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Article review
Mechanism of formation outer membrane vesicles of bacteria: A review

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A B S T R A C T

Pathogenic and non-pathogenic bacteria secrete outer membrane vesicles (OMV) throughout normal growth. The formation of outer membrane vesicles is an important biological process. The vesicle membranes of bacteria are created when a little part of the membrane stands out from the envelope of the cell, then it is released. This review aimed to focus on OMV formation and its application as vaccines. This study focused on research related to the development of outer membrane vesicles and their use as vaccines. It depended on 13 studies and reviews (2010-2025). The studied databases comprised in PubMed, Scopus, and Web of Science.

Most studies showed that OMVs play vital roles in pathogenesis, stress response, and immunomodulation. Many factors for instance, temperature and exposure to toxic elements, need an adaptation for the cell of bacteria to survive in different circumstances. Inconclusion, according to the examined evidence, OMVs are multiple function nanostructures that serve purposes beside bacterial survival, encompassing immunological regulation, horizontal transfer of genes, and medicinal delivery. Future studies must emphasize standardized techniques for OMV separation, comprehensive compositional analysis, and clinical studies assessing the safety and immunogenicity in people.

1. Introduction

Extracellular vesicles (EVs) are a diverse group of membrane structures produced by cells across three domains of life: eukaryotic cells, archaea, and bacteria. Bacterial extracellular vesicles play a significant role in interactions between bacteria themselves and between bacteria and hosts during infection [1]. Outer membrane vesicles (OMVs) represent spherical membrane structures liberated from outer membrane of bacteria. Membrane vesicles of bacteria have been found for over sixty years; outer membrane vesicle biogenesis represents a poorly described process. In cells, outer membrane vesicles for eukaryotes have a role in storage, trafficking, and cellular component digestion according to function and location. Outer membrane vesicles enable bacteria to excrete insoluble molecules with soluble matter [2].

Outer membrane vesicles play various roles in symbiosis and pathogenesis. Outer membrane vesicles are contribute to antibiotic impedance, nutrient uptake, cells communication, stress responses to stress

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and transfer of nucleic acid. Outer membrane vesicles are filled with nucleic acid, proteins, and different compounds that act as "multi-objective carriers" liberated into the environment [3].
Outer membrane vesicles are considered nanocarriers that deliver virulence factors to host tissues [4]. OMVs play essential roles in immunomodulation and the establishment of the gut microbiota [5]. In addition, they could be considered as a defense mechanism by carrying away toxic compounds after exposure to stressful conditions [6] as shown in Figure 1.
Researchers have proposed an important role for outer membrane vesicles (OMVs) in the homeostasis of microorganisms [7]. The vesicles can be distinguished by their shape, size (between 30 nm and 1 μm), and cellular location based on the function that they perform [8]. Interestingly, Pseudomonas aeruginosa can use OMVs to disrupt the way host cells transport substances by causing the breakdown of the cystic fibrosis transmembrane conductance regulator (CFTR) with a toxin called Cif that is carried in the OMVs [9].
Outer membrane vesicle composition and vesiculation are affected by the growth environment, temperature, quorum sensing, and bacteria growth phases [10]. Several gram-negative bacteria release natural outer membrane vesicles during growth or due to the stress of the environment (e.g. nutrient loss, antibiotic exposure, and oxidation), contributing to inducing bacterial fitness. They can merge into biofilms encouraging biofilm formation by contributing pivotal nutrients [11].
It was supposed that gram-negative bacteria can make outer membrane vesicles; recently it has been conveyed that some gram-positive bacteria produce extracellular vesicles similar to outer membrane vesicles, although they are less characterized [12].
Outer membrane vesicles can regulate the host immune system as a result of the immunogenic protein and glycans existing on the surface, providing them with natural adjuvant properties and suggesting outer membrane vesicles can be used as in vaccination programs. It revealed that intrinsic adjuvant action induces both inherent and adaptive immunity responses [13].

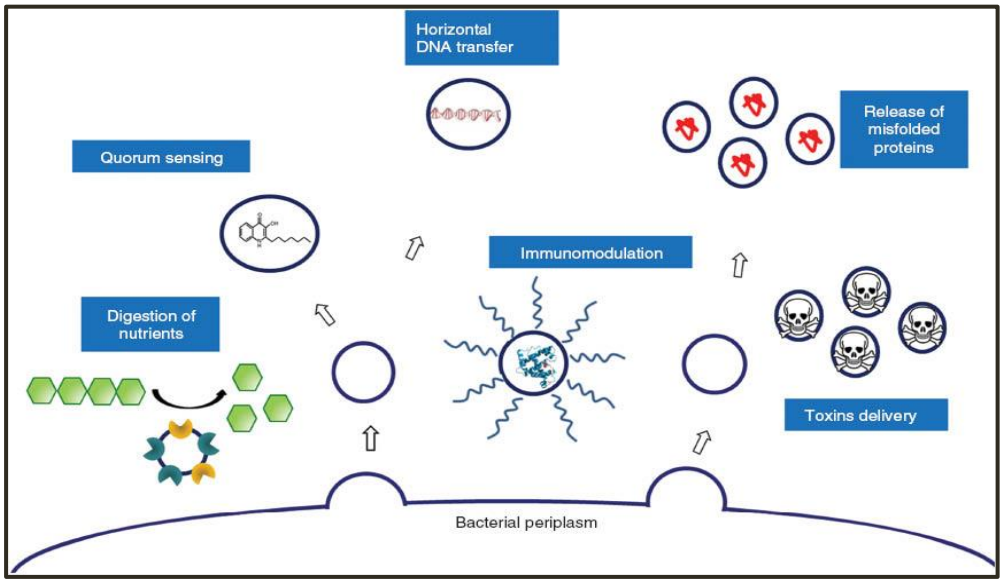

Fig.1. The role of outer membrane vesicles in bacteria, which are loaded with multiple molecules, including DNA, toxins, misfolded proteins, and quorum sensing signals.

2.Methodology

The study was carried out using a systematic literature search. This study focused on research related to the development of outer membrane vesicles and their use as vaccines. It depended on 13 studies and reviews the years (2010-2025). The studied databases comprised in PubMed, Scopus, and Web of Science. Search terms encompassed 'outer membrane vesicles', 'OMV biogenesis', 'OMV composition', and 'OMV vaccinations'. Studies were considered if they inclusion (1) examined the methods, composition, or applications of bacterial OMV production; (2) underwent peer review. Studies were rejected if they concentrated exclusively on eukaryotic vesicles, were opinion pieces lacking data, or were abstracts without comprehensive results. Data from each study were retrieved

and classified into themes: biogenesis mechanisms, environmental impacts, molecular composition, and therapeutic/vaccine applications.

3.Litrature review

3.1. Outer membrane vesicle (OMV) Biogenesis

OMVs are small blisters arising from bacteria's outer membrane. OMV development may need energy due to the growth of bacteria cells to excrete OMV [14]. Secretion of OMVs is an important process that permits bacteria to interact with their environment [5]. The composition of OMV has been identified, and the description of outer membrane composition, like LPS and outer membrane proteins, assured the outer membrane origin [15]. Biogenesis of vesicles is the selective method ; specific cell compositions are chosen carefully as loads to conduct a different cellular function. The peptidoglycan fragment accumulation in the periplasmic area could exert a pressure of turgor strong sufficient to curve the outer membrane and produce outer membrane vesicles (Figure 2A) [16]. On the other hand, the peptidoglycan fragment encapsulated inner part of the outer membrane to secrete as a virulence factor. Outer membrane vesicles originated in outer membrane regions with relaxing outer membrane-peptidoglycan interaction and proteins that support this interaction precluded from the outer membrane vesicle (Figure 2B). O antigen role in outer membrane vesicle biogenesis was investigated in many strains of *P. aeruginosa* that exhibit various phenotypes of LPS on their surface including (A+B+, A-B+, A+B- and A-B-) [17]. Outer membrane vesicles originated in regions where the B-band moiety is plentiful, Outer membrane bending to free the charge discording produced by the O antigen charged with negative (Figure 2C) [15].

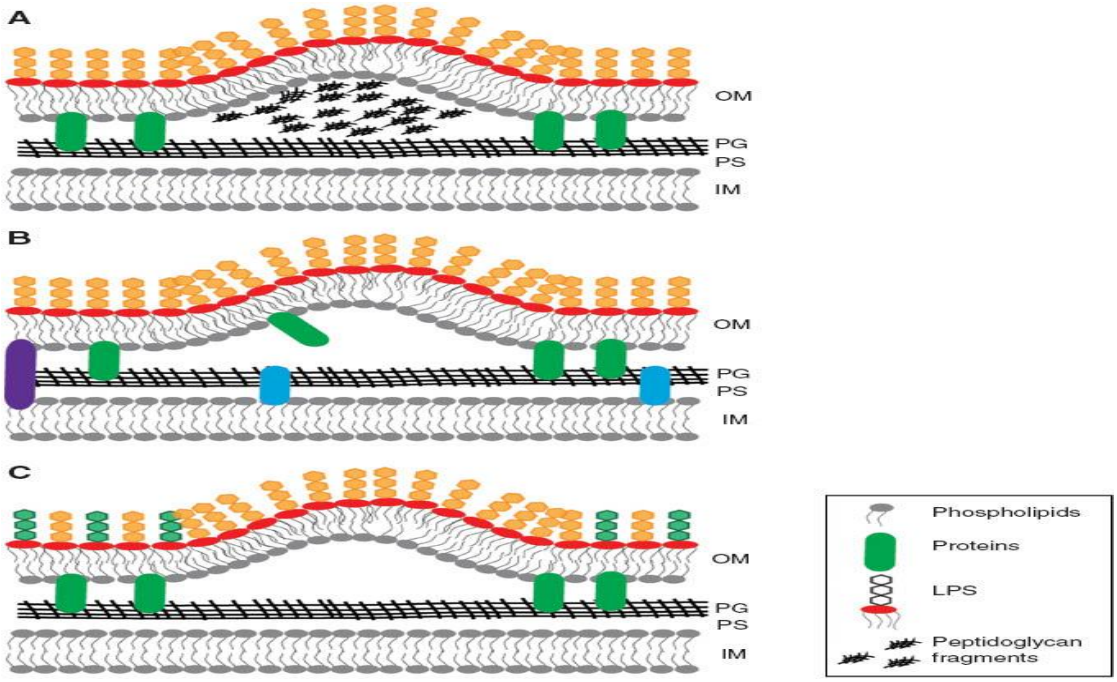

Fig. 2. Outer membrane vesicle (OMV) formation models. (A) Peptidoglycan fragments accumulation, (B) Outer membrane-peptidoglycan interaction, (C) O antigen charged repulsion with negative charged (orange) and (green) the neutral O-antigen excluded. OM (outer membrane) PG(peptidoglycan layer)PS(periplasmic space). IM (inner membrane).

3.2. Composition of OMV

Analyses of outer membrane vesicle composition have shown that vesicles contain differences in virulence factors, including proteins, adhesins, lipopolysaccharide, enzymes, glycerophospholipids,

toxins, outer membrane proteins, and periplasmic components [15]. Many outer membrane vesicles are enriched in envelope composition, as well as cytosolic and inner membrane proteins[16]. Biochemical and proteomic analyses showed that OMVs have several bacterial compositions,such as RNA, DNA, proteins, LPS, enzymes, and peptidoglycan [17, 18]. The compositions of OMVs are summarized in Table 1[19].

Table 1: Compositions of OMVs (Outer membrane vesicles) and description

Composition	Description
Membrane structure	Composed of a lipid bilayer and analogous to the outer membrane of bacteria
Lipid	LPS, phospholipid and lipid-associated membrane
Proteins	Various transmembrane and outer membrane proteins , that included channel proteins, enzymes, and transporters.
Nucleic acid	RNA, DNA, and included genes transfer horizontally
Signalling molecules and cytotoxins	Carried signalling molecules, toxin, and bioactive molecules
Periplasmic protein	Consists of proteins in periplasmic space of bacteria
Function and size of OMVs	Transport materials, immunological regulation, transfer of genes; size ranged 20-400nm

Toxic matters are related to outer membrane vesicles from various pathogenic bacteria; vesicles of Pseudomonas aeruginosa have been shown to contain both monomers of flagellin and DNA CpG [15,17]. Purified vesicles can act as a transmission mechanism for virulence factors by reacting with both eukaryotic and prokaryotic cells [20], as shown in Figure 3.

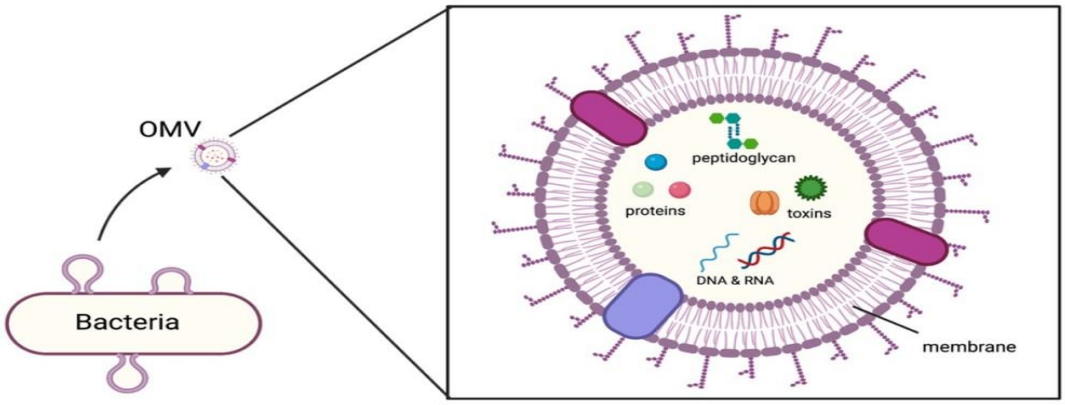

Fig. 3. Outer membrane vesicle components, such as protein and genetic materials

Moreover, gram-negative bacteria release OMVs, which are important for virulence, communication, and interactions between the host and the pathogen. The amount of content may change depending on how bacteria grow (for example, when they are stressed or in a biofilm state). Some OMVs have genes that make them resistant to antibiotics or toxins that make infections worse [21], as shown in Table 2.

Table.2. Bacterial species and their outer membrane vesicles (OMVs) content

Bacterial Species	OMVs Content	Key Functions/Effects
Escherichia coli	Lipopolysaccharides (LPS), proteins (OmpA, OmpC), DNA, RNA, toxins	Immune modulation, biofilm formation, virulence delivery
Pseudomonas aeruginosa	LPS, virulence factors (exotoxin A, elastase), quorum-sensing molecules	Host cell damage, immune evasion, antibiotic resistance
Neisseria meningitidis	LPS (endotoxin), outer membrane proteins (PorA, PorB), DNA	Meningitis induction, immune system activation
Helicobacter pylori	VacA toxin, CagA protein, urease, LPS	Gastric inflammation, peptic ulcers, carcinogenesis
Salmonella Typhimurium	LPS, flagellin, SPI-1/SPI-2 virulence factors	Host cell invasion, systemic infection promotion
Vibrio cholerae	Cholera toxin (CTX), hemagglutinin protease, LPS	Diarrhea induction, host colonization
Acinetobacter baumannii	LPS, β-lactamases, efflux pump proteins	Antibiotic resistance, biofilm formation, immune evasion
Porphyromonas gingivalis	Gingipains, LPS, fimbriae proteins	Periodontitis, inflammation, bone resorption
Bacteroides fragilis	Polysaccharide A (PSA), proteases, LPS	Commensal-host interaction, immune tolerance induction
Mycobacterium tuberculosis	Lipoproteins (LpqH), glycolipids, ESX secretion system proteins	Immune evasion, macrophage manipulation

3.3. Outer membrane vesicles originated from mechanical disturbance

When bacterial membranes are physically disturbed in ways like using EDTA, vortexing, or sonication, it can lead to more outer membrane vesicles being produced. Lipopolysaccharide (LPS)molecules are negatively charged, and calcium molecules in the membrane save LPS from repulsion. Calcium chelators used for the isolation (like EDTA) weaken the bacteria's membrane, resulting in outer membrane vesicles. This results in outer membrane vesicles that are closely similar to native bacterial membranes and induce an immunity response [22]. Sonication of bacterial fragments and whole bacteria causes these components to merge and form an outer membrane vesicle. This led to the appearance of nonmembrane composition included in outer membrane vesicles [23], also, the bacterial vertexing would have an identical effect. The nonmembrane composition introduced into outer membrane vesicles using these methods may raise antigenicity, but at the same time reduce safety [19].

4. Outer membrane vesicles applied as a vaccine

The article analyzed the utilization of outer membrane vesicles in vaccinations. A comparison analysis with prior studies indicates that OMV-based vaccinations exhibit superior immunogenicity relative to pure antigen vaccines in animal models (e.g., OMV-based Neisseria vaccines induced a 2–3-fold augmentation in bactericidal antibodies). Nevertheless, some research warn of possible reactogenicity stemming from residual LPS levels, indicating the necessity for detoxification procedures[24].

Outer membrane vesicles are a natural means by which bacteria can deliver substances, such as nucleic acids, proteins, and lipids, to nearby cells and biofilms. The primary use of OMVs is to present antigens for vaccination purposes. This approach was first developed to protect against N. meningitidis serogroup B. Different bacterial types have subsequently produced OMVs to deliver antigens from either homologous or heterologous strains. As mentioned earlier, it is also possible to express non-bacterial antigens and have them appear on the surface of OMVs after they fuse with a carrier protein[25] .

There are two approved OMV vaccines: VAMENGOC-BCTM and BexseroTM, which both protect against the harmful *N. meningitidis* serogroup B strain (MenB).Vaccines targeting various meningococcal serogroups of Neisseria have been available for some time; however, developing a vaccine for MenB has proven difficult due to the significant variability of the primary antigen, PorA, among different strains. Conventional vaccine strategies have proven ineffective due to the low immunogenicity of PorA and its structural similarity to foetal neural tissue. Outer membrane vesicles (OMVs) can be derived from the specific strain responsible for a meningitis outbreak, demonstrating effectiveness in instances of clonal outbreaks [22].

VA-MENGOC-BCTM was the inaugural licensed OMV vaccine, receiving approval for use in Cuba in 1987 after years of elevated disease rates caused by a single serotype of MenB (B4:P1.15). The vaccine encompassed serogroup C, with an estimated efficacy of 85% . OMV vaccines were later utilized during MenB outbreaks in Norway, New Zealand, and France. The vaccine administered during the Norwegian and French (MenBVac) outbreaks exhibited a rapid decrease in immune response over time; however, it unexpectedly provided broader protection against various Neisserial strains [11,26]. The MenB epidemic in New Zealand reached its peak in 2001. A mass immunization campaign utilizing the OMV vaccine MeNZBTM occurred between 2004 and 2006, with an estimated efficacy of 75% [26].

Currently, the only OMV-based vaccine authorised by the FDA and EMA is BexseroTM, manufactured by GlaxoSmithKline in Brentford, the United Kingdom. The vaccine is administered intramuscularly to individuals aged 10 to 25 years. This vaccine utilized the MeNZBTM OMV vaccine used during the New Zealand outbreak and is formulated with three additional recombinant proteins (rMenB) identified through reverse vaccinology. The vaccine is projected to protect 66–91% of MenB strains globally [22]. BexseroTM has demonstrated cross-protective effects against N. gonorrhoeae [11].

Lastly, this contribution aims to integrate many bacterial systems and clarify the mechanisms of gaining evidence from OMV production in the context of its immunomodulatory and therapeutic potential. This review continues to be more comprehensive than previous ones by organizing the findings around themes, among which biogenesis mechanisms, environmental factors, molecular cargo, and translational applications stand out, focusing after all on more than one bacterial species or one functional element. The article also emphasizes the OMV processes in traditional infections like *Neisseria meningitidis* and merges them with the recent findings from *Acinetobacter baumannii*, which is considered an opportunistic pathogen, thus showing how vesiculation strategies can be conserved and adaptable at the same time. This report will provide perspectives for the development of broad-spectrum OMV-based therapies. All these factors contribute to the lack of OMV purification standards.

Limitations: This review is limited to the collective studies that stem from unequal bacterial species, purification methods, and analytical approaches. This lack of uniform standards leads to the heterogeneity problem. Due to diverse measurement systems and standards for reporting results,

quantitative comparisons become more complex. Because of their nascent stage, applications such as the use of OMV in cancer immunotherapy and gene delivery lack sufficient literature.

5. Conclusion

Outer membrane vesicles are the consequence of a selective and directed cellular process. Many studies doubt the presence of a true vesiculation method in bacteria. The vesiculation displays bacterial cells the capacity to face insoluble and soluble stress outcomes; the production of vesicles offers a mechanism for the cell to save itself from tension circumstances. Nevertheless, much information about OMV biogenesis remains unanswered. Gram-negative bacteria release OMVs, which are important for virulence, communication, and interactions between the host and the pathogen. The amount of content may change depending on how bacteria grow (for example, when they are stressed or in a biofilm state). Some OMVs have genes that make them resistant to antibiotics or toxins that make infections worse. According to the examined evidence, OMVs are multiple function nanostructures that serve purposes beside bacterial survival, encompassing immunological regulation, horizontal transfer of genes, and medicinal delivery. Future studies must emphasize standardized techniques for OMV separation, comprehensive compositional analysis, and clinical studies assessing the safety and immunogenicity in people.

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آلية تكوين حويصلات الغشاء الخارجي للبكتيريا: مراجعة

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الكلمات المفتاحية

الغشاء الخارجي، الحويصلات، التكوين الحيوي

الملخص

تُفرز البكتيريا المُمرضة وغير المُمرضة حويصلات الغشاء الخارجي (OMV) خلال نموها الطبيعي. يُعد تكوين حويصلات الغشاء الخارجي عملية بيولوجية مهمة. تتكون أغشية حويصلات البكتيريا عندما يبرز جزء صغير من الغشاء من غلاف الخلية، ثم يتم إطلاقه. هدفت هذه المراجعة إلى التركيز على تكوين حويصلات الغشاء الخارجي وتطبيقاتها كلقاحات. ركزت هذه الدراسة على الأبحاث المتعلقة بتطوير حويصلات الغشاء الخارجي واستخدامها كلقاحات. اعتمدت على 13 دراسة ومراجعة (2010-2025). تضمنت قواعد البيانات المدروسة PubMed وScopus وWeb of Science. أظهرت معظم الدراسات أن حويصلات الغشاء الخارجي لها أدوارًا حيوية في التسبب بالمرض والاستجابة للإجهاد وتنظيم المناعة. تتطلب العديد من العوامل، مثل درجة الحرارة والتعرض للعناصر السامة، تكيفًا لخلية البكتيريا للبقاء على قيد الحياة في ظروف مختلفة. في الختام، وفقًا للأدلة المدروسة، تُعدّ OMVs هياكل نانوية متعددة الوظائف، تخدم أغراضًا إلى جانب بقاء البكتيريا، وتشمل التنظيم المناعي، والنقل الأفقي للجينات، وتوصيل الأدوية. يجب أن تُركّز الدراسات المستقبلية على تقنيات موحدة لفصل OMVs، والتحليل التركيبي الشامل، والدراسات السريرية التي تُقيّم السلامة والمناعة لدى الأشخاص..

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