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Recombinant Lactic Acid Bacteria as Potential Oral Vaccine Vectors against Multidrug-Resistant Acinetobacter Baumannii

Hadeel M. Khalaf[1], Nurul Izza Ismail[2*], Hasan A. Aal Owaif [3], Muna E. Raypah[4] and Amira Suriaty Yaakop[5]

[1-2,4]School of Biological Sciences, Universiti Sains Malaysia, 11800 USM, Penang, Malaysia
[1]Department of Molecular and Medical Biotechnology, College of Biotechnology, Al-Nahrain University, Baghdad, Iraq
[3]Department of Applied Biology, College of Biotechnology, Al-Nahrain University, Baghdad, Iraq
[4]Centre for Artificial Intelligence & Data Science, Universiti Malaysia Pahang, Al-Sultan Abdullah, 26300 Gambang, Malaysia

*Corresponding author: Nurul Izza Ismail (e-mail: nurul.ismail@usm.my).

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Abstract Multidrug-resistant Acinetobacter baumannii (MDR-AB) is an important healthcare-associated pathogen for which preventive vaccine strategies remain under investigation. Recombinant lactic acid bacteria (LAB) are being explored as vectors for oral antigen delivery, but their relevance to MDR-AB requires careful distinction between direct and indirect evidence. This scoping review summarized English-language publications retrieved from PubMed, Scopus, and Google Scholar for the period January 2018 to December 2024. Out of the 213 articles found initially, 46 articles were included in the review and classified into three themes: LAB strain (n = 23), recombinant oral vaccines (n = 7), and *A. baumannii* antigen and vaccine (n = 16). LABs contributed to knowledge about strain properties, vector development, and mucosal antigen delivery, which included vaccines against bacteria other than *A. baumannii*. On the other hand, studies of *A. baumannii* have analyzed antigens such as outer membrane proteins, adhesions, and outer membrane vesicles mainly through the use of non-laboratory-adapted (non-LAB) vaccination methods. These two strands of research provide complementary evidence for the development of an oral recombinant LAB vaccine for *A. baumannii* but cannot demonstrate the direct efficacy of the vaccine. Research challenges are involved in antigen choice, stability of the construct, antigen expression, immunogenicity at both mucosal and systemic level, standardization of the challenge method and safety study. Therefore, while recombinant lactic acid bacteria (LABs) represent a promising approach to oral vaccines, their protective efficacy against MDR-AB is not yet proven.

Key Words: Acinetobacter Baumannii, Oral Vaccines, Lactic Acid Bacteria, Mucosal Immunity, Outer Membrane Vesicles

INTRODUCTION

The increasing global burden of multidrug-resistant (MDR) bacterial infections has intensified interest in preventive strategies that complement antimicrobial stewardship and reduce healthcare-associated transmission. Carbapenem-resistant Acinetobacter baumannii (CRAB) is a high-priority Gram-negative pathogen because of its extensive resistance and capacity to persist in healthcare environments [1-2].

There is an enormous possibility of vaccinating that can support infection prevention efforts and lower the need for antimicrobial treatment [3-4]. However, the creation of vaccines against *A. baumannii* poses many challenges in terms of antigenic variation, unknown cross-strain immunity and the lack of protective correlates of immunization in experimental models. Vaccines under clinical study include subunit vaccines directed to outer membrane proteins (OMPs), adhesins and biofilm-associated factors, as well as outer membrane vesicle (OMV) and multiepitope vaccine constructs [5-6].

Within such an approach, some strains of lactic acid bacteria (LAB), including strains of Lactococcus lactis and related genera, were investigated as vectors for the delivery of recombinant antigens and modulators of immune response [7-9]. Some of the strains have an experience in food use, have some evidence of compatibility with fermentation-based production processes, and show some evidence of capability to deliver antigens by the mucosal route, nevertheless, none of these features can guarantee the safety of the strain under investigation [10-11]. Evidence derived from the research of pathogens unrelated to LAB provides grounds for the possibility of antigen/immunomodulator expression and delivery with the use of LAB strains [9,12]. These findings justify evaluating LAB as a potential platform for future *A. baumannii* vaccines while keeping platform feasibility separate from pathogen-specific efficacy.

The rationale for this review is the genetic tractability of selected LAB strains and the possibility of expressing heterologous antigens intracellularly, secreting them, or displaying them on the cell surface. Experimental studies involving pathogens other than *A. baumannii* have measured mucosal and systemic immune responses to selected recombinant constructs. These findings support further platform development but do not demonstrate universal safety, superiority to other delivery systems, or protection against *A. baumannii*. Accordingly, this scoping review integrates evidence on recombinant LAB delivery with separate, pathogen-specific studies of *A. baumannii* antigens and non-LAB vaccine platforms.

MATERIALS AND METHODS

This scoping review followed the methodological framework of the Joanna Briggs Institute (JBI) and was reported with reference to the PRISMA extension for scoping reviews (PRISMA-ScR) [13]. The review question was: 'What preclinical evidence supports recombinant lactic acid bacteria (LAB) as potential oral vaccine carriers for multidrug-resistant Acinetobacter baumannii?' PubMed, Scopus, and Google Scholar were searched for English-language publications dated January 2018 to December 2024. Search terms included 'lactic acid bacteria', 'recombinant LAB', 'oral vaccine', 'edible vaccine', 'vaccine development', and 'Acinetobacter baumannii', combined with Boolean operators. No geographical restrictions were applied.

Eligibility criteria included (a) studies investigating LAB strains and properties relevant to potential oral antigen delivery, (b) studies evaluating recombinant LAB-based oral vaccination, antigen delivery, or immunological responses, including work targeting pathogens other than *A. baumannii*, and (c) preclinical investigations of *A. baumannii* antigens or vaccine candidates, including OMPs, OMVs, fimbriae, and adhesins. Only relevant review articles that met the established criteria were considered. Articles not written in English, duplicate entries, and those not within the review's scope were excluded. Recombinant LAB research involving pathogens different from the platform was used to evaluate the feasibility of the platform, while those involving *A. baumannii* using other platforms were used for antigen identification and reporting of protection results.

In total, the search strategy yielded 213 records from PubMed (75 articles), Scopus (52 articles), and Google94 articles in full text, 48 articles were excluded according to the eligibility criteria, leaving 46 articles that were included (Figure 1). For the purpose of narrative synthesis, the included articles were further divided into the categories of LAB strains (n = 23), recombinant oral vaccines (n = 7), and *A. baumannii* antigens or vaccines (n = 16). These categories were used to distinguish bacterial-vector characteristics, antigen-delivery evidence, and pathogen-specific antigen and protection data.

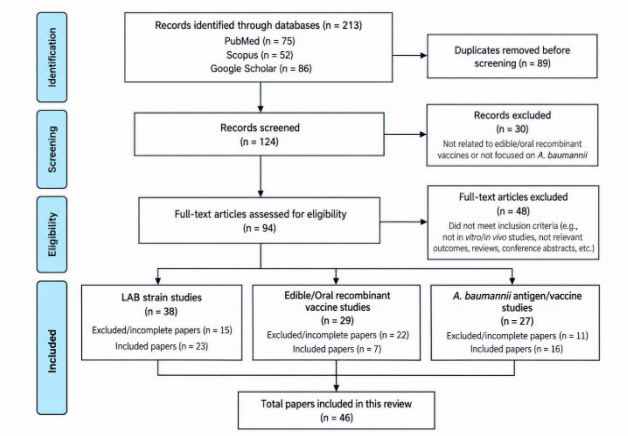


Figure 1: Flowchart of Record Identification, Screening, Full-Text Assessment, And Inclusion in This Scoping Review

Scholar (86 articles). Excluding 89 duplicate studies left 124 articles that were screened for relevance in their title and abstracts, 30 of which were excluded. After assessing

Study characteristics were charted using a form adapted from JBI guidance. Extracted fields included the delivery platform, LAB strain, antigen, experimental model, route of administration, measured immunological outcomes, and study limitations. Data were organized in Microsoft Excel and synthesized narratively to map the available preclinical evidence, experimental constraints, and remaining gaps. The purpose was evidence mapping rather than an assessment of comparative vaccine efficacy.

Consistent with the mapping objective of a scoping review, no formal risk-of-bias or certainty-of-evidence assessment was conducted [13,14]. Because the included studies differed in design, delivery platform, and outcome measurement, their experimental models, immunological endpoints, and reported limitations were described rather than combined into an efficacy estimate. The strength and directness of the evidence were interpreted in relation to the review question, without assigning formal certainty ratings.

RESULT AND DISCUSSION

LAB and the Strains Used in Vaccine-Related Research

LAB comprise a diverse group of Gram-positive, predominantly fermentative bacteria, including species assigned to Lactococcus, Lactobacillus and related reclassified genera, Pediococcus, Leuconostoc, Streptococcus, Enterococcus, and Weissella. Studies have isolated and characterized LAB strains from diverse sources to investigate properties potentially relevant to live antigen delivery (Table 1). Some strains produce organic acids and other inhibitory compounds with antimicrobial activity, however, strain isolation or antimicrobial activity alone does not establish vaccine-delivery suitability. Selected strains have been explored for mucosal immunomodulation [15-16], probiotic-associated applications [17-18], antibacterial effects [19-21], and recombinant protein delivery [8,22].

Table 1: Characteristics of LAB Strains and The Directness of Evidence Relevant to Potential Oral Antigen Delivery

Strain	LAB species / identification	Isolation source	Specific outcome reported	Relevance to oral vaccine and citation audit	Reference
ZFM54	Lacticaseibacillus paracasei subsp. paracasei	Cheese	Survived simulated gastric acid (pH 3) and 0.3% bile, acute oral-toxicity assessment in mice reported no adverse effects.	Indirect: probiotic/oral-safety properties, not antigen delivery or vaccine protection.	Qureshi *et al.* [24]
NZ3900	Lactococcus lactis	Fermented milk	Viability of recombinant NZ3900 in optimised fermented milk reached 9.64 log10 CFU/mL.	Indirect: food-grade formulation feasibility, no measured GI persistence or vaccine efficacy.	How *et al.* [25]
Challis	Streptococcus gordonii	Not applicable	The cited paper designed a multi-epitope vaccine AGAINST S. gordonii using in-silico approaches.	Multi-epitopes vaccine	Nasir *et al.* [2]
NS8	Lactobacillus helveticus	Not applicable	oral administration of L. helveticus NS8 effectively alleviates AD severity in mice by suppressing the Th2 immune response.	Oral demonstration	Rong *et al.* [26]
B11W	Lactobacillus helveticus	Whey	Isolated and taxonomically characterised among dairy fermentation microbiota.	No oral administration, gut viability, antigen expression or vaccine efficacy established.	Baruzzi *et al.* [27]
B12W	Weissella viridescens	Whey	Dairy isolate identified, citrate-fermenting activity was reported.	Fermentation property only, oral-vaccine suitability not established.	Baruzzi *et al.* [27]
B15Z	Lactobacillus delbrueckii subsp. bulgaricus	Whey and cheese	Prominent during Scamorza fermentation, proteolytic activity documented.	Technological suitability for dairy fermentation, not gastrointestinal or vaccine efficacy.	Baruzzi *et al.* [27]
B1S	Limosilactobacillus fermentum	Ripened cheese	Taxonomically identified among cheese-fermentation isolates, citrate fermentation reported.	No direct oral or vaccine evaluation identified in its primary source.	Baruzzi *et al.* [27]
B22S	Lacticaseibacillus paracasei subsp. paracasei	Ripened cheese	Taxonomic characterisation and milk-protein proteolytic activity reported.	No strain-specific oral vaccination outcome.	Baruzzi *et al.* [27]
B24W	Lactobacillus gasseri	Whey	Isolated and taxonomically identified in dairy microbiota.	Isolation does not establish gut survival or suitability as a vaccine carrier.	Baruzzi *et al.* [27]
B26W	Lactobacillus helveticus	Whey	Isolation and milk-protein proteolytic activity documented.	Fermentation evidence only, no oral vaccine assessment.	Baruzzi *et al.* [27]
B2C	Limosilactobacillus fermentum	Curd	Isolated and identified from cheese-processing samples.	No oral-vaccine-related experimental outcome established.	Baruzzi *et al.* [27]
B30W	Lactobacillus gasseri	Whey	Isolated and identified among dairy-fermentation microbiota.	No evidence for oral antigen delivery or vaccine-mediated protection.	Baruzzi *et al.* [27]
B4W	Limosilactobacillus fermentum	Whey	Isolated and identified from natural whey microbiota.	No oral vaccine or in-vivo GI outcome shown in cited isolation work.	Baruzzi *et al.* [27]
B63Z	Lactobacillus delbrueckii subsp. delbrueckii	Cheese	Taxonomically characterised in dairy fermentation samples.	No demonstrated oral viability or vaccine-carrier efficacy.	Baruzzi *et al.* [27]
C11Z	Streptococcus thermophilus	Cheese	Taxonomic identification and strain typing were reported.	Fermentation isolate only, not proven to be an oral vaccine vector.	Baruzzi *et al.* [27]
WYH	Lactiplantibacillus plantarum	Traditional Chinese fermented vegetables (Jiangshu)	Demonstrated tolerance to acidic conditions and bile salts. Oral administration in mice for acute and 28-day repeated-dose toxicity assessments showed no mortality or significant adverse effects under the tested conditions.	Indirect evidence of gastrointestinal stress tolerance and oral administration, no recombinant antigen delivery or vaccine-mediated protection was evaluated.	Ou *et al.* [28]
KAU007	Lactiplantibacillus plantarum	Idli	KAU007 extracts were investigated for antiviral/antimicrobial effects against non-*A. baumannii* pathogens.	Indirect non-vaccine evidence, not recombinant oral antigen carriage.	Rather *et al.* [29]
KLDS6.0930	Enterococcus durans	Traditional naturally fermented cream	Genomic and phenotypic safety evaluation, including 28-day repeated oral dosing in rats, no adverse organ or routine blood/serum effects reported.	Indirect oral safety evidence, Enterococcus requires strain-specific safety scrutiny, no vaccine testing.	Li *et al.* [30]
Strain	LAB species / identification	Isolation source	Specific outcome reported	Relevance to oral vaccine and citation audit	Reference
LOC1	Lactiplantibacillus plantarum	Fresh tea leaves	Altered LPS/TLR4-triggered inflammatory gene expression in RAW264.7 murine macrophages.	Indirect in-vitro immunomodulation, no antigen-specific oral vaccine test.	Suzuki *et al.* [31]
OLL1073R-1	Lactobacillus delbrueckii subsp. bulgaricus	Yogurt culture	Consumption of yogurt fermented with this strain enhanced seasonal-influenza vaccine antibody titres in healthy adults.	Indirect immunomodulation, neither recombinant LAB antigen carriage nor *A. baumannii* protection.	Hemmi *et al.* [32]

Table 1: Continued

Strain	LAB species / identification	Isolation source	Specific outcome reported	Relevance to oral vaccine and citation audit	Reference
SBT2171	Lactobacillus helveticus	Dairy culture	Heat-killed SBT2171 reduced experimental autoimmune encephalomyelitis severity in mice after INTRAPERITONEAL administration.	Not oral, demonstrates immunomodulation, not vaccine-delivery efficacy.	Yamashita *et al.* [33]
LAB18S	Enterococcus durans	Soft cheese (Minas Frescal cheese)	Demonstrated tolerance to acidic conditions (pH ≥3.0) and bile salts (up to 15 mg/mL). Genomic analysis identified no plasmids or detected virulence- and antimicrobial resistance-associated genes in the investigated strain.	Indirect evidence of gastrointestinal stress tolerance and preliminary probiotic suitability, no recombinant antigen delivery or vaccine-mediated protection was evaluated.	Comerlato *et al.* [34]

The suitability of LAB as vaccine vectors is strain- and context-dependent. Zhai *et al.* [23] reported antigen-specific systemic and intestinal mucosal responses in mice after oral administration of recombinant Lactococcus lactis NZ9000 expressing the S1 antigen of porcine deltacoronavirus, with or without the M-cell-targeting ligand Co1. In contrast, Sun *et al.* [12] investigated an *A. baumannii* Ata-derived peptide fused to cholera toxin B using a non-LAB immunization approach. These findings address distinct questions: LAB-mediated delivery against an unrelated virus and antigen-specific protection against *A. baumannii*. Gastrointestinal survival and persistence must be measured for each intended LAB strain and construct, they cannot be inferred for all entries in Table 1.

Choice of suitable LAB strain is important for development of vector systems, yet safety and immunoprotective properties of each strain/construct pair have to be validated separately. Heterogeneous data gathered in experiments reported in Table 1 cover the areas of strain isolation, probiotic and oral tolerance research, yet these facts do not confirm their eligibility as a vector of recombinant oral vaccines. Strain characterization has to involve its identification, genetic compatibility, antigen presentation, virulence potential, horizontal gene transfers and persistence [10]. Adherence to epithelia can affect antigen availability in some constructs [35-36], while colonization and persistence depend on both strain and host [17,21]. Strains of lactic acid bacteria used as vectors of vaccines can be isolated either from diet-related sources of fermented food products or from host-adapted strains [37-38].

Genetics of Lactococcus lactis is relatively well-known, nevertheless, its previous utilization as a component of some products used in the food industry does not imply the safety of its recombinant forms [39]. L. lactis has been used as a vector for the expression of selected immunomodulating proteins and antigens, among which was the TTFC (tetanus toxin fragment C) [40-42]. Some experiments using different cargo proteins, among which was TTFC, resulted in varying immune responses depending on the particular construct and delivery system [41-45]. Such findings from non-*A. baumannii* systems support platform immunogenicity where measured, not antibody-mediated clearance, immune memory, or protection against *A. baumannii*.

Recombinant LAB as Antigen Delivery Platforms

An oral LAB-based vaccine strategy requires coordinated selection of the antigen, expression system, and delivery formulation. Although genetic-engineering methods permit antigen localization at different cellular sites, construct stability, antigen accessibility, and measured immune responses vary across systems. Any improvement in immunogenicity must therefore be demonstrated for the specific construct and experimental model [8,40,46].

Recombinant LAB investigated as experimental mucosal vectors include Lactococcus lactis and Lactiplantibacillus plantarum. In studies of unrelated pathogens, selected constructs have elicited measured mucosal or systemic responses, whereas resistance to gastrointestinal degradation varies by strain, antigen, and formulation [8, 46-47]. By contrast, the cited *A. baumannii* study evaluated native OMVs in a murine challenge model rather than LAB-mediated delivery [48]. LAB may also possess strain-specific immunomodulatory properties, but their molecular contributions to vaccine responses remain incompletely characterized. Outcomes from non-*A. baumannii* LAB models [23-49] cannot be extrapolated to protection against *A. baumannii*, and clinical efficacy of such a recombinant LAB vaccine has not been established.

Recombinant LAB can be engineered to express single antigens, fusion proteins, or multiepitope constructs. Such designs may broaden antigen presentation, but improved stability, immunogenicity, or protection must be verified experimentally for each formulation [52-53]. This distinction is particularly important for *A. baumannii*, for which antigenic heterogeneity complicates the selection of broadly protective targets (Table 2).

Three main types of antigen localization techniques are used for recombinant LABs: intracellular localization, secretion, and cell wall anchorage [15,46,54]. Intracellular localization could protect the antigen before secretion, however, cell rupture is necessary. Secreted antigens are better exposed to mucosal environment but can be degraded. Cell wall anchored antigens could increase access, however, their immune activity needs comparison to intracellular antigens within one study model [15-16]. Studies with different pathogens examined antigen-specific responses to IgA or IgG antibodies to certain LAB constructs [16,23,49], these responses cannot be regarded as functions and protection from *A. baumannii* infection independently. Native *A.*

Table 2: Recombinant LAB Studies: Delivery System, Antigen or Cargo, Experimental Model, Measured Outcomes, And Relevance to *A. baumannii*

Reference and evidence type	Recombinant LAB / cargo	Target pathogen or indication	Experimental model / route	Main reported outcomes
Choi *et al.* [7] Technology / platform study DOI: 10.1128/mbio.01197-23	Engineered Limosilactobacillus reuteri, luminescent tagging of therapeutic proteins (leptin, IL-22, IFN-β).	No vaccine antigen or pathogen, recombinant-therapeutic tracking.	In vitro assays, mouse oral gavage and intestinal/fecal detection.	Detected engineered LAB and products by luminescence, no vaccine-specific immune outcome.
Lin *et al.* [50] Oral immunotherapy (non-vaccine) DOI: 10.1002/btm2.10533	Lactococcus lactis secreting hsCD80 or CTB–hsCD80.	Tumor immunotherapy: colorectal tumors/adenoma and melanoma.	In vitro assays and orally treated tumor-bearing mice.	Increased activated/IFN-γ+ T cells, tumor suppression and survival benefit in some models, intestinal cargo uptake.
Zhai *et al.* [23] Oral recombinant vaccine DOI: 10.1016/j.virol.2022.12.010	Recombinant Lactococcus lactis NZ9000 expressing PDCoV S1 ± Co1 targeting ligand.	Porcine deltacoronavirus (PDCoV), S1 antigen.	Orally immunized mice.	Systemic and intestinal mucosal antigen-specific antibodies, Co1 M-cell targeting evaluated.
Xu *et al.* [49] Oral recombinant vaccine DOI: 10.1186/s13071-022-05597-w	Lactiplantibacillus plantarum NC8 displaying Tsgal.	Trichinella spiralis, galectin antigen.	BALB/c mice, oral immunization and parasite challenge.	Serum IgG/IgA, intestinal sIgA and cytokine responses, reduced worm and muscle-larval burdens.
Oh *et al.* [15] Oral recombinant vaccine model DOI: 10.1016/j.vaccine.2021.06.010	Recombinant Lactiplantibacillus plantarum, BmpB model-antigen constructs.	Model antigen BmpB, *A. baumannii* not studied.	In vitro experiments and mouse oral-delivery model.	Compared expression/localization and M-cell uptake, mucosal antigen responses.
Fatehi *et al.* [51]	Lactococcus lactis	Brucella lumazine synthase (BLS)	In vitro and in vivo (BALB/c mice)/ oral administration	Increased antigen-specific IgG1 and IgG2a, increased IFN-γ, TNF-α, IL-4 and IL-10 responses
Ma *et al.* [47] Oral recombinant vaccine DOI: 10.1186/s12934-018-0861-7	Lacticaseibacillus casei L393 expressing PEDV COE with Co1/DCpep ligands.	Porcine epidemic diarrhea virus (PEDV), core neutralizing epitope (COE).	Mice, oral vaccination, systemic/mucosal assays.	Serum IgG, neutralizing antibodies and mucosal sIgA, enhanced by targeting peptides.

* LAB: Lactic acid bacteria, CTB: Cholera toxin B subunit, hsCD80: Human soluble CD80, PDCoV: Porcine deltacoronavirus, PEDV: Porcine epidemic diarrhea virus, COE: Core neutralizing epitope, DCpep: Dendritic-cell-targeting peptide, sIgA: Secretory IgA

baumannii OMVs and LABs producing certain proteins from OMVs are different methods for delivering antigens. The expression, proper folding, and localization of a particular OMV protein in LABs will need to be validated independently before the evaluation of its protective activities [55-56]. The figure below shows a hypothetical approach instead of an experimental example of a LAB vaccine.

On the whole, the possibility of using recombinant LAB for antigen presentation depends on the choice of antigen, its expression levels, localization, and gut delivery. These factors require specific adjustment and testing before oral vaccine trials in multidrug-resistant *A. baumannii* can be evaluated.

The gut microbial community can influence mucosal immune response and, therefore, can affect the efficacy of oral delivery of recombinant lactic acid bacteria (LAB) [57-58]. The structure of the microbial community can influence the environment in which the introduced strain will find itself and, thus, may influence its viability, longevity, and immunization. However, these effects are situation-dependent and cannot be viewed as an enhancement of vaccine efficacy without proper measurements. Most of the data are obtained using experimental models, and additional research on this issue is necessary to establish the clinical significance of these findings.

Acinetobacter Baumannii

Acinetobacter baumannii is a Gram-negative opportunistic pathogen associated with healthcare- acquired infections, particularly among vulnerable patients [1,59]. Figure 2 schematically depicts selected features of the bacterial envelope and virulence-associated systems relevant to antigen selection. The organism can persist in healthcare environments and may display resistance to multiple antibiotic classes [2]. Experimental vaccine candidates have been investigated, but clinical protective efficacy has not been established. Although certain LAB-derived products have shown antibacterial effects against *A. baumannii* in experimental settings, antibacterial activity should not be interpreted as vaccine-mediated protection. Antigen selection remains a problem because of pathogen variability and the fact that the pathogen targets may vary, which limits cross-strain coverage. Therefore, Table 3 can be seen as heterogenous pre-clinical data related to particular antigen targets and non-laboratory adapted delivery mechanisms.

Figure 2 Schematic representation of the Acinetobacter baumannii cell envelope and selected virulence-associated systems (not to scale). Adapted with modifications from Aparna *et al.* [60] and Shadan *et al.* [61]. Abbreviations: BamA, beta-barrel assembly machinery protein A, Bap, biofilm-associated protein, T2SS, type II secretion system, T6SS, type VI secretion system, OMPs, outer membrane proteins, AceI, Acinetobacter chlorhexidine efflux protein, ABC, ATP-binding cassette, RND, resistance-nodulation-division, PLD, phospholipase D, Fe, iron, Zn, zinc, LOS, lipooligosaccharide. Permission or another valid reuse basis is required for any original graphic components taken from the Springer source [60].

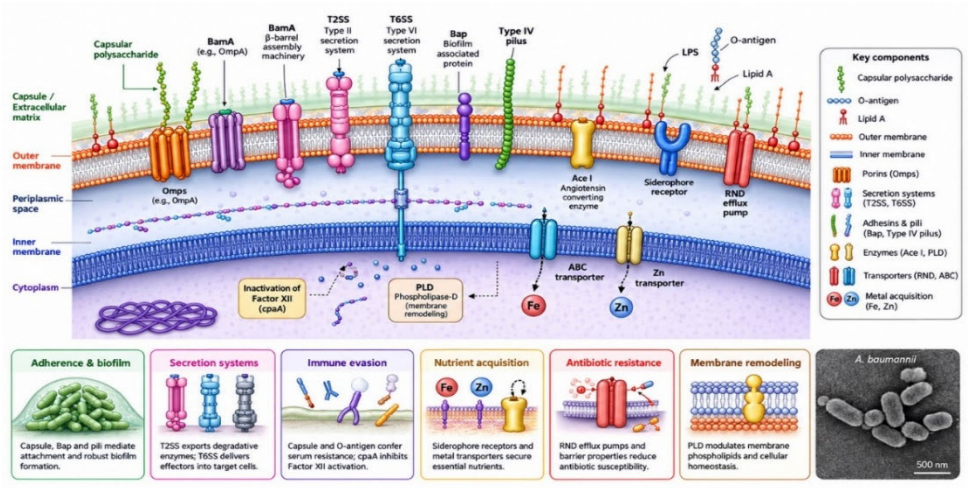


Figure 2: *A. baumannii* Cell Architecture

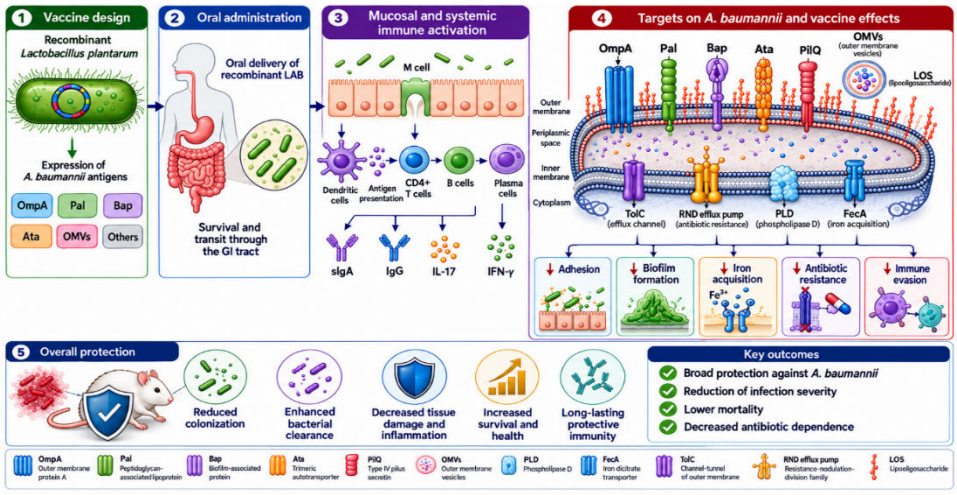


Figure 3: Mechanism of Recombinant LAB-Based Edible Vaccine Against *Acinetobacter Bumannii*

Potential LAB-Based Oral Vaccine Targets Among *A. baumannii* Outer Membrane Proteins

Over the past decade, research has concentrated on single-component vaccines targeting specific Outer Membrane Proteins (OMPs) of *A. baumannii* (Figure 2). Several OMPs have been identified as promising immunogens, including OmpW, OmpA, Oma87, BauA, Omp34, Ata, CipA, PBP-7/8, OprD/Occ, CarO, PKF, AbOmpA, and OmpK/Omp22 (Table 3). These proteins are surface-exposed, making them accessible to the host immune system, and many are directly associated with virulence or resistance mechanisms [65]. Additionally, capsular polysaccharide (CPS) has been considered as a potential antigen [77]. However, the high variability of CPS (over 100 capsule loci discovered) poses a great challenge in making a universal vaccine [78, 79]. OMP vaccines have shown promise in pre-clinical studies. Since OMP is present on the surface of bacteria, it has several epitopes and can stimulate antibodies in large amounts. However, since the production of some OMPs (such as CarO, CipA, OprD/Occ, and PBP-7/8) is reduced when developing resistance, they are not good candidates for universal vaccine targets [68-70]. Despite this variability, OMP is promising for a vaccine due to its role in important biological processes like adhesion, survival, immune evasion, and virulence [1,59]. Preclinical studies have demonstrated different degrees of protection against *A. baumannii* infection after active immunization using recombinant protein antigens. Bolourchi *et al.* [71] studied PKF, AbOmpA, and a mixture thereof in C57BL/6 mice infected intraperitoneally with the ATCC 19606 strain of *A. baumannii* at twice the median lethal dose (2 × LD50). Seven days after infection, the survival rates were 83.3%, 66.6%, and 85.71%, respectively, as compared to 0% for the control group. In turn, Akbari *et al.* [65] tested hybrid antigens including surface loops of BauA and Omp34 in a murine model of sepsis infected with the *A. baumannii* ABI022 strain. Immunization with BauA loop 7, Omp34 loop 3, and a combination of both constructs resulted in 42.86%, 42.86%, and 71.43% protection, respectively. Furthermore, OMP-targeted vaccines may be complemented by LAB

Table 3: Preclinical Studies of Candidate A. Baumannii Antigens and Non-LAB Vaccine or Virulence-Associated Experimental Systems

Vaccine Immunogens (Antigens)	Adjuvant	Ratio	Days	Dose	Route	Model	Strains	Recombinant protein expressions	Animal (In vivo)	Weight	Period	Challenge	Ref.
OmpW and OmpA			120 h	10 µL		G. mellonel la larvae	A. baumannii Acb-1	E. coli	Larvae	220-260 mg	24 h, 48 h	A. baumannii Acb-1	Schmitt *et al.* [62]
Oma87	Freund's complete	1:1 (v/v)	0, 14, 28, and 42	20 µg	s.c.	Murine sepsis	A. baumannii ATCC 19606	E. coli BL21 (DE3)	Mice	22–25 g	4 days	A. baumannii ATCC 19606	Rasooli *et al.* [63]
Omp34	Freund's complete		14, 28, and 42	20 µg	s.c.	Murine sepsis	A. baumannii ATCC 19606	E. coli strain ATCC 2566	Mice	20–25 g	24 h, 48 h	A. baumannii ATCC 19607	Golestani *et al.* [64]
BauA, Omp34	Freund's complete	1:1 (v/v)	1, 14, and 28	20 µg	s.c.	Murine sepsis	A. baumannii ATCC 19607	E. coli strain ER 2566	Mice	20–25 g	24 h	A. baumannii ABI022	Akbari *et al.* [65]
Omp34	Freund's complete	1:1 (v/v)	1, 2, 14	20 µl	i.p.	Murine pneumonia	A. baumannii (Kh60)	E. coli BL21 (DE3)	Mice	3–27 g	24–48 h	A. baumannii (Kh60)	Mesbahi Moghaddam *et al.* [66]
Ata	Aluminum	1:1 (v/v)	14 days	40 µg	i.p.	Murine pneumonia	A. baumannii strain ATCC 17978	E. coli BL21(DE3) /pET30a	Mice		0 h, 12 h, 24 h, and 7 days	A. baumannii strain ATCC 17978	Sun *et al.* [12]
Ata	Freund's complete	1:1 (v/v)	0, 14, 28, and 42	21 µg	i.p.	Murine sepsis pneumo nia	A. baumannii ATCC 19609	E. coli strain Top10	Mice	20–25 g	0, 4, and 24 h	A. baumannii ATCC 19606	Hatefi Oskuei *et al.* [67]
CipA and PBP-7/8	MPL	1:1 (v/v)	0, 14, 28, and 42	30 µg	s.c.	Sepsis	A. baumannii ATCC 19606	E. coli BL21 (DE3)	Mice		18 h, 24 h, 2 and 3 days	A. baumannii ATCC 19606	Badmasti *et al.* [68]
OprD/Occ		1:1 (v/v)	14, 32	20 µg	i.p.	C57	DT- Ab022 and DT- Ab057	E. coli TOP10	Mice		40 h	A. baumannii ST2/KL22	Hua *et al.* [69]
CarO		1:1 (v/v)	7 days	0.5 mL	i.p.	Sepsis murine	Carbape nem-resistant A. baumannii (CRA B)	ΔcarO	Mice	18–20 g	18–20 h	Carbapenem-resistant A. baumannii (CRAB)	Labrador-Herrera *et al.* [70]
PKF, AbOmpA	Aluminu m	1:1 (v/v)	0, 14, 28, 42 and 56	500 µl	i.p.	C57/BL Murine	A. baumannii ATCC 19606	E. coli BL21 (DE3)	Mice		18 h, day 56th, 7 days	A. baumannii ATCC 19606	Bolourchi *et al.* [71]
OmpK/Omp22	Freund's complete	1:1 (v/v)	14 and 21	20 µg	s.c.	Sepsis	A. baumannii ATCC 19608	pCold-K/22	Mice		24 h, 72 h	A. baumannii	Guo *et al.* [72]
CAM87009.1	Freund's complet e	1:1 (v/v)	0, 14, 21, and 28	101 µg	s.c., i.p.	Murine	A. baumannii fimbr iae	E. coli	Mice			A. baumannii fimbriae	de Freitas *et al.* (2021) [73]
Type I pili, CsuA/B, and FimA	Freund's complete	1:1 (v/v)	0, 13, 27, and 42	10 µg	s.c., i.p.	Sub-lethal murine sepsis	A. baumannii ATCC 19,606	E. coli BL21 (DE3)	Mice	22·28 ±1·9	14 h, 24 h	A. baumannii ATCC 19,606	Ramezanaliza deh *et al.* [74]
BAYE2132	Freund's complete	1:1 (v/v)	3	100 µg	s.c.	Murine pneumonia	A. baumannii ATCC 19606	E. coli ER2566 and pET28-a	Mice	20-25 g	24 h, 48 h	A. baumannii ATCC 19606	Mahmoudi *et al.* [75]
Blp1 and Bap	PBS and Freund's complete		0, 14, 28, 32, and 42	2 µg	i.m.	Sepsis murine	A. baumannii	E. coli ArcticExpress (DE3)	Mice		24 h	A. baumannii	Skerniškytė *et al.* [76]

metabolism, which promotes biofilm disruption, nuclease activity, and immune modulation. Probiotic LAB have also been shown to produce biosurfactants capable of eradicating A. baumannii biofilms [77]. This dual action direct bacterial antagonism combined with antigen-specific immunity underscores the potential of potential LAB-based oral vaccines targeting OMPs and OMV-associated antigens.

Potential LAB-Based Oral Vaccine Targets Among *A. baumannii* Fimbriae and Adhesin Proteins

The polysaccharide capsule and surface-associated structures of *A. baumannii* influence its interactions with host tissues and environmental surfaces. Fimbriae, pili, and adhesins have been implicated in biofilm formation and epithelial attachment [73,80-81]. These functions make selected proteins potential antigen candidates, but their surface exposure, conservation, and protective immunogenicity must be established for each target. Antibacterial or anti-adhesion effects reported for unrelated LAB strains cannot be assumed to confer vaccine-specific protection.

Blp1 has been investigated as an adhesin-associated protein potentially involved in *A. baumannii* biofilm formation and adhesion, the interpretation of gene-deletion findings depends on the experimental strains and assays used [76]. FilF, Ata, and NucAb have also been considered as potential antigenic components of multiepitope strategies. However, any claim of protection by a particular construct requires its own documented challenge outcomes. Antibody binding or inhibition of adhesion in vitro does not demonstrate in-vivo protection unless directly tested. Thus, adhesin and fimbrial targets merit further evaluation, but their suitability for LAB-based oral vaccination remains hypothetical [82].

Immunogenicity Considerations for Potential LAB-Based Oral Vaccines Against *A. baumannii*

Figure 3 highlights the proposed approach to study selected surface antigens of *A. baumannii* through recombinant Lactic Acid Bacteria (LAB). It is important to state that the illustration is not considered evidence of an experimentally validated oral vaccine. Previous studies conducted on *A. baumannii* OMVs have proven immune response and decrease in bacterial burden upon challenge [48,56,83]. Nevertheless, immune mechanisms which provide such effects have not been identified until now and cannot be specified without the help of specific tests. OMV components derived from pathogens can activate pathways of the innate immune system, yet, every pathway mediated by cytokines or receptors in the case of vaccines must be confirmed by the cited researches [55].

Native OMVs from Acinetobacter baumannii contain multiple membrane-associated components, whereas recombinant LAB are Gram-positive vectors that lack an outer membrane and do not naturally produce *A. baumannii*-type OMVs. LAB can be engineered to express heterologous proteins intracellularly, secrete them, or present them on their surfaces using appropriate expression and anchoring systems [16,84]. Hwang *et al.* [85] demonstrated surface presentation and mucosal immune responses using a recombinant LAB construct directed at an unrelated pathogen. OMV-associated proteins such as OmpA could therefore be investigated as candidate cargoes, but their expression, folding, localization, immunogenicity, and protective activity in a LAB-based oral system remain to be established.

For proposed LAB oral vaccines targeting *A. baumannii*, mucosal IgA and systemic IgG are potential immunogenicity endpoints, not evidence of established protection. In native *A. baumannii* OMV studies, immune responses and bacterial burdens were measured directly after challenge. Higham *et al.* [83] reported reduced airway colonization and systemic dissemination after intranasal OMV immunization, whereas systemic antibody responses induced by intramuscular immunization did not necessarily predict protection. These findings concern native OMVs rather than LAB-expressed antigens. Durable memory and the roles of individual cytokines, including IL-1, IL-4, and IL-7, should not be inferred without the corresponding cellular, functional, and longitudinal measurements [48, 56].

Figure 3. Proposed, unvalidated recombinant LAB-based oral delivery strategy for selected Acinetobacter baumannii antigens. The stages of LAB engineering, oral administration, mucosal recognition, and potential immune responses are illustrative, reduced colonization, improved survival, long-term immune memory, and clinical benefit have not been demonstrated for the specific LAB–*A. baumannii* construct depicted. Conceptual background adapted from Morris *et al.* [86] (DOI: 10.3389/fmicb.2019.01601, CC BY 4.0) and Smiline Girija and Ganesh [87] (DOI: 10.1007/s00203-021-02721-9). Abbreviations: LAB, lactic acid bacteria, OMVs, outer membrane vesicles, OmpA, outer membrane protein A, Pal, peptidoglycan-associated lipoprotein, Bap, biofilm-associated protein, Ata, Acinetobacter trimeric autotransporter, PilQ, type IV pilus secretin, LOS, lipooligosaccharide, RND, resistance-nodulation-division, TolC, outer membrane efflux channel, PLD, phospholipase D, FecA, ferric-dicitrate transport receptor, sIgA, secretory immunoglobulin A, IgG, immunoglobulin G, IL-17, interleukin-17, IFN-γ, interferon gamma. Rights for any graphical material reused from [87] must be confirmed.

Functional Relevance of Antibody Responses

Antigen-specific IgA and IgG titres quantify immunogenicity but are not, on their own, measures of antibody-mediated bacterial killing or protective efficacy. In an *A. baumannii*-specific study, Higham *et al.* [83] measured antibody responses alongside respiratory and systemic bacterial burdens following native OMV vaccination and challenge, intranasal vaccination reduced bacterial colonization and dissemination, while systemic antibody induction after intramuscular vaccination did not guarantee protection. These are directly observed outcomes in a non-

LAB OMV model, not evidence that an LAB-based oral vaccine protects against *A. baumannii* or that a particular antibody effector mechanism has been proven.

In general, mucosal immunology, secretory IgA (sIgA) can contribute to immune exclusion and restriction of microbial adhesion, but these functions should not be presumed from an increased sIgA titre alone [15, 16]. Recombinant LAB studies targeting unrelated pathogens have reported antigen-specific mucosal antibody responses, including the PDCoV S1 model of Zhai *et al.* [23]. In an *A. baumannii*-specific model, Higham *et al.* [83] measured mucosal antibody responses and reduced bacterial burdens after native OMV vaccination. Neither observation, without an IgA-specific functional or depletion experiment, demonstrates that sIgA itself prevented *A. baumannii* colonization.

A. baumannii antigen studies using non-LAB immunogens have reported antibody responses and challenge outcomes, for example, Bolourchi *et al.* [71] and Akbari *et al.* [65] investigated recombinant protein antigens in murine models. IgG can participate in Fc-dependent phagocytosis and complement-associated clearance according to general immunological principles, but those mechanisms should be described as proposed for a given vaccine unless opsonophagocytic killing, complement activity, or another direct functional assay was performed. Increased IgG concentrations, decreased bacterial burdens, and survival outcomes are distinct measurements and cannot by themselves identify the mechanism responsible for protection.

Antigen localization is a vaccine-design variable that may affect antigen accessibility and measured immune responses in individual recombinant LAB systems [15-16]. Evidence from surface-display studies involving unrelated pathogens supports platform feasibility, not functional antibody superiority or *A. baumannii*-specific protection. *A. baumannii* OMVs of native origin contain many different components of bacteria, nevertheless, the extent to which these components are recognized, are opsonized or involved in complement-mediated killing and the protection against challenge by the pathogen needs to be determined separately for each individual formulation of OMVs and cannot be extrapolated based on OMV composition only [55, 56].

Synthesis of Evidence Strength and Translational Relevance

The evidence mapped in this review differs in directness, experimental outcome, and translational relevance. Recombinant LAB studies targeting porcine deltacoronavirus or Trichinella spiralis, including those of Zhai *et al.* [23], Ma *et al.* [47], and Xu *et al.* [49], demonstrated antigen delivery and measured mucosal or systemic immune responses in non-*A. baumannii* models. These studies support platform feasibility, not protection against *A. baumannii*. In contrast, Bolourchi *et al.* [71] and Akbari *et al.* [65] reported challenge outcomes following immunization with pathogen-specific recombinant antigens in mice, while studies of native *A. baumannii* OMVs reported immune and bacterial-burden endpoints [48, 56, 83]. These pathogen-specific data provide more direct evidence for antigen selection, but they do not demonstrate efficacy of an oral LAB delivery system. Furthermore, antibody titres must be distinguished from functional antibody assays, bacterial clearance, and survival endpoints. Overall, the combined strategy remains preclinical and hypothetical, it requires construct-specific expression and stability testing, pathogen-specific functional immunity assays, reproducible challenge experiments, and safety evaluation before clinical translation.

Future Experimental Priorities

Development of recombinant LAB-based oral vaccine candidates against multidrug-resistant Acinetobacter baumannii requires a staged response to experimental and translational gaps [4,59]. Initial work should assess construct stability during propagation and storage, confirm antigen expression and intracellular, secreted, or surface-associated localization, and compare the immunogenicity of these formats [15,16]. Oral dose, immunization schedule, formulation stability, and survival under gastric and bile conditions should then be optimized and measured directly [17].

Antigen prioritization should address genetic and antigenic heterogeneity across *A. baumannii* strains. The conserved OMPs, fimbriae, adhesins, and a selected group of OMV-associated proteins can be considered as vaccine candidates, either individually or in combinations, but cross-protection can be established only empirically, not based on the conservation of an antigen or by complexity of a construct [35]. The immune response should discriminate between mucosa-specific secretory IgA and systemic IgG responses. The endpoints such as opsonophagocytic killing, serum bactericidal killing, complement activation, interference with bacterial adherence or biofilm development, bacterial load, and survival rate need to be assessed individually. Correlates of protection need to be established.

Efficacy of protection must be assessed through standardized and reproducible animal model challenges featuring defined strains of bacteria, defined dose of challenge, route of infection, control group, and predetermined endpoint. Independent validation with tests conducted on clinically important and genetically heterogeneous strains of bacteria is required for evaluation of consistency and range of the observed protection. Differences between results in the laboratory setting and clinically important outcomes can limit extrapolation to human subjects [4, 88].

Development needs to emphasize on manufacturing scalability, batch-to-batch consistency, product quality and shelf-life stability. Taking into consideration that recombinant LAB are living and genetically engineered microorganisms, there will be a need for assessing genetic containment, horizontal gene transfer, gastrointestinal

survival, shedding and risks to vulnerable groups. The requirements related to environmental containment, manufacturing and human clinical testing have to be taken into account. This is important before implementing the LAB oral approach.

CONCLUSIONS

The evidence summarized here suggests that recombinant LAB warrant investigation as potential oral vaccine vectors against multidrug-resistant *A. baumannii* infections. Studies involving other pathogens indicate that selected recombinant LAB can function as mucosal antigen-delivery vectors and elicit immune responses. However, genetic tractability and a history of food use do not establish the safety of engineered live vectors. Suitability for further development requires strain-, construct-, host-, and context-specific assessment of genetic stability, horizontal gene transfer, gastrointestinal persistence and shedding, and risks in vulnerable populations.

Native *A. baumannii* OMVs contain multiple immunogenic bacterial components and have elicited measurable antibody responses and reduced bacterial burdens in selected preclinical challenge studies [48, 56, 83]. These outcomes do not, without direct investigation, establish long-lasting B- or T-cell memory or define the antibody or cytokine mechanisms of protection. Further research must identify suitable antigens, compare adjuvant and delivery strategies, perform pathogen-specific functional assays, and evaluate durability and protective efficacy in standardized challenge models before considering translation to humans.

Further work should prioritize antigen conservation and cross-strain coverage, determine how microbiota interactions influence delivery and antigen exposure, and directly compare mucosal and systemic immune responses in standardized models. Each recombinant LAB construct also requires evaluation of genetic stability, horizontal gene transfer, persistence, shedding, and safety in vulnerable hosts before clinical investigation.

Multidrug-resistant *A. baumannii* remains a major healthcare threat. Recombinant LAB-based oral vaccine candidates warrant further research, but their clinical applicability is uncertain and depends on reproducible protective efficacy, stable antigen expression, acceptable biosafety profiles, and compliance with the regulatory requirements applicable to live genetically modified organisms.

Author Contributions

Hadeel M. Khalaf: Conceptualization, Data Curation, Investigation, Writing-Original Draft. Nurul Izza Ismail, Hasan A. Aal Owaif, Amira Suriaty Yaakop: Methodology, Supervision, Validation, Visualization, Writing-Review & Editing. Muna E. Raypah: Writing-Review & Editing.

Data Availability

Data will be made available upon request.

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