the genus
Acinetobacter, or any of its metabolic products. The genus Acinetobacter produces diverse pathologies for example but not limited to, bacteremia, meningitis, urinary tract infections, skin and soft tissue infections, surgical site infections and pneumonia. For these reasons, one of the more preferred forms, diseases produced by organisms of the genus Acinetobacter are selected from a list that consists of bacteremia, meningitis, urinary tract infections, skin and soft tissue infections, surgical site infections and pneumonia.

The medicaments and compositions of the invention can be used alone or in combination with other medicaments or compositions for the treatment of diseases produced by organisms from the genus Acinetobacter.

Both the medicaments and the compositions of the invention can also include pharmaceutically acceptable vehicles or excipients

The medicaments and compositions of the invention may be used either alone or in combination with other medicaments or compositions for the treatment or prevention of diseases caused by organisms of the genus Acinetobacter.

The term “excipient” makes reference to a substance that helps in the absorption of the elements of the composition of the medicaments of the invention, stabilizing said elements, activating or helping the preparation of the medicament such that it provides consistency or flavours that make it more palatable. The excipients can maintain the ingredients together, like for example is the case with starches, sugars, cellulose, sweetners, coloring agents, the function of protecting the medicament, for example isolating it form air and/or humidity, the function of filling the pill, capsule or any other form of presentation, for example, the case of dibasic calcium phosphate, the function for facilitating dissolution of the components and their absorption in the intestine, without excluding other types of excipients described in this paragraph.

The vehicle, in the same way as the excipient, is a substance that is used in the medicament to dilute any of the components of the present invention to a desired volume or weight. The pharmaceutically acceptable vehicle is an inert substance or of similar action to any of the elements of the present invention.
The function of the vehicle is to facilitate the incorporation of other elements, permit better dosing and administration and give consistency and form to the medicament. When the form of presentation is liquid, the pharmaceutically acceptable vehicle is the diluent.

The adjuvants and pharmaceutically acceptable vehicle that can be used in the composition of the invention are those vehicles known by experts in the field.

In this report, the term “adjuvant” refers to any agent that does not poses antigenic activity in and of itself, that can be used to stimulate the immune system to increase the response to a vaccine. There are many adjuvants, for example but not limited to, aluminium phosphate, aluminium hydroxide, toll-like receptor agonists, cytokines, squaline, Freunds incomplete and complete adjuvants. In a preferred form of this aspect of the invention, the adjuvant is selected for a list that consists of aluminium phosphate, aluminium hydroxide, toll-like receptor agonists, cytokines, squaline, Freunds incomplete and complete adjuvants. In a still more preferred form of this aspect of the invention, the adjuvant is aluminium phosphate.

As used here, the term “active ingredient”, “active substance” or “pharmaceutically active substance” or “pharmaceutically active ingredient” refers to any component that potentially provides pharmacological activity or other different effect in the diagnosis, cure, alleviation, treatment or prevention of a disease or that affects the structure or function of the human or animal body. The term includes those components that promote a chemical change in the elaboration of the drug and are present in the same and a modified form that provides specific activity or the effect.

An eighth aspect of the invention refers to the composition of the invention for conferring protection against an infection caused by A. baumannii en a mammal, or alternatively, the use of the composition of the invention in the elaboration of a medicament for conferring protection against an infection caused by A. baumannii in a mammal.

Another aspect of the invention refers to the fusion protein or peptide of the invention or the composition of the first or second aspect of the invention or the
pharmaceutical composition of the invention that can be administered once or various, such as two, three, four, five, six, seven, eight, nine or ten or more times. There are no particular limitations relative to the quantity of the active ingredient per dose.

An additional aspect of the present invention refers to a composition that consists of an antibody or fragment thereof that is capable of binding to SEQ ID NO: 27 or SEQ ID NO: 28 or a fusion protein as defined in the second aspect or the fusion protein of the invention, wherein preferably said composition is a pharmaceutical composition, preferably a vaccine, and wherein said pharmaceutical composition is used in the treatment or prevention of an infection caused by \textit{A. baumannii}.

A \textit{ninth aspect} of the invention refers to an antibody or an active fragment thereof obtainable by immunization of a mammal with the composition of the first or second aspect of the invention or with the fusion protein of the invention, preferably said antibody or active fragment consists of a composition in which preferably said composition is a pharmaceutical composition and said pharmaceutical composition is used as a therapy, particularly for the treatment of infections caused by \textit{A. baumannii}.

\textit{METHOD FOR PREPARING AN ACINETOBACTER CELL FOLLOWING THE INVENTION}

A \textit{tenth aspect} of the invention refers to a method for preparing a cell of \textit{A. baumannii} as described above.

\textit{Acinetobacter}

In agreement with this aspect, this method consists of the steps to (i) provide a bacterial cell deficient in LPS, particularly a cell of \textit{Acinetobacter}, (ii) insert a recombinant nucleic acid molecule in said bacterial cell, encoding said nucleic acid molecule a fusion peptide that consists of (a) at least on domain of the polypeptide wherein said domain is capable of producing an immune response in mammal and (b) a domain of escape for the phagolysosome, and (iii) culture the obtained cell in agreement with step (ii) in adequate conditions. Preferably,
a cell capable of expressing said nucleic acid is obtained. More preferably, the cell is a cell of *A. baumannii*.

In agreement with the additional aspect, this method consists of step of (i) providing a bacterial cell deficient in LPS, particularly an *Acinetobacter* cell, (ii) inserting a recombinant nucleic acid molecule in said bacteria, encoding said nucleic acid molecule a peptide or polypeptide for escape from the phagolysosome, and (iii) culture the obtained cell in agreement with step (ii) in adequate conditions.

If desired, the method of the present invention consists of inserting at least one recombinant nucleic acid molecule in the bacterial cell, said molecule encoding a peptide or polypeptide capable of producing an immune response in mammals.

It is understood that “infection” in the present invention is that pathology generated by the invasion or colonization of any host tissue by any organisms of the genus *Acinetobacter*, preferably *Acinetobacter baumannii*.

It is understood that “soft tissue” in the present invention, is all non-bony tissue of an organism.

The term “prevention” as understood in the present invention consists of avoiding the appearance of damage whose cause is cells of the genus *Acinetobacter*, or any derivative or metabolic product of the same.

The term “antigen” in the report refers to a molecule (generally a protein or polysaccharide) that can induce the formation of antibodies. There many different types of molecules that can act as antigens, such as proteins, peptides, polysaccharides, and more rarely other molecules such as nucleic acids.

The term “resistance” refers to any mechanism of defense developed by bacteria against a drug. The mechanisms of resistance acquired and transmitted by bacteria are the most important and consist primarily of: the production of enzymes that inactivate antibiotics, appearance of modifications that impede the arrival of the drug to its target and/or alteration of the target
itself. One bacterial strain can develop various resistance mechanisms against one or many antibiotics and in the same way an antibiotic can be inactivated by distinct mechanisms from diverse bacterial species.

The terms “polynucleotide” and “nucleic acid” are used interchangeably, referring to polymeric forms of nucleotides of any length, both of DNA and RNA.

The terms “amino acid sequence”, “peptide”, “oligopeptide”, “polypeptide” and "protein” are used interchangeably, and refer to a polymeric form of amino acids of any length that can be chemically or biochemically modified.

During the description of the claims, the word “comprising” and its variants does not intend to exclude other technical characteristics, additives, components or steps. For those expert in the state of the art, other objects, advantages and characteristics of the invention are released in the section of the description and practice of the invention. The following examples and drawings are provided as illustrations, and are not intended to limit the present invention.

**DESCRIPTION OF THE FIGURES**

**Figure 1. Stability and endotoxin content of IB010.** (A) Genomic DNA from three independent cultures of ATCC 19606 and IB010 was extracted and amplified using primers specific for the lpxD gene. The band corresponding to approximately 1000 Kb corresponds to the intact lpxD gene, whereas the faster migrating band corresponds to the lpxD gene with a deletion of 462 nucleotides. (B) Endotoxin levels of ATCC 19606 and IB010 determined by the Limulus Amebocyte Assay. Bars represent the median values of three independent cultures, and error bars represent the standard error of the mean. EU; endotoxin units.

**Figure 2. Antibody response to immunization with IB010.** Serum samples were collected from ATCC 19606 vaccinated, IB010 vaccinated and control mice before vaccination (Day 0) and at day 7 and 21 after the first immunization, and levels of antigen specific total IgG (A) and IgM (B) were measured by ELISA (n = 8 mice/group). IgG1 (C) and IgG2c (D) levels were measured in 21-day serum were measured by ELISA in ATCC 19606 vaccinated, IB010 vaccinated and control mice. In all panels box and whisker
plots represent the interquartile ranges and ranges, respectively, and horizontal lines represent median values. * p < 0.05 compared to levels in control mice at the same time point, # p < 0.05 compared to 7-day samples from the same experimental group, † p < 0.05 compared to 21-day samples in ATCC 19606 vaccinated mice.

**Figure 3. Effect of vaccination on tissue bacterial loads.** Immunized and control mice were infected with 2.0 x 10^6 cfu (300 x LD_{50}) of the ATCC 19606 strain and spleen bacterial loads were determined 12 hours post-infection (n = 8 mice/group). Data points represent bacterial loads from individual mice, and horizontal lines represent median values from groups of mice. * p < 0.05 compared to control mice.

**Figure 4. Effect of vaccination on post-infection pro-inflammatory cytokine levels.** Immunized and control mice were infected with 2.0 x 10^6 cfu (300 x LD_{50}) of the ATCC 19606 strain and serum levels of IL-1β, TNF-α, and IL-6 were determined (n = 8 mice/group). Data points represent cytokine levels from individual mice, and horizontal lines represent median values from groups of mice. * p < 0.05 compared to control mice, # p < 0.05 compared to ATCC 19606 vaccinated mice.

**Figure 5. Effect of vaccination on survival in a mouse model of disseminated A. baumannii infection.** Vaccinated and control mice were infected with 2.25 x 10^8 cfu (340.9 x LD_{50}) of the ATCC 19606 strain (A) or 1.05 x 10^6 cfu (2.18 x LD_{50}) of the A. baumannii clinical isolate Ab-154 (B), and survival was monitored over the following 7 days (n=8 mice/group). * p < 0.05 compared to control mice.

**Figure 6. Protein profile of the A. baumannii OMVs without LPS.** The strains ATCC 19606 and IB010 for the production of OMVs after culturing 24, 48 and 72 hours. After the purification of OMVs the amount of protein was quantified using Bradford method and 10 mcg of the protein was visualized in a 10% polyacrylamide gel with Coomassie stain.

**Figure 7. Effect of 2,2 Bipyridyl, iron chelator, in the protein profile of OMVs.** The strains ATCC 19606 and IB010 were used to analyze the effect of
2,2 bipyridyl (BIP) on the protein profile of the OMVs after culturing for 24 hours. In the case of the ATCC 19606 strain a concentration of 200 mcM was used while in the case of IB010 100 and 150 mcM was used. After the purification of OMVs the amount of protein was quantified using Bradford method and 10 mcg of the protein was visualized in a 10% polyacrylamide gel with Coomassie stain.

**Figure 8. Effect of 2,2 Bipyridyl, iron chelator, on the production of OMVs.** The strains ATCC 19606 and IB010 were used to analyze the effect of 2,2 bipyridyl (BIP) on the production of the OMVs after culturing for 24 hours. In the figure the total protein content of the OMVs is shown after treatment with Bip and measuring the concentration with Bradford.

**Figure 9. Visualization of OMVs.** OMVs purified form ATCC 19606 were fixed using glutaraldehyde at 1.6% and stained with osmium tetroxide and lead and uranium and visualized by electron microscopy.

**Figure 10. Visualization of purified OMVs.** OMVs purified form IB010 were fixed using glutaraldehyde at 1.6% and stained with osmium tetroxide and lead and uranium and visualized by electron microscopy.

**Figure 11. Protein profile of OMVs without LPS.** The strains IB010 and 167R were used to produce OMVs and the amount of protein was quantified from each sample. Ten mcg of the protein was visualized in a 10% polyacrylamide gel with Coomassie stain.

**EXAMPLES OF THE INVENTION**

**Example 1**

**Ethics Statement**

All experiments involving the use of animals were approved by the University Hospital Virgen del Rocio Committee on Ethics and Experimentation (Evaluation code: 2013PI/296). In all experiments, efforts were made to minimize suffering, and any animals appearing moribund during the course of experimentation were immediately euthanized using thiopental.
**Bacterial strains.** *A. baumannii* ATCC 19606 is an antibiotic susceptible reference strain. An LPS-deficient derivative of ATCC 19606 was obtained by plating an overnight culture of ATCC 19606 on Mueller Hinton agar containing 10 mg/l of colistin, as described previously (Clinical Laboratory Standards Institute 2013) Strains with mutations in the genes involved in LPS biosynthesis were identified by sequencing the *lpxA*, *lpxC* and *lpxD* genes of the colistin resistant mutants that were present after overnight growth at 37 °C. A strain with a large deletion in the *lpxD* gene was identified and designated IB010. Resistance to colistin was confirmed by broth microdilution according to Clinical Laboratory Standard Institute guidelines [23]. Absence of LPS was confirmed by measuring the endotoxin levels of three independent cultures of each strain using the QCL-1000 Limulus Ameocyte Assay (Lonza) according to the manufacturer’s instructions. The Ab-154 strain is a previously characterized *A. baumannii* clinical isolate (Gautam, 1997. *J. Clin. Microbiol.* 35, 2977-2980).

**Vaccine preparation and mouse immunization.** The IWC vaccines (both LPS-containing and LPS-deficient) were prepared as described based on a previously described method (Moffatt et al., 2010. *Antimicrob. Agents Chemother.* 54, 4971-4977). Briefly, the ATCC 19606 and IB010 strains were grown in Mueller- Hinton broth to OD_{600} of 0.8. In the case of IB010, 10 μg/ml of colistin were added to the culture. In order to confirm that no reversion to wild type occurred during growth of IB010, three independent cultures of ATCC 19606 and IB010 were grown, and genomic DNA was isolated from each culture using the QIAmp DNA Mini Kit (Qiagen). The *lpxD* specific primers 5' GCTAATTGGTGAAGGTAGTC 3' and 5' GACGAATCGTGTGAATCTGC 3' were used to amplify genomic DNA from the cultures in order to confirm that the deletion in *lpxD* of IB010 was present after growth.

For vaccine preparation, bacteria were washed extensively in phosphate buffer saline before inactivation in 0.5 M formalin for 18 h with shaking at room temperature. Complete inactivation of the bacteria was confirmed by plating on blood agar. The concentration of inactivated cells was adjusted to 1x10^{10} cells/ml and combined 1:1 (v/v) with the aluminium-based adjuvant, Alhydrogel 2% (w/v) (InvivoGen). Vaccination was carried out in 6 to 8-week-old, female C57BL/6 mice by intramuscular injection of 100 μl of the vaccine into each
quadriiceps muscle on days 0 and 14. Control mice were injected similarly with a mixture of phosphate buffer saline and adjuvant.

**Mouse model of A. baumannii infection.** A mouse model of sepsis previously developed by our group and used for the evaluation of vaccines against A. baumannii was used to characterize the efficacy of the (Batson et al., 1950. J. Exp. Med. 91, 219-229; Rodríguez-Hernández et al., 2000. J. Antimicrob. Chemother. 45, 493-501). This model produces a disseminated infection after intraperitoneal instillation of the inoculum, typically resulting in death within 24 to 48 hours. For preparation of the inocula, A. baumannii strains were grown for 18 h at 37°C in Mueller-Hinton broth cultures and adjusted to the appropriated concentration in physiological saline as described previously (Moffatt et al., 2010. Antimicrob. Agents Chemother. 54, 4971-4977; Martin et al., 1998. J. Immunol. Methods 212, 187-192). Bacterial concentrations of the inocula were determined by plating on blood agar. Mice were infected on day 21 (one week after the second immunization) by intraperitoneal injection with 0.5 ml of the bacterial suspension and survival was monitored for 7 days.

**Spleen bacterial loads and serum cytokine levels.** Post-infection bacterial loads were determined in vaccinated and control mice 12 h after infection. Mice were euthanized with an overdose of thiopental and after collection of blood samples from the retro-orbital sinus, spleens were aseptically removed, weighed and homogenized in 2 ml of physiological saline. Serial log dilutions were plated on blood agar plates for bacterial quantification. Serum levels of interleukin-1β (IL-1β), tumor necrosis factor alpha (TNF-α), and interleukin-6 (IL-6) were determined in mice at 12 h post-infection using BD OptEIA mouse kits (BD Biosciences).

**Enzyme-linked immunosorbent assays (ELISAs).** For indirect enzyme-linked immunosorbent assays (ELISAs), 96-well plates were coated with $5 \times 10^7$ bacterial cells/well in phosphate buffer saline by incubating at 4 °C overnight. ELISAs were performed using sera collected on days 0, 7 and 21 as described previously [28]. Antibody titers were measured against the strain which was used to immunize the mouse, and were defined as the dilution in which
spectrophotometric readings were at least 0.1 units above background wells (wells containing no serum).

**Statistical analysis.** Antibody titers, bacterial loads, and cytokine levels were compared using the Kruskal-Wallis H test and the Mann-Whitney U test for independent samples, and the Friedmann and Wilcoxon tests for dependent samples. The Bonferroni correction was applied when appropriate. Survival data were compared using the log-rank test. All statistics were performed using SPSS version 15.0 software (SPSS Inc.), and a p value of ≤ 0.05 was considered significant.

**Results**

**Selection of an LPS-deficient strain for vaccine development.** Growth of ATCC 19606 in the presence of 10 μg/ml colistin resulted in numerous colistin-resistant derivatives with mutations in the lpxA, lpxC and lpxD genes (data not shown). One of these strains, IB010, contained a large deletion of 462 nucleotides in the lpxD (nucleotides 104-565) gene and was chosen for further use in vaccine studies. We reasoned that on the basis that the strain contained a large deletion, this strain would be less likely to revert to wild type during growth than strains containing single nucleotide changes or small deletions in the LPS biosynthesis genes. Broth microdilution experiments demonstrated that the minimum inhibitory concentration of the ATCC 19606 strain was ≤ 0.25 μg/ml and >128 μg/ml for IB010, demonstrating that, similar to results described previously, mutations in lpxD can result in resistance to colistin (Moffatt *et al.*, 2010. *Antimicrob. Agents Chemother.* 54, 4971-4977). In order to ensure that the IB010 was genetically stable during growth, genomic DNA from three independent cultures of ATCC 19606 and IB010 were amplified with lpxD-specific primers to confirm that the deletion was present. As shown in Figure 1A, a band corresponding to the mutated lpxD gene of IB010 containing a deletion of 462 nucleotides was present after amplification from all IB010 cultures indicating that no reversion had occurred. Phenotypic loss of LPS and reduction in endotoxin levels were characterized by the Limulus Amebocyte Assay for ATCC 19606 and IB010, and demonstrated that mutation in the lpxD
gene resulted in a dramatic reduction in endotoxin levels to > 1 EU per 10^6 cells (Figure 1B).

**Antibody response to the LPS-deficient IWC vaccine.** Formalin treatment of ATCC 19606 and IB010 resulted in no viable bacteria, indicating complete bacterial inactivation. In order to quantify the antibody response produced by immunization with inactivated IB010, indirect ELISAs were performed using sera collected from negative control mice (immunized with PBS and adjuvant) and mice vaccinated with 1 x 10^9 inactivated IB010 cells. As a positive control, one group of mice was immunized with 1 x 10^9 inactivated ATCC 19606 cells on the basis that we have previously shown that immunization with these cells induces a robust immune response and produces protective immunity against experimental infection (McConnell y Pachón, 2010. *Vaccine* 29, 1-5). As shown in Figure 2A, immunization with inactivated IB010 elicited detectable levels of antigen-specific total IgG in all mice seven days after a single intramuscular administration, and these antibody levels were significantly increased upon boosting with a second administration of the vaccine (p = 0.03 Wilcoxon test). Total IgG titers in mice receiving two administrations of inactivated IB010 vaccine were similar to titers in mice receiving the vaccine containing inactivated wild type cells (p = 0.726 Mann Whitney U test). Control mice had no detectable antigen-specific IgG at any point. In contrast, IgM levels were similar between mice immunized with the inactivated IB010 vaccine and mice receiving inactivated wild type cells seven days after a single administration (p = 0.186 Mann Whitney U test), however seven days after a second immunization there was no detectable antigen-specific IgM in IB010-vaccinated mice whereas all mice immunized with inactivated wild type cells had detectable levels of IgM (Figure 2B).

Levels of the IgG subtypes IgG1 and IgG2c, the IgG2a homolog in C57BL/6 (Martin et al., 1998. *J. Immunol. Methods* 212: 187-192), were determined in 21-day serum (Figure 2C and D). Both groups of mice receiving the inactivated vaccines had significant levels of IgG1 and IgG2c compared to control mice (p < 0.001; Mann-Whitney U test). Interestingly, IgG1 titers were significantly higher in IB010-vaccinated mice compared to ATCC 19606-vaccinated mice (p = 0.003; Mann-Whitney U test), whereas IgG2c titers were similar between these
groups. These results indicate that both Th1 and Th2 responses are elicited by the inactivated IB010 vaccine similar to what was previously shown for the inactivated ATCC 19606 vaccine (McConnell y Pachón, 2010. *Vaccine* 29, 1-5).

**Effect of vaccination on post-infection bacterial loads.** In order to characterize the effect of vaccination on post-infection tissue bacterial loads, we employed a mouse model previously developed by our group for the characterization of vaccine for preventing infection by *A. baumannii* (McConnell *et al.*, 2011. *Infect. Immun.* 79, 518-526; McConnell *et al.*, 2011. *Vaccine* 29: 5705-5710; McConnell y Pachón, 2010. *Vaccine* 29, 1-5). This model rapidly produces a disseminated infection in which bacteria are detected in distal organs as soon as one hour post-infection [16]. Vaccinated and control mice were infected with 2.0 x 10⁶ cfu (300 x LD₅₀) of the ATCC 19606 strain, and 12 hours after infection spleen bacterial loads were determined (Figure 3). IB010 vaccination reduced the number of bacteria in spleens approximately 1000-fold compared to control mice (p < 0.05; Mann-Whitney U test). Spleen bacterial loads in IB010 vaccinated mice were not significantly different than in mice immunized with inactivated ATCC 19606 cells.

**Effect of vaccination on post-infection serum cytokine levels and survival.**

In order to characterize the effect of immunization with the inactivated LPS deficient vaccine on cytokine levels, sera were collected from vaccinated and control mice 12 h post-infection and the levels of IL-1β, IL-6 and TNF-α were determined (Figure 4). Levels of all three cytokines were significantly lower in both groups of vaccinated mice than in control mice (p = 0.003 for IL-1β, IL-6 and TNF-α; Mann-Whitney U test), suggesting that vaccinated mice did not experience the pro-inflammatory cytokine release associated with the development of septic shock.

Vaccine efficacy was tested by infecting immunized and control mice with 2.25 x 10⁶ cfu (340.9 x LD₅₀) of the ATCC 19606 strain seven days after the second immunization, and survival was monitored over seven days (Figure 5). All mice vaccinated with the IB010 vaccine were protected from challenge, whereas all control mice died within 48 hours (P<0.001; log-rank test). As expected, all mice
immunized with the ATCC 19606 strain survived challenge, similar to results that were previously reported (McConnell et al., 2011. *Infect. Immun.* 79, 518-526; McConnell et al., 2011. *Vaccine* 29: 5705-5710; McConnell y Pachón, 2010. *Vaccine* 29, 1-5). In order to determine if vaccination with IB010 could protect against heterologous challenge with an unrelated strain, immunized and control mice were infected with 1.05 x 10^6 cfu (2.18 x LD50) of the previously characterized *A. baumannii* clinical isolate Ab-154 [29]. Once again, all immunized mice survived challenge whereas control mice succumbed to infection within 48 hours (p < 0.001; log-rank test), indicating that immunization with IB010 can provide cross protection against challenge with a heterologous strain.

In conclusion, these results provide important information regarding the development of a vaccine for the prevention of infections caused by *A. baumannii* based on whole bacterial cells lacking LPS. These results may also provide insights into the possibility of developing vaccines for other bacterial species based on strains lacking LPS.

**Example 2**

This example relates to the development of a vaccine against *A. baumannii* based on OMVS purified from said cultures of mutants without LPS.

To carry out this objective, the strains ATCC 19606T and its mutant without LPS IB010, which was generated in our laboratory from the ATCC 19606T strain and contains a deletion of 462 nucleotides between positions 103 and 565 of the gene *lpxD*.

Upon realizing the purification of the OMVs of said strains the following protocol was used:

- Strains are refreshed on blood agar or MHBII plates with colistin at 10 mcg/ml and grown overnight at 37 °C.

- A liquid culture is used to growth ATCC 19606 or IB010. They are cultured with aeration at 180 rpm at 37 °C overnight.
- The next day cultures of 50 or 100 ml or 1L in MHB are made and incubated overnight at 37 °C with aeration (180 rpm)
- After incubation, the cells are centrifuged at 4000 rpm during 30 min at 4 °C
- Next, the supernatant is filtered with a 0.22 micron filter.

- Afterwards, the OMVs are precipitated by ultracentrifugation for 90 minutes at 30000 rpm at 4 °C

Finally, the pellet is resuspended in PBS and the absence of viable bacteria is confirmed by plating. The OMVs are stored at -80°C

(Protocol adapted from McConnell MJ et al. 2011 Aug 5;29(34):5705-10)

Using the previous protocol different purifications of OMVs have been performed.

- Purifications of OMVs of the strains ATCC 19606 and IB010 for the production of OMVs after culturing 24, 48 and 72 hours. After the purification of OMVs the amount of protein was quantified using Bradford method and 10 mcg of the protein was visualized in a 10% polyacrylamide gel with Coomassie stain.

- In addition, purification have been performed with OMVs from the strain ATCC 19606 and IB010 in the presence and absence of Bip, which is an iron chelator, with the objective of verifying if the presence of the chelator resulted in the increased expression of proteins related with iron metabolism, for example, siderophore receptors. In this case, OMVs were purified for the quantification of proteins and for Coomassie staining of acrylamide gels and for the visualization of OMVs by electron microscopy.

Finally, OMVs were purified from the LPS mutant of a clinical isolate of A. baumannii Ab-167 which contains an ISAb1 insertion in the lpxC gene.

Proteins were quantified and visualized on acrylamide gels by Coomassie staining. And proteins were quantified by Bradford and 2D Quant kit.

**Example 3. Purification of outer membrane proteins.**

*A. baumannii* ATCC 19606 was grown in 1 liter of Mueller-Hinton broth to an optical density at 600 nm (OD600) of 0.6, and pelleted bacteria were
resuspended in 10 ml of 10 mM phosphate buffer, pH 7.2, and lysed by sonication. Unlysed cells were removed by centrifugation at 4,000 x g for 5 min, and the supernatant was centrifuged at 20,000 _ g for 1 h to pellet cell envelopes. Inner membranes were selectively solubilized with 5 ml of 2% N-laurylsarcosinate by incubation at 37°C for 30 min. The insoluble fraction was pelleted by centrifugation at 20,000 _ g for 1 h and then washed with 2 ml of 62.5 mM Tris-Cl, pH 6.8.

Endotoxin was extracted from the preparation by use of a cold detergent wash step in which proteins were resuspended in 5% SDS and incubated at 4°C for 10 min. SDS and endotoxin were subsequently removed by precipitating in methanol chloroform and resuspended in PBS.

Addition of the adjuvant

The purified proteins at a concentration of 500 mcg/ml were mixed with aluminum phosphate adjuvant at a 1:1 ration.

Clauses

1. An Acinetobacter cell deficient in LPS.

2. The Acinetobacter cell according to the preceding claim, obtained through partial or complete inactivation of one or various of the nucleic acids encoding the endogenous LPS biosynthesis genes.

3. The Acinetobacter cell according to the preceding claim, wherein the genes are selected from lpxA, lpxB and/or lpxC, or any combination thereof.

4. The Acinetobacter cell according to any one of the preceding claims, wherein the cell is obtained by deletions, and/or insertions of one or various nucleotides in the coding sequences of the genes.

5. The Acinetobacter cell according to any one of the preceding claims, wherein the cell is an attenuated Acinetobacter cell.

6. A composition comprising:
a) a cell according to any one of claims 1-2, and
b) a nucleic acid molecule, and/or a polypeptide.

7. The composition according to the preceding claim, wherein the nucleic acid molecule is recombinant and the polypeptide is recombinant.

8. The composition according to any one of claims 6-7, wherein the polypeptide is selected from:
   a) the peptide sequence SEQ ID NO: 27 (putative ferric siderophore receptor (A. baumannii ATCC 17978; accession number YP_001084684)) or a fragment thereof, wherein the fragments are biologically active fragments, and preferably selected from the list consisting of SEQ ID NO: 1 to SEQ ID NO: 11, or any of its combinations, or sequences having at least 85% sequence identity with peptide sequences SEQ ID NO: 1 to SEQ ID NO: 11, and/or
   b) the peptide sequence SEQ ID NO: 28 (putative ferric hydroximate siderophore receptor (A. baumannii ATCC 17978; accesión número YP_001084696)) or a fragment thereof, where the fragments are biologically active fragments, and preferably selected from the list consisting of SEQ ID NO: 12 to SEQ ID NO: 23, or any of its combinations, or sequences having at least 85% sequence identity with peptide sequences SEQ ID NO: 12 to SEC ID NO: 23.

9. The composition according to any one of claims 6-8, further comprising the amino acid sequence SEQ ID NO: 28 and the amino acid sequence SEQ ID NO: 27.

10. The composition according to any one of claims 6-9 further comprising a fusion protein comprising at least 2, preferably 3, more preferably 4 amino acid sequences form the list SEQ ID NO: 1 to SEQ ID NO: 23 or a variant of these sequences having at least 85% sequence identity with SEQ ID NO. 1 to SEQ ID NO: 23.
11. The composition according to claim 10, wherein the fusion protein further comprises the amino acid sequence SEQ ID NO: 24 or the amino acid sequence SEQ ID NO: 25.

12. The composition according to any one of claims 6-11, wherein the composition comprises a nucleotide sequence capable of transcribing an amino acid sequence as described in any one of claims 8-11.

13. The composition according to claim 12, wherein the nucleotide sequence is the SEQ ID NO: 26.

14. The composition according to any one of claims 6-13, further comprising an expression vector comprising the nucleotide sequence according to any one of claims 12-13.

15. The composition according to any one of claims 6-14, further comprising outer membrane vesicles deficient in LPS, or cells according to any one of claims 1 to 5.

16. The composition according to any one of claims 6-14, further comprising at least one of the purified outer membrane proteins of A. baumannii with amino acid sequence SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47; SEQ ID NO: 48; SEQ ID NO: 49; SEQ ID NO: 50; SEQ ID NO: 51; SEQ ID NO: 52; SEQ ID NO: 53; SEQ ID NO: 54; SEQ ID NO: 55; SEQ ID NO: 56; SEQ ID NO: 57; SEQ ID NO: 58; SEQ ID NO: 59; SEQ ID NO: 60; SEQ ID NO: 61; SEQ ID NO: 62; SEQ ID NO: 63; SEQ ID NO: 64; SEQ ID NO: 65; SEQ ID NO: 66; SEQ ID NO: 67; SEQ ID NO: 68; SEQ ID NO: 69; SEQ ID NO: 70; SEQ ID NO: 71; SEQ ID NO: 72; SEQ ID NO: 73; SEQ ID NO: 74; SEQ ID NO: 75; SEQ ID NO: 76; SEQ ID NO: 77; SEQ ID NO: 78; SEQ ID NO: 79; SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, or combinations thereof.

17. An outer membrane vesicle that is deficient in LPS.
18. The outer membrane vesicle according to claim 17, obtained from a cell according to any one of claims 1-5.

19. The composition according to any one of claims 6-15, wherein the cells or the outer membrane vesicles are designed to produce the amino acid sequences according to any one of claims 8-11 or 16 and/or comprising the nucleotides or nucleic acids according to any one of claims 12-13.

20. A nucleotide sequence capable of transcribing any fusion protein according to any one of claims 10-11.

21. An expression vector comprising a nucleotide or a nucleic acid according to any one of claims 12-13.

22. The composition according to any one of claims 7 to 16 and 19 that is a pharmaceutical composition.

23. The composition according to claim 22, further comprising a pharmaceutically acceptable vehicle.

24. The composition according to any one of claims 22 or 23, further comprising another active ingredient.

25. The composition according to any one of claims 22-24, further comprising an adjuvant.

26. The composition according to any one of claims 7 to 16, 19 to 22 and 25, wherein the composition is a vaccine.

27. The composition according to any one of claims 7 to 16, 19 to 22, and 25 for use as a medicament.

28. The composition according to any one of claims 7 to 16, 19 to 22, and 25 for the prevention, improvement or the treatment of an infection caused by A. baumannii in un mammal.

29. The composition according to any one of claims 7 to 16, 19 to 22, and 25 for conferring protection against infection caused by A. baumannii in a mammal.
30. An antibody or active fragment thereof obtained by immunization of a mammal with the composition according to any one of claims 7 to 16, 19 to 22 and 25.

31. The antibody or fragment thereof of the previous claim, wherein the composition is a pharmaceutical composition and wherein said composition is used in therapy, particularly for the treatment and prevention of infection caused by *A. baumannii*.

**Additional clauses**

1. - An *Acinetobacter* cell deficient in LPS obtained by partial or complete inactivation of one or various cellular nucleic acid molecules that encode endogenous LPS biosynthesis genes.

2. - The *Acinetobacter* cell according to the preceding claim, wherein the genes are selected from *lpxA*, *lpxB* and/or *lpxC*, or any of their combinations.

3. - A composition comprising:
   a) a cell according to any one of claims 1-2, and
   b) a recombinant nucleic acid molecule, and/or a recombinant polypeptide.

4. - The composition according to claim 3, wherein the polypeptide is selected from:
   c) the peptide sequence SEQ ID NO: 27 (putative ferric siderophore receptor (*A. baumannii* ATCC 17978; accession number YP_001084684)) or a fragment thereof, wherein the fragments are biologically active fragments, and preferably selected from the list consisting of SEQ ID NO: 1 to SEQ ID NO: 11, or any of its combinations, or sequences having at least 85% sequence identity with peptide sequences SEQ ID NO: 1 to SEQ ID NO: 11, and/or
   d) the peptide sequence SEQ ID NO: 28 (putative ferric hydroximate siderophore receptor (*A. baumannii* ATCC 17978; accession number YP_001084696)) or a fragment thereof, where the fragments are biologically active fragments, and preferably selected from the list
consisting of SEQ ID NO: 12 to SEQ ID NO: 23, or any of its combinations, or sequences having at least 85% sequence identity with peptide sequences SEQ ID NO: 12 to SEC ID NO: 23.

5. - The composition according to any one of claims 3-4, wherein the composition comprises a nucleotide sequence capable of transcribing an amino acid sequence described in claim 4, preferably SEQ ID NO: 26.

6. - The composition according to any one of claims 3-5, further comprising an outer membrane vesicle deficient in LPS, or cells according to any one of claims 1 and 2.

7. - The composition according to any one of claims 3-6, further comprising at least one of the proteins purified from the outer membrane of A. baumannii SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48; SEQ ID NO: 49; SEQ ID NO: 50; SEQ ID NO: 51; SEQ ID NO: 52; SEQ ID NO: 53; SEQ ID NO: 54; SEQ ID NO: 55; SEQ ID NO: 56; SEQ ID NO: 57; SEQ ID NO: 58; SEQ ID NO: 59; SEQ ID NO: 60; SEQ ID NO: 61; SEQ ID NO: 62; SEQ ID NO: 63; SEQ ID NO: 64; SEQ ID NO: 65; SEQ ID NO: 66; SEQ ID NO: 67; SEQ ID NO: 68; SEQ ID NO: 69; SEQ ID NO: 70; SEQ ID NO: 71; SEQ ID NO: 72; SEQ ID NO: 73; SEQ ID NO: 74; SEQ ID NO: 75; SEQ ID NO: 76; SEQ ID NO: 77; SEQ ID NO: 78; SEQ ID NO: 79; SEQ ID NO: 80; SEQ ID NO: 81; SEQ ID NO: 82; SEQ ID NO: 83; SEQ ID NO: 84; SEQ ID NO: 85; SEQ ID NO: 86; SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, or any combination thereof.

8. An outer membrane vesicle deficient in LPS.

9. The outer membrane vesicle according to claim 8, obtained from a cell as described in any one of claims 1-2.

10. The composition according to any one of claims 3-7, wherein the cells or the outer membrane vesicles are designed to produce the polypeptide sequences
according to any one of claims 4 and 7, and/or comprising the nucleic acid sequences of claim 5.

11. The composition according to any one of claims 3-7 and 10, wherein the composition is a pharmaceutical composition.

12. The composition according to any one of claims 3-7, 10 and 11 for use as a medicament.

13. The composition according to any one of claims 3-7, 10 and 11 for use in the prevention, improvement or treatment of an infection caused by *A. baumannii* in a mammal.

14. An antibody or active fragment thereof obtained by immunization of a mammal with the composition according to any one of claims 3 to 7, 10 and 11.

15. The antibody or the active fragment thereof according to the preceding claim, wherein the composition is a pharmaceutical composition, and where said composition is used in therapy, particularly for the treatment or prevention of an infection caused by *A. baumannii*. 
CLAIMS

1. A vaccine composition suitable for the prophylactic treatment of an infection caused by an *Acinetobacter baumannii* strain in a mammal, which comprises:
   a. an *Acinetobacter baumannii* strain deficient in lipopolysaccharide (LPS) characterized by the partial or complete inactivation of one or various cellular nucleic acid molecules that encode endogenous LPS; and/or
   b. an outer membrane vesicle (OMV) derived from an *Acinetobacter baumannii* strain deficient in lipopolysaccharide (LPS) as defined in paragraph a) above.

2. The vaccine composition of claim 1, wherein the *Acinetobacter baumannii* strain deficient in lipopolysaccharide (LPS) is characterized by the partial or complete inactivation of the genes selected from the list consisting of LpxA, LpxB and/or LpxC.

3. The vaccine composition of any of claims 1 or 2, wherein said vaccine further comprises a recombinant polypeptide selected from the list consisting of:
   a. An aminoacid sequence SEQ ID No 27 (putative ferric siderophore receptor (A. baumannii ATCC 17978; accession number YP_001084684)) or a fragment thereof, wherein the term fragment is understood herein as biologically active fragments selected from the list consisting of SEQ ID No 1 to SEQ ID NO 11 or any combination thereof, or an amino acid sequence having at least 85% identity with any of sequences SEQ ID NO 1 to SEQ ID NO 11; and/or
   b. An amino acid sequence SEQ ID No 28 (putative ferric hydroximate siderophore receptor (A. baumannii ATCC 17978; accession number YP_001084696)) or a fragment thereof, wherein the term fragment is understood herein as biologically active fragments selected from the list consisting of SEQ ID NO 12 to SEQ ID NO 23 or any combination thereof, or sequences that have at least 85% identity with the amino acids SEQ ID NO 12 to SEC ID NO 23.

4. The vaccine composition of any of claims 1 to 3, that further comprises a purified outer membrane protein sequence of *A. baumannii* selected from the list consisting of: SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO:
44. SEQ ID NO: 45, SEQ ID NO: 46; SEQ ID NO: 47; SEQ ID NO: 48; SEQ ID NO: 49; SEQ ID NO: 50; SEQ ID NO: 51; SEQ ID NO: 52; SEQ ID NO: 53; SEQ ID NO: 54; SEQ ID NO: 55; SEQ ID NO: 56; SEQ ID NO: 57; SEQ ID NO: 58; SEQ ID NO: 59; SEQ ID NO: 60; SEQ ID NO: 61; SEQ ID NO: 62; SEQ ID NO: 63; SEQ ID NO: 64; SEQ ID NO: 65; SEQ ID NO: 66; SEQ ID NO: 67; SEQ ID NO: 68; SEQ ID NO: 69; SEQ ID NO: 70; SEQ ID NO: 71; SEQ ID NO: 72; SEQ ID NO: 73; SEQ ID NO: 74; SEQ ID NO: 75; SEQ ID NO: 76; SEQ ID NO: 77; SEQ ID NO: 78; SEQ ID NO: 79; SEQ ID NO: 80; SEQ ID NO: 81; SEQ ID NO: 82; SEQ ID NO: 83; SEQ ID NO: 84; SEQ ID NO: 85; SEQ ID NO: 86; SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, or any combination thereof.

5. The vaccine composition of any of claims 1 to 4, wherein said vaccine further comprises the fusion recombinant polypeptide of SEQ ID NO 24 or 25 or the fusion recombinant polypeptide coded by the nucleic acid molecule of SEQ ID NO 26.

6. The vaccine composition of any of claims 1 to 5, wherein the Acinetobacter baumannii strain deficient in lipopolysaccharide (LPS) comprises or is transformed, transduced or transfected with a nucleotide sequence capable of coding for any of the amino acid sequences as defined in any of claims 3 to 5 so that such strain is capable of producing the exogenous expression of any of said amino acid sequences.

7. The vaccine composition of any of claims 1 to 5, wherein said vaccine composition further comprises a vector, such as viral vector, a plasmid or an expression cassette comprising a nucleotide sequence capable of coding for any of the amino acid sequences as defined in any of claims 3 to 5 and expressing said amino acid sequences.

8. The vaccine composition of any of claims 1 to 5 or 7, wherein said Acinetobacter baumannii strain deficient in lipopolysaccharide (LPS) is inactivated.

9. The vaccine composition of any of the precedent claims, wherein the Acinetobacter baumannii strain deficient in lipopolysaccharide (LPS) is derived from ATCC strain 19606.

10. The vaccine composition of any of claims 1 to 8, for use in the prophylactic treatment of an infection caused by A. baumannii in a mammal, preferably a human.
11. A vaccine composition comprising an antibody (monoclonal or polyclonal) or a fragment thereof selected from the list consisting of Fab, Fab', Fab'-SH, Fv, scFv, F(ab')2, Vhh, nanobody and diabody, capable of specifically binding the *Acinetobacter baumannii* strain deficient in lipopolysaccharide (LPS) and/or the outer membrane vesicle (OMV) derived therefrom as defined in any of claims 1 or 2.

12. A vaccine composition comprising an isolated antibody (monoclonal or polyclonal) or a fragment thereof selected from the list consisting of Fab, Fab', Fab'-SH, Fv, scFv, F(ab')2, Vhh, nanobody and diabody, obtained or obtainable after immunizing a mammal with the vaccine composition as defined in any of claims 1 or 2.

13. The vaccine composition of any of claims 11 or 12, for use in the therapeutic treatment (after infection) of an infection caused by *A. baumannii* in a mammal, preferably in a human.

14. An *Acinetobacter baumannii* strain deficient in lipopolysaccharide (LPS) as defined in any of claims 1 or 2, transformed, transduced or transfected with a nucleotide sequence capable of coding for any of the amino acid sequences as defined in any of claims 3 to 5 so that such strain is capable of producing the exogenous expression any of said amino acid sequences.

15. An *Acinetobacter baumannii* strain deficient in lipopolysaccharide (LPS) as defined in any of claims 1 or 2, for use as a medicament.

16. A method for the production of antibodies or fragments thereof, preferably selected from the list consisting of Fab, Fab', Fab'-SH, Fv, scFv, F(ab')2, Vhh, nanobody and diabody, which comprises:

a. Selecting an antibody or a fragment thereof, preferably from an antibody library;

b. Using an *Acinetobacter baumannii* strain deficient in lipopolysaccharide (LPS) and/or an outer membrane vesicle (OMV) derived therefrom, as an antigenic target;
c. Selecting those antibodies or fragments thereof having affinity or binding affinity or capable of specifically binding such strain and/or OMV; and

d. Producing those antibodies or fragments thereof identified in step c) above

17. Use of an *Acinetobacter baumannii* strain deficient in lipopolysaccharide (LPS) and/or an outer membrane vesicle (OMV) derived therefrom, for the production of antibodies or fragments thereof capable of binding such strain and/or OMV.
Fig. 3

Bacterial Load (log$_{10}$ cfu/g)

- Adjuvant
- Control
- ATCC 19606
- IB010 Vaccine
- Vaccine
Fig. 5

A

Survival (%)

ATCC 19606

Adjuvant Control

ATCC 19606 Vaccine

IB010 Vaccine

Day

B

Survival (%)

Ab-154

Adjuvant Control

IB010 Vaccine

Day
### Total Protein (μg)

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Fig. 11

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INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N1/00 A61K35/74
ADD. A61K39/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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X Further documents are listed in the continuation of Box C. X See patent family annex.

* Special categories of cited documents :
A* document defining the general state of the art which is not considered to be of particular relevance
E* earlier application or patent but published on or after the international filing date
L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
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Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
S* document member of the same patent family

Date of the actual completion of the international search

27 August 2015

Date of mailing of the international search report

14/09/2015

Name and mailing address of the ISA/
European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk,
Tel. (+31-70) 340-3040,
Fax. (+31-70) 340-3016

Authorized officer
van Heusden, Miranda

Form PCT/ISA/210 (second sheet) (April 2005)
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<td>WO 2014/048976 A1 (VAXDYN SL [ES]; SERVICIO ANDALUZ DE SALUD [ES]; UNIV SEVILLA [ES]) 3 April 2014 (2014-04-03) claims 20-23; example 3; sequences 27,28 page 9, line 24 - page 10, line 7</td>
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A claims 20-23; example 3; sequences 27,28 page 9, line 24 - page 10, line 7

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Form PCT/ISA/210 (continuation of second sheet) (April 2008)

page 2 of 4
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<td>GOEL VIKAS KUMAR ET AL: &quot;Monoclonal antibodies against the iron regulated outer membrane Proteins of Acinetobacter baumannii are bactericidal&quot;, BMC MICROBIOLOGY, BIOMED CENTRAL LTD, GB, vol. 1, no. 1, 9 August 2001 (2001-08-09), page 16, XP021014761, ISSN: 1471-2180, DOI: 10.1186/1471-2180-1-16</td>
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<td>ES 2 366 735 A1 (FUNDACION PUBLICA ANDALUZA PARA LA GESTION DE LA INVESTIGACION EN SALUD)</td>
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<td>25 October 2011 (2011-10-25) claims 1,6,9,10; table 1</td>
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<td>WO 2013/040478 A2 (LOS ANGELES BIOMED RES INST [US]; IBRAHIM ASHRAF S [US]; YEAMAN MICHAEL) 21 March 2013 (2013-03-21) claim 49; example III; sequence 21</td>
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<td>MICHAEL J. MCCONNELL ET AL: &quot;Acinetobacter baumannii: human infections, factors contributing to pathogenesis and animal models&quot;, FEMS MICROBIOLOGY REVIEWS, 1 June 2012 (2012-06-01), pages n/a-n/a, XP055150121, ISSN: 0168-6445, DOI: 10.1111/j.1574-6976.2012.00344.x the whole document</td>
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<td>MERITXELL GARCÍA-QUINTANILLA ET AL: &quot;Immunization with Lipopolysaccharide-Deficient Whole Cells Provides Protective Immunity in an Experimental Mouse Model of Acinetobacter baumannii Infection&quot;, PLOS ONE, vol. 9, no. 12, 8 December 2014 (2014-12-08), page e114410, XP055204634, DOI: 10.1371/journal.pone.0114410 page 6 - page 8; figures 2,3</td>
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