Bacterial Extracellular Vesicles and Liver Diseases: A Survey

Abstract

Bacterial extracellular vesicles (BEVs), particularly Outer Membrane Vesicles (OMVs), are emerging as critical players in the pathogenesis of liver diseases through their intricate interactions with the microbiome-liver axis. These nano-sized particles, released by bacteria, are instrumental in modulating immune responses and facilitating cellular communication, thereby influencing liver pathology. This survey systematically explores the multifaceted roles of BEVs, focusing on OMVs, in liver disease mechanisms. It highlights how OMVs contribute to liver disease progression by delivering virulence factors and bioactive molecules that enhance bacterial survival and pathogenicity. The survey also delves into the microbiome-liver axis, emphasizing the bidirectional communication that influences liver health and disease. Recent advances in OMV research are discussed, showcasing methodological innovations that offer deeper insights into OMV-host cell interactions and their potential as therapeutic targets. Despite significant progress, challenges remain in understanding the precise mechanisms of OMV cargo delivery and their selective targeting, underscoring the need for further research. The therapeutic potential of BEVs is vast, with applications extending to infectious disease management, cancer therapy, and liver disease treatment. Continued exploration of BEV mechanisms and interactions with host systems is crucial for developing innovative strategies to combat liver diseases and improve patient outcomes.

1 Introduction

1.1 Structure of the Survey

This survey is systematically organized to explore the role of bacterial extracellular vesicles (BEVs), particularly Outer Membrane Vesicles (OMVs), in liver diseases. The **Introduction** highlights the significance of BEVs in liver pathogenesis and their interaction with the microbiome-liver axis. The subsequent section, **Background and Definitions**, provides essential concepts, detailing BEVs, OMVs, liver diseases, and the microbiome-liver axis.

The survey progresses to the **Role of Bacterial Extracellular Vesicles in Liver Diseases**, examining the contributions of these vesicles, especially OMVs, to liver disease pathogenesis. The section on the explores the intricate interactions between the gut microbiome and liver function, emphasizing the influence of bioactive extracellular vesicles (BEVs). This exploration underscores the gut microbiota's role in modulating liver immunology and its implications for various liver diseases, including alcohol-associated liver disease (ALD), nonalcoholic fatty liver disease (NAFLD), and autoimmune liver disease (AILD). By reviewing current research, this section elucidates the mechanisms through which gut dysbiosis contributes to liver pathology and highlights potential therapeutic interventions that leverage microbiome-liver interactions [1, 2].

In the **Mechanisms of Pathogenesis** section, the survey discusses the specific ways OMVs contribute to liver disease, including interactions with host cells, immune modulation, and the delivery of

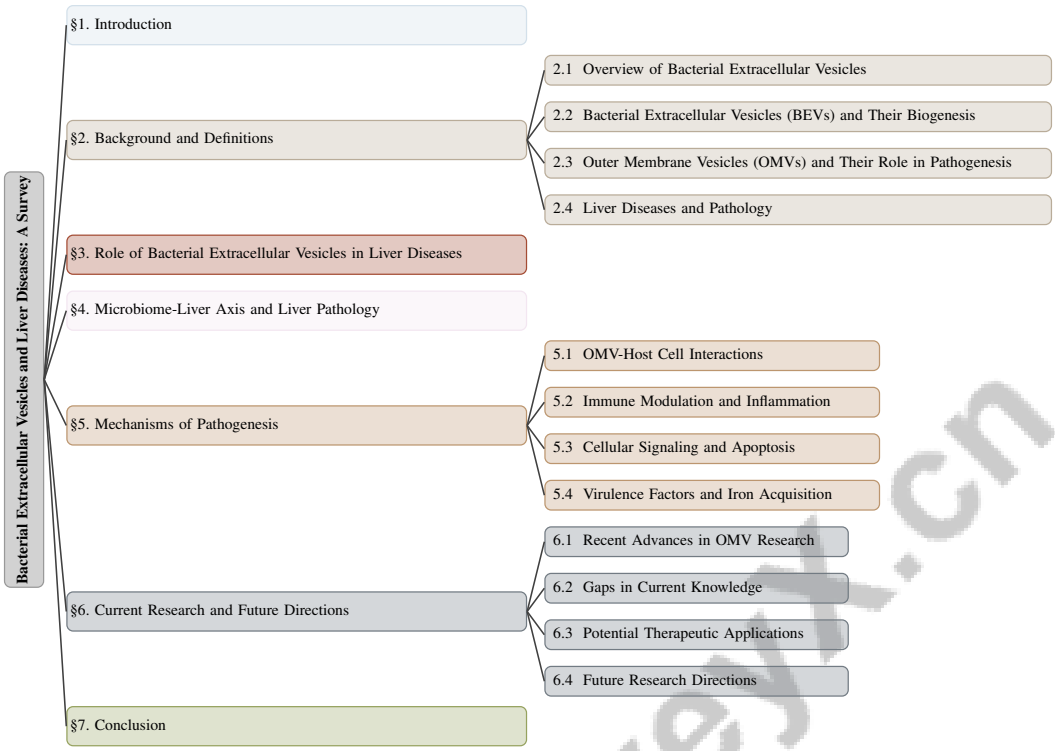

Figure 1: chapter structure

virulence factors. The following section, **Current Research and Future Directions**, highlights recent advances, identifies gaps in knowledge, and suggests potential therapeutic applications and future research trajectories.

The **Conclusion** synthesizes the key points, reiterating the critical role of BEVs in liver disease pathogenesis and their potential as therapeutic targets. This structured approach facilitates a comprehensive exploration of the relationships between BEVs and liver diseases, emphasizing their roles in intercellular communication, immune modulation, and pathogenicity. By elucidating the mechanisms through which BEVs influence host responses and disease progression, this research lays a solid groundwork for developing innovative diagnostic tools and therapeutic strategies for liver-related health issues [3, 4, 5, 6].The following sections are organized as shown in Figure 1.

2 Background and Definitions

2.1 Overview of Bacterial Extracellular Vesicles

Bacterial extracellular vesicles (BEVs) are nanoscale, membrane-bound particles released by both Gram-negative and Gram-positive bacteria, playing a pivotal role in intercellular communication and host-pathogen dynamics. They are integral to the dissemination of bacterial components, such as lipopolysaccharides (LPS), which provoke immune responses and systemic inflammation [7]. BEV biogenesis involves membrane protrusion and detachment, encapsulating biomolecules like proteins, nucleic acids, and metabolites [8]. This encapsulation allows BEVs to deliver virulence factors, modulating bacterial and host cellular processes.

BEVs impact the microbiome-liver axis by modulating immune responses and cellular signaling pathways, significantly affecting liver health [9]. Dysbiosis, characterized by gut microbiota imbalances, modifies BEV production and composition, influencing liver pathology. The cargo of BEVs, including proteins and genetic material, is linked to disease processes, such as carcinogenesis, highlighting their potential role in cancer development [4]. Understanding BEVs' characteristics and functions is crucial for unraveling their roles in health and disease, particularly liver diseases, where they may serve as biomarkers and therapeutic targets.

2.2 Bacterial Extracellular Vesicles (BEVs) and Their Biogenesis

BEVs emerge from bacteria through intricate biogenesis pathways, essential for microbial communication and pathogenesis. The formation involves membrane protrusion and detachment, encapsulating diverse biomolecules such as proteins, lipids, nucleic acids, and metabolites [3]. Environmental factors and bacterial physiological states influence vesicle composition and function [5]. For instance, *Escherichia coli* produces outer membrane vesicles (OMVs) in a temperature-dependent manner, affecting their biophysical properties and biological roles [10].

BEV biogenesis is categorized based on pathways and host cell interactions, illustrating their diverse roles in health and disease [4]. The type VI secretion system (T6SS) is crucial for iron acquisition via interactions between effectors like TseF and OMVs, emphasizing BEVs' role in nutrient acquisition and microbial survival [11]. BEVs also facilitate intercellular communication by transferring signaling molecules and genetic material between bacterial cells and hosts, influencing immune modulation and cellular apoptosis [12].

OMVs are categorized by function and potential applications, such as antibiotic delivery, highlighting their role in inter-bacterial communication [13]. Isolation techniques like ultracentrifugation (UC) and ultradiafiltration (UF) have been compared for efficiency, enhancing understanding of vesicle yields and properties across bacterial strains [14]. Despite progress, challenges remain in elucidating BEV interactions with host cells due to rapid OMV entry and cargo delivery [15]. Ongoing research explores BEVs as biomarkers and therapeutic targets, particularly in liver diseases, offering promising intervention avenues [6].

2.3 Outer Membrane Vesicles (OMVs) and Their Role in Pathogenesis

Outer Membrane Vesicles (OMVs) are critical in the pathogenesis of various diseases due to their involvement in bacterial virulence and host cell interactions. Originating from Gram-negative bacteria's outer membrane, OMVs encapsulate bioactive molecules, such as proteins, LPS, and nucleic acids, enhancing pathogenic potential [15]. Their biophysical characteristics, influenced by origin and environmental conditions, are vital in determining host cell interactions and disease outcomes [10].

OMVs facilitate virulence factor dissemination, enhancing bacterial survival and pathogenicity within the host [14]. For example, the effector protein TseF, secreted by T6SS, interacts with OMVs containing Pseudomonas quinolone signal (PQS) for iron acquisition, crucial for bacterial survival and virulence [11]. Additionally, OMVs' inflammatory nature can induce robust immune responses, including pro-inflammatory cytokine production, potentially leading to conditions such as sepsis [16]. This ability underscores OMVs' significance in mediating disease and exacerbating inflammatory conditions [17].

The dual membrane structure of Gram-negative bacteria contributes to inherent antibiotic resistance, complicating therapeutic strategies [13]. This resistance is exacerbated by OMVs' role in the horizontal transfer of antibiotic resistance genes. Despite their importance in pathogenesis, studying OMVs is challenging due to difficulties in isolating and characterizing these vesicles, hindering understanding of host cell interactions [6]. Advances in isolation and characterization techniques are essential for overcoming these challenges and leveraging OMVs as targets for innovative therapeutic interventions.

2.4 Liver Diseases and Pathology

Liver diseases encompass a broad spectrum of conditions affecting liver function, potentially leading to severe health complications. Recent research focuses on metabolic dysfunction-associated steatotic liver disease (MASLD), characterized by hepatic lipid accumulation and its progression to more severe liver diseases [18]. MASLD can evolve into non-alcoholic steatohepatitis (NASH), marked by inflammation and fibrosis, with progression from MASLD to NASH being critical due to potential evolution into cirrhosis or hepatocellular carcinoma (HCC) [19, 20].

The gut microbiota's role in liver diseases has gained considerable attention, particularly in non-alcoholic fatty liver disease (NAFLD), alcohol-associated liver disease (ALD), and autoimmune liver disease (AILD) [2]. The gut-liver axis is a crucial pathway through which gut-derived factors,

including BEVs, influence liver pathology. Dysbiosis, or gut microbiota imbalances, is implicated in these liver diseases' pathogenesis, contributing to inflammation and liver damage progression [7].

The impact of bacterial pathogens and their OMVs on liver health is an emerging interest area. For instance, OMVs from *Porphyromonas gingivalis*, primarily associated with periodontal disease, have been linked to systemic diseases like NAFLD, highlighting the interconnectedness of oral and systemic health [21]. This underscores the importance of understanding bacterial infections' systemic implications and their vesicles in liver pathology.

The study of liver diseases increasingly emphasizes their multifactorial nature, revealing how metabolic dysfunction, gut microbiota dysbiosis, and immune responses interact to contribute to liver disorders' pathophysiology, including ALD, NAFLD, and AILD. This focus on the gut-liver axis is crucial for developing targeted therapeutic strategies for liver diseases [1, 2]. A comprehensive understanding of these interactions is essential for identifying biomarkers for early detection and progression of liver diseases.

In recent studies, the role of Outer Membrane Vesicles (OMVs) has garnered significant attention, particularly concerning their implications in liver disease pathogenesis and immune modulation. As illustrated in Figure 2, this figure highlights the multifaceted impact of OMVs on disease progression, their therapeutic potential, and their ability to enhance pathogen virulence. Such insights are crucial for understanding the complex interactions at play in liver diseases and may pave the way for novel therapeutic strategies.

3 Role of Bacterial Extracellular Vesicles in Liver Diseases

3.1 Outer Membrane Vesicles and Liver Disease Pathogenesis

Outer Membrane Vesicles (OMVs) are increasingly recognized for their role in liver disease pathogenesis, particularly in how they mediate bacterial-host interactions that influence disease progression. Notably, periodontal pathogens like *Porphyromonas gingivalis* utilize OMVs to disseminate virulence factors, impacting both periodontal and liver conditions [21]. Their significance is especially evident in the progression of non-alcoholic fatty liver disease (NAFLD) to more severe conditions such as non-alcoholic steatohepatitis (NASH) and hepatocellular carcinoma (HCC). As the prevalence of NAFLD rises, effective diagnostic and therapeutic strategies become crucial [20]. The multifactorial etiology of NAFLD complicates its pathogenesis, with OMVs potentially exacerbating liver inflammation and fibrosis through immune modulation and cellular apoptosis [19].

OMVs from various bacterial species deliver bioactive molecules that alter host cellular functions. For example, *Neisseria gonorrhoeae* uses OMVs to transport PorB to macrophage mitochondria, inducing dysfunction and apoptosis, thereby facilitating immune evasion [12]. These interactions underscore OMVs' capacity to modulate immune responses in the liver, exacerbating disease pathology. The size and composition of OMVs are crucial in determining their pathogenic potential, as their size influences host cell entry and protein composition impacts bacterial pathogenesis [17]. Understanding these properties is essential in the context of liver diseases.

This figure illustrates the role of Outer Membrane Vesicles (OMVs) in liver disease pathogenesis, highlighting their impact on disease progression, properties influencing their pathogenic potential, and their therapeutic applications Figure 3. OMVs' ability to encapsulate and deliver hydrophobic molecules, coupled with their stability and selective targeting potential, positions them as promising vehicles for therapeutic interventions in liver diseases [13]. Advances in isolation techniques, such as ultradiafiltration, which yields smaller vesicle sizes, are vital for optimizing OMV applications in therapy and research [14]. Current research highlights the potential of bacterial extracellular vesicles, including OMVs, as modulators of immune responses and vehicles for therapeutic agents, opening new avenues for liver disease treatment [4]. Understanding OMV involvement in liver disease development is crucial for devising innovative strategies to combat these conditions and enhance patient outcomes.

3.2 OMVs in Immune Modulation and Pathogen Virulence

Outer Membrane Vesicles (OMVs) play a pivotal role in modulating immune responses and enhancing pathogen virulence, significantly contributing to infectious disease pathogenesis. Secreted by

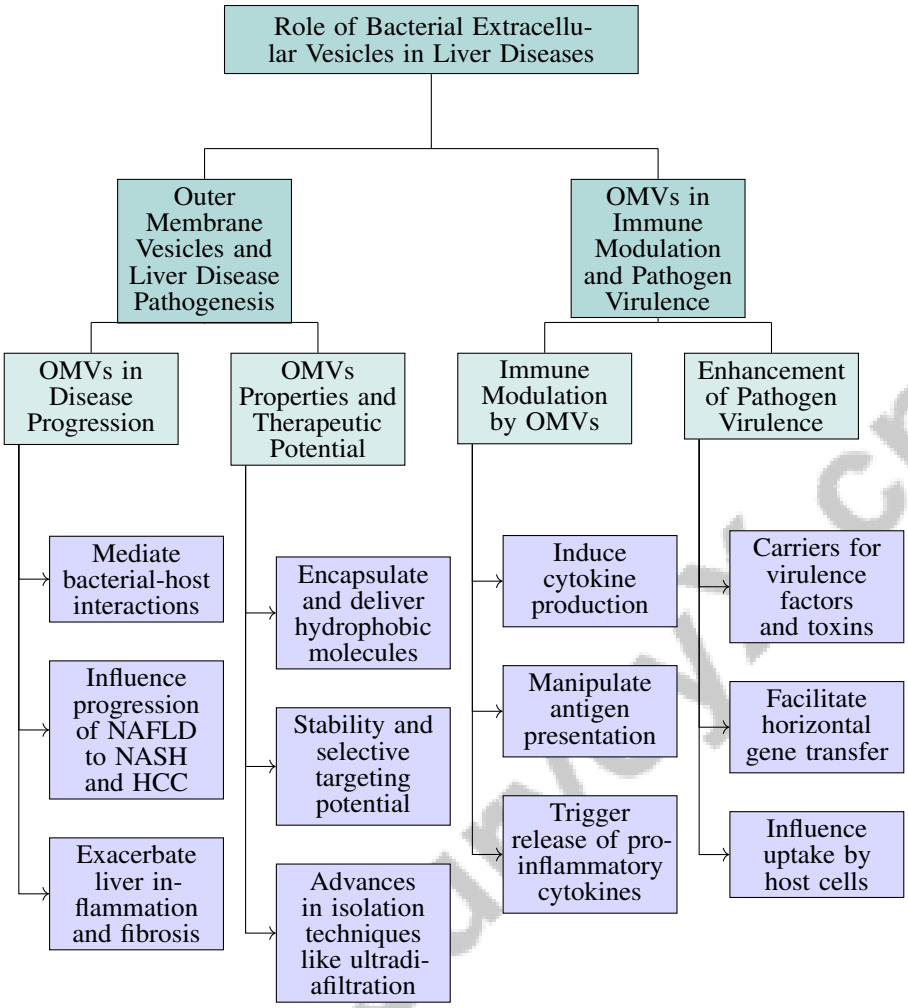

Figure 2: This figure illustrates the role of Outer Membrane Vesicles (OMVs) in liver disease pathogenesis and immune modulation, highlighting their impact on disease progression, therapeutic potential, and enhancement of pathogen virulence.

Gram-negative bacteria, these vesicles deliver bioactive molecules such as proteins, lipopolysaccharides (LPS), and nucleic acids, interacting with host immune systems in complex ways [15]. The immunomodulatory effects of OMVs arise from their ability to induce cytokine production, manipulate antigen presentation, and modulate immune cell functions, facilitating immune evasion and pathogen persistence [16].

OMVs interact with macrophages and dendritic cells, triggering the release of pro-inflammatory cytokines like TNF-, IL-6, and IL-1, contributing to systemic inflammation and disease progression [17]. For instance, OMVs from *Neisseria gonorrhoeae* induce mitochondrial dysfunction and apoptosis in macrophages, impairing the host's immune response [12], illustrating how OMVs can alter immune cell functions, promoting bacterial survival and virulence.

Furthermore, OMVs enhance bacterial virulence by serving as carriers for virulence factors and toxins, protecting them from degradation and facilitating targeted delivery to host cells, thereby increasing pathogenic potential [14]. The biophysical properties of OMVs, including size and lipid composition, influence their uptake by host cells and subsequent immune responses [10]. Understanding these structural characteristics is crucial for elucidating pathogen-host interactions.

Additionally, OMVs facilitate horizontal gene transfer, including the dissemination of antibiotic resistance genes, posing significant challenges in treating bacterial infections [13]. This function not

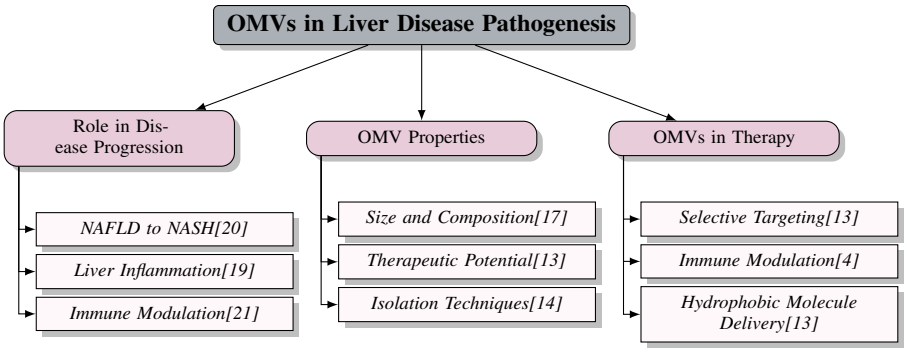

Figure 3: This figure illustrates the role of Outer Membrane Vesicles (OMVs) in liver disease pathogenesis, highlighting their impact on disease progression, properties influencing their pathogenic potential, and their therapeutic applications.

only enhances bacterial survival in hostile environments but also complicates therapeutic interventions, necessitating the development of novel strategies to target OMVs and their cargo.

4 Microbiome-Liver Axis and Liver Pathology

4.1 Microbiome-Liver Axis

The microbiome-liver axis is a dynamic interaction between gut microbiota and the liver, crucial for liver health and disease pathogenesis. This bidirectional system facilitates the transfer of gut-derived metabolites, microbial products, and inflammatory mediators to the liver via portal circulation, impacting chronic liver diseases such as hepatitis B and C, alcoholic liver disease, and non-alcoholic fatty liver disease (NAFLD) [1, 2]. The gut microbiota, acting as a "virtual metabolic organ," modulates immune responses and metabolic processes, highlighting its significance in liver physiology and pathology. Maintaining this axis's integrity is essential for liver function, influencing metabolism, immune response, and detoxification.

Dysbiosis, an imbalance in gut microbiota, leads to the translocation of bacterial products like lipopolysaccharides (LPS) and bacterial extracellular vesicles (BEVs) into portal circulation, triggering inflammatory responses in the liver and exacerbating NAFLD progression to non-alcoholic steatohepatitis (NASH) [19]. Therapeutic strategies targeting the microbiome-liver axis aim to restore gut microbiota balance and reduce liver inflammation through probiotics, prebiotics, and synbiotics, which enhance beneficial microbial populations and decrease gut permeability [19]. These interventions offer potential for improving liver health and preventing disease progression.

Understanding the microbiome-liver axis is pivotal for developing effective therapies and identifying biomarkers for early liver disease detection. Hepatology research increasingly acknowledges this axis's clinical potential, suggesting innovative management strategies for liver diseases, including NAFLD, alcohol-associated liver disease (ALD), and autoimmune liver disease (AILD). This evidence underscores the intricate relationship between gut microbiota and liver function, indicating that targeted interventions like fecal transplantation could significantly enhance patient outcomes [9, 1, 20, 2].

4.2 Influence of Gut Microbiota and Dysbiosis

The gut microbiota, a diverse microbial community in the gastrointestinal tract, is vital for host health by regulating metabolic, immune, and barrier functions. Dysbiosis, an imbalance in gut microbiota, is linked to various liver diseases, including NAFLD, ALD, and AILD [2]. This imbalance increases intestinal permeability, allowing bacterial products such as LPS and BEVs to enter portal circulation, triggering hepatic inflammation and liver damage [7].

In NAFLD, dysbiosis is associated with progression from simple steatosis to NASH, marked by inflammation and fibrosis. Altered gut microbiota in NAFLD patients leads to increased production of short-chain fatty acids (SCFAs) and other metabolites, influencing lipid metabolism and inflammatory

pathways in the liver [19]. Specific bacterial taxa correlate with liver disease severity, suggesting gut microbiota composition as a potential biomarker for disease progression and treatment response.

In ALD, dysbiosis exacerbates liver injury by promoting endotoxin translocation that activates hepatic Kupffer and stellate cells, resulting in inflammation and fibrosis. Disruption of gut barrier integrity and increased circulating endotoxins are critical in ALD progression, underscoring the need for a balanced gut microbiota for liver health [2].

Therapeutic interventions targeting gut microbiota, such as probiotics, prebiotics, and fecal microbiota transplantation, aim to restore microbial balance and reduce liver inflammation. These approaches show promise in modulating gut-liver interactions and improving liver function, highlighting the potential of microbiome-targeted therapies in managing liver diseases [19].

The influence of gut microbiota and dysbiosis on liver diseases highlights the importance of understanding the gut-liver axis in hepatic condition pathogenesis and treatment. Ongoing research in gut microbiota modulation is crucial for developing innovative strategies to prevent and treat liver diseases, given the significant role the gut-liver axis plays in these conditions [1, 8, 9, 2, 7].

4.3 Gut-Liver Axis as a Bi-Directional Communication Pathway

The gut-liver axis functions as a sophisticated bidirectional communication system, crucial for maintaining homeostasis and influencing liver disease pathogenesis. This axis facilitates the exchange of metabolites, microbial products, and immune signals between the gut and liver, primarily through the portal vein, allowing gut-derived substances to reach the liver directly. Conversely, the liver influences gut function and microbial composition via bile acids and other metabolites, establishing a dynamic feedback loop essential for metabolic regulation and immune homeostasis [2].

Maintaining the gut-liver axis's integrity is vital for liver health; disruptions can lead to bacterial component translocation like LPS and BEVs into the liver, triggering hepatic inflammation and contributing to liver pathology [7]. These microbial products activate hepatic immune cells, such as Kupffer cells, resulting in pro-inflammatory cytokine production and the progression of liver diseases like NAFLD and ALD [19].

Bile acids, synthesized in the liver and released into the intestine, play a significant role in modulating gut microbiota composition and function. They act as signaling molecules influencing metabolic pathways and immune responses, affecting both gut and liver health. Dysregulation of bile acid metabolism can disrupt gut microbiota, leading to dysbiosis and exacerbation of liver diseases [2].

Therapeutic strategies targeting the gut-liver axis focus on restoring microbial balance and enhancing gut barrier function to prevent harmful substance translocation. Approaches such as probiotics, prebiotics, and bile acid modulators aim to support bidirectional communication and maintain gut-liver axis integrity, offering promising avenues for liver disease prevention and treatment [19].

A comprehensive understanding of the gut-liver axis as a bidirectional communication pathway, involving intricate endocrine and immunological mechanisms, is essential for developing effective therapeutic interventions and identifying biomarkers. These advancements can significantly enhance early detection and management of liver diseases like chronic hepatitis, alcoholic liver disease, and NAFLD by leveraging insights from gut microbiota modulation and its impact on liver pathophysiology [20, 1, 8, 2, 7]. As research progresses, the potential to leverage this axis in clinical practice continues to expand, underscoring its importance in liver health and disease.

Figure 4 illustrates the key aspects of the gut-liver axis, highlighting the bidirectional communication pathways, the effects of disruptions on liver health, and potential therapeutic strategies.

4.4 Therapeutic Interventions Targeting the Microbiome-Liver Axis

Therapeutic interventions targeting the microbiome-liver axis represent a promising approach for managing liver diseases. These strategies focus on modulating gut microbiota and enhancing gut barrier function to mitigate liver inflammation and prevent disease progression. Probiotics, live microorganisms that confer health benefits, have shown potential in reducing liver inflammation and improving liver function by modulating immune responses and enhancing gut barrier integrity [2].

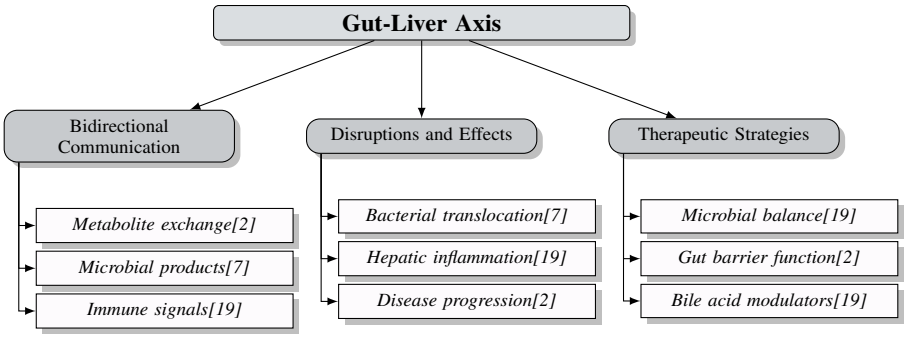

Figure 4: This figure illustrates the key aspects of the gut-liver axis, highlighting the bidirectional communication pathways, the effects of disruptions on liver health, and potential therapeutic strategies.

Prebiotics, non-digestible compounds that stimulate the growth of beneficial gut bacteria, are crucial for maintaining healthy gut microbiota and preventing dysbiosis-related liver diseases. The combination of probiotics and prebiotics, known as synbiotics, offers synergistic effects that further enhance gut health and support liver function [19]. These interventions aim to reduce the translocation of bacterial products such as LPS and BEVs into the liver, thereby decreasing hepatic inflammation and fibrosis.

Fecal microbiota transplantation (FMT) is an emerging therapeutic strategy involving the transfer of stool from a healthy donor to a patient with dysbiosis-related liver disease. FMT has shown promise in restoring microbial diversity and function, leading to improvements in liver health and metabolic parameters, highlighting the potential of directly manipulating gut microbiota for therapeutic benefits in liver diseases [2].

Bile acid modulators, which influence bile acid metabolism and signaling, are also being investigated for their role in modulating the gut-liver axis. Bile acids serve as signaling molecules that regulate metabolic pathways and immune responses; their dysregulation can contribute to liver pathology. Modulating bile acid composition and signaling can help restore gut microbiota balance and reduce hepatic inflammation, offering a novel therapeutic avenue [19].

Therapeutic interventions targeting the gut microbiome-liver axis present substantial opportunities for enhancing liver health and mitigating the progression of various liver diseases, including NAFLD, ALD, and AILD. This emerging research field underscores the intricate relationship between gut microbiota and liver function, emphasizing the potential for innovative treatment strategies such as fecal transplantation, probiotics, and prebiotics to restore microbial balance and improve liver outcomes. As evidence accumulates from preclinical and clinical studies, these interventions could redefine therapeutic approaches to chronic liver conditions [1, 2]. As research in this area advances, these strategies may become integral components of clinical practice, offering new hope for patients with liver-related conditions.

5 Mechanisms of Pathogenesis

Category	Feature	Method
Cellular Signaling and Apoptosis	OMV Influence on Signaling	TC-OMVs[10], OMV-T[12], NVs[16], CAOM[14]
Virulence Factors and Iron Acquisition	Iron Acquisition Mechanisms	GEHRA[15], TseF-IA[11]

Table 1: This table provides a comprehensive summary of the methods employed to investigate the influence of Outer Membrane Vesicles (OMVs) on cellular signaling, apoptosis, and iron acquisition mechanisms. It lists the categories, specific features studied, and the corresponding methods or techniques used, highlighting the breadth of approaches in understanding bacterial pathogenesis through OMVs.

In exploring pathogenesis mechanisms, Outer Membrane Vesicles (OMVs) are recognized as pivotal mediators in host-bacteria interactions. Far from being mere byproducts, OMVs actively influence

disease dynamics and host cellular functions, with significant implications for disease progression. Table 1 presents a detailed summary of the methods used to study the impact of OMVs on cellular signaling and iron acquisition, underscoring their role in bacterial pathogenesis.

5.1 OMV-Host Cell Interactions

OMVs are critical in mediating bacteria-host cell interactions, significantly influencing disease pathogenesis. They deliver bioactive molecules such as proteins, lipopolysaccharides (LPS), and nucleic acids to host cells [15]. The size and lipid composition of OMVs determine their uptake and intracellular trafficking [17], making it essential to understand these mechanisms to elucidate how OMVs modulate host functions and disease progression.

OMVs engage with immune cells, delivering components like PorB to mitochondria, inducing apoptosis and impairing immune responses [12]. This manipulation of apoptosis pathways facilitates immune evasion, enhancing bacterial survival. Hybrid reporter assays have shed light on OMV entry kinetics and cargo release, emphasizing the complexity of OMV-host interactions [15].

Biophysical properties, including thermodynamics, significantly influence OMV interactions with host cells. Temperature variations can alter lipid bilayer organization, affecting OMV stability and fusion with host membranes [10]. Their immunogenic nature can also trigger inflammatory responses, contributing to conditions like sepsis [16].

Effective OMV isolation methods are crucial for preserving vesicular integrity, essential for accurately assessing OMV-host interactions [14]. Advances in production techniques aim to enhance yield and reduce immunogenicity, improving their potential as therapeutic delivery vehicles [13].

Understanding OMV-host cell interactions is vital for developing therapeutic strategies targeting OMV-mediated pathways in infectious and inflammatory diseases, where OMVs deliver virulence factors, modulate immune responses, and influence pathogenesis. Exploiting OMV properties can lead to effective treatments against antibiotic resistance and related health challenges [15, 16, 13, 6].

5.2 Immune Modulation and Inflammation

OMVs are significant modulators of immune responses and inflammation, impacting disease pathogenesis. Secreted by Gram-negative bacteria, OMVs contain bioactive molecules that interact with host immune systems to alter inflammatory pathways [15]. They induce pro-inflammatory cytokine production, affect antigen presentation, and modulate immune cell functions, including those of macrophages and dendritic cells [16].

As depicted in Figure 5, this figure illustrates the role of Outer Membrane Vesicles (OMVs) in immune modulation, highlighting their activation of immune responses, characteristics influencing pathogenicity, and potential therapeutic applications. OMVs activate immune responses by engaging pattern recognition receptors (PRRs) on immune cells, leading to cytokine secretion such as TNF-, IL-6, and IL-1 [17]. This can trigger systemic inflammation, exacerbating conditions like sepsis and chronic inflammatory diseases. For instance, OMVs from *Neisseria gonorrhoeae* induce mitochondrial dysfunction and apoptosis in macrophages, impairing host immune response and facilitating bacterial survival [12].

Lipid composition and size of OMVs are crucial in determining their immunogenicity and ability to modulate immune responses, influencing host cell uptake and inflammatory response [10]. OMVs enhance bacterial pathogenicity by modulating host immune defenses and promoting immune evasion [14].

OMVs can also facilitate horizontal transfer of antibiotic resistance genes, complicating treatment efforts and underscoring the need for novel therapeutic strategies targeting OMV-mediated immune modulation [13]. Advances in OMV isolation and characterization are vital for elucidating their interaction with the immune system and developing OMV-based therapeutic interventions [6].

OMVs' role in immune modulation and inflammation highlights their potential as therapeutic targets, especially in combating infections and mitigating inflammatory responses associated with bacterial diseases [13, 3, 4, 16, 6]. Continued research into OMV-host interactions and their impact on immune responses is crucial for developing strategies to reduce pathogenic effects and improve clinical outcomes in inflammatory and infectious diseases.

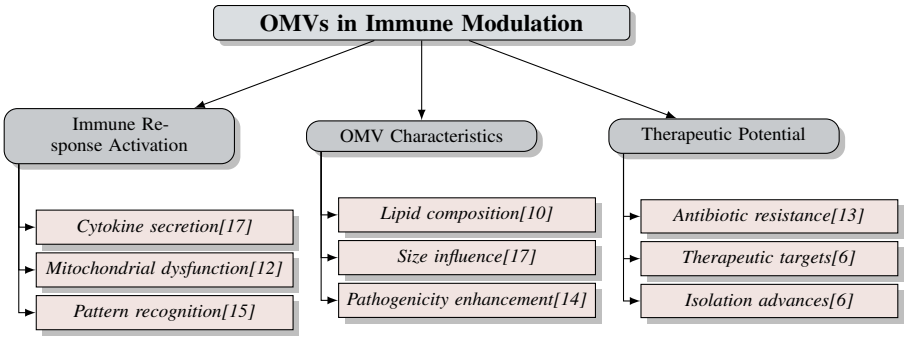

Figure 5: This figure illustrates the role of Outer Membrane Vesicles (OMVs) in immune modulation, highlighting their activation of immune responses, characteristics influencing pathogenicity, and potential therapeutic applications.

5.3 Cellular Signaling and Apoptosis

OMVs are integral in modulating cellular signaling pathways and inducing apoptosis, key processes in bacterial pathogenesis. They serve as delivery vehicles for bioactive molecules, including proteins, LPS, and nucleic acids, influencing host cell signaling pathways and cellular fate [15]. These interactions can activate signaling cascades that promote cell survival or induce apoptosis, depending on the infection context.

OMVs from pathogens like *Neisseria gonorrhoeae* carry PorB, targeting mitochondria in macrophages, leading to dysfunction and apoptosis [12]. This manipulation of apoptosis pathways facilitates immune evasion and enhances bacterial survival, providing a strategic advantage for bacteria.

The size and lipid composition of OMVs influence their interactions with host cell membranes and signaling pathways activation. Thermodynamic properties affect their stability and fusion with host membranes, modulating apoptotic signal delivery [10]. Their immunogenic nature can trigger inflammatory responses, contributing to disease pathogenesis [16].

Advances in OMV isolation and characterization are essential for understanding molecular interactions with host signaling pathways. Techniques like ultradiafiltration enable production of OMVs with defined sizes and compositions, facilitating studies on their effects on signaling and apoptosis [14]. Insights from this research are crucial for developing OMV-based therapeutic interventions targeting specific pathways to mitigate pathogenic effects.

OMVs' impact on cellular signaling and apoptosis underscores their significance in bacterial pathogenesis and potential as targets for novel therapeutic strategies. Ongoing research into how OMVs modulate host signaling and induce apoptosis is vital for developing targeted interventions. Understanding OMV delivery of virulence factors, such as PorB to mitochondria, could lead to innovative strategies disrupting pathogenic processes and enhancing clinical outcomes for infectious diseases [12, 13].

5.4 Virulence Factors and Iron Acquisition

OMVs are crucial in delivering virulence factors and facilitating iron acquisition, essential for bacterial pathogenesis. They enhance bacterial survival and pathogenicity by modulating host cellular processes and evading immune responses. The bacterial cell wall composition, particularly O antigen presence, influences OMV entry routes and efficiency, affecting virulence factor delivery [15].

Iron acquisition is vital for bacterial growth and survival, serving as a cofactor for numerous cellular processes. OMVs secrete proteins like TseF, linking Pseudomonas quinolone signal (PQS) signaling with type VI secretion system (T6SS)-mediated secretion and receptor engagement [11]. This mechanism highlights OMVs' strategic role in securing essential nutrients from the host, enhancing bacterial virulence and persistence.

The interplay between OMVs and iron acquisition pathways highlights bacterial pathogenesis complexity, where OMVs deliver virulence factors and facilitate nutrient uptake. This dual role is relevant

in liver diseases, where iron homeostasis disruptions can exacerbate disease progression. While progress has been made in understanding these mechanisms, questions remain about causal relationships between gut microbiota changes and liver disease progression, such as NAFLD, and long-term effects of therapeutic approaches targeting these pathways [20].

OMVs' ability to deliver virulence factors and facilitate iron acquisition is critical in bacterial pathogenesis, presenting potential therapeutic intervention targets. Ongoing research into complex interactions between gut microbiota and liver immunology is essential for formulating strategies to mitigate bacterial infections' adverse effects on host health, particularly regarding rising liver diseases like NAFLD, ALD, and AILD. Understanding the gut-liver axis and its dysregulation may lead to innovative therapeutic approaches, including gut microbiota modulation through fecal transplantation, probiotics, and prebiotics, potentially improving liver health outcomes [8, 1, 2].

6 Current Research and Future Directions

6.1 Recent Advances in OMV Research

Recent progress in Outer Membrane Vesicles (OMVs) research has significantly advanced our understanding of their roles in pathogenesis and therapeutic applications. High-sensitivity techniques have been developed to study OMV-host cell interactions, providing real-time insights into these complex dynamics [15]. These techniques enhance our understanding of OMV-mediated delivery of bioactive molecules to host cells, influencing cellular responses and disease progression. Innovations in isolating and analyzing bacterial extracellular vesicle-associated lipopolysaccharides (LPS) have further clarified OMVs' role in systemic immune activation and inflammation, particularly in liver diseases [7].

As illustrated in Figure 6, recent advances in OMV research encompass a variety of techniques and applications, including studies on OMV-host cell interactions, the identification of biomarkers for liver disease, and the potential of OMVs in cancer diagnostics and therapy. Significant advancements in liver disease research have identified new biomarkers and therapeutic targets for non-alcoholic fatty liver disease (NAFLD), facilitating the development of diagnostic tools essential for early detection [20]. Research on *Neisseria gonorrhoeae* has elucidated OMVs' role in modulating host immune responses, suggesting potential therapeutic interventions [12]. Additionally, the influence of OMV size on host cell entry mechanisms and protein cargo composition has been explored, revealing critical aspects of OMV function [17].

Bacterial extracellular vesicles (BEVs) have emerged as potential biomarkers for cancer diagnosis and play roles in immunotherapy and vaccine development, highlighting their clinical versatility [4]. BEVs facilitate the delivery of virulence factors and toxins, influencing disease mechanisms and paving the way for innovative therapeutic strategies. Their application in antibiotic delivery addresses challenges in combating antibiotic resistance, while their role in mediating immune responses presents new intervention opportunities in inflammatory conditions and cancer therapies [13, 3, 4, 16, 6].

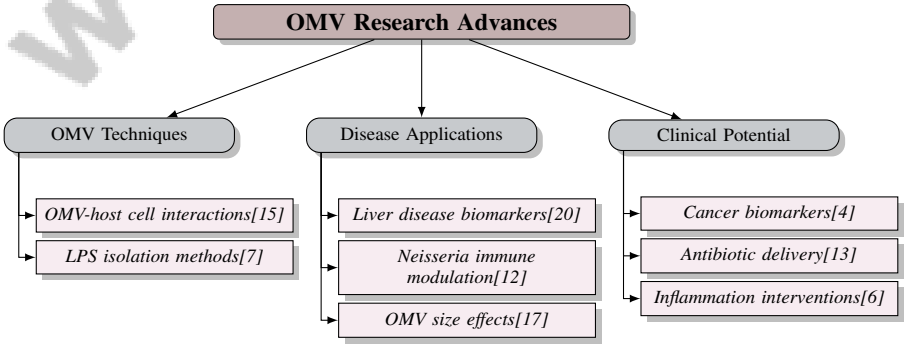

Figure 6: This figure illustrates the recent advances in Outer Membrane Vesicles (OMVs) research, focusing on OMV techniques, disease applications, and clinical potential. It highlights key areas such as OMV-host cell interaction studies, liver disease biomarker identification, and the role of OMVs in cancer diagnostics and therapy.

6.2 Gaps in Current Knowledge

Despite advances in understanding bacterial extracellular vesicles (BEVs) and their implications in liver diseases, several critical gaps persist. A significant challenge is the incomplete understanding of BEVs' contribution to oncogenesis and the variability of these effects based on the host's microbiome composition, complicating targeted therapy development [4]. This underscores the need for personalized medicine approaches, considering genetic factors in treatment response and the long-term safety of emerging therapies [19].

The mechanisms of OMV cargo delivery and factors influencing selective targeting remain inadequately explored [13]. Understanding these processes is vital for harnessing OMVs' potential as therapeutic delivery vehicles, enhancing specificity and efficacy in clinical applications. Additionally, the impact of different OMV isolation methods on vesicle morphology and yield poses challenges for downstream analyses, necessitating standardized protocols for consistency and reliability [14].

In liver disease contexts, the lack of extensive clinical trials establishing causative relationships between gut microbiota changes and liver disease outcomes represents a significant gap [1]. Exploring the mechanisms through which the gut microbiome influences liver pathology, particularly in hepatobiliary diseases, is essential for advancing our understanding of the microbiome-liver axis and developing effective diagnostic and therapeutic strategies. These gaps highlight the urgent need for in-depth investigations to clarify the interactions among BEVs, the gut microbiome, and liver diseases, particularly regarding immune modulation and pathophysiological processes [1, 9, 2, 4].

6.3 Potential Therapeutic Applications

The therapeutic potential of bacterial extracellular vesicles (BEVs), particularly Outer Membrane Vesicles (OMVs), is increasingly recognized in liver diseases and beyond. OMVs offer promising avenues for developing novel therapeutic strategies due to their natural capacity to deliver bioactive molecules and modulate immune responses. Targeting OMV delivery mechanisms may enhance immune responses, particularly in infections such as those caused by *Neisseria gonorrhoeae*, where OMVs are crucial for immune evasion [12].

Mesenchymal stem cell-derived nanovesicles (MSC-derived NVs) show potential as therapeutic agents for septic patients, emphasizing vesicle-based therapies in managing inflammation-related liver diseases [16]. These NVs could mitigate systemic inflammation and improve liver function, offering a novel approach to treating inflammatory liver conditions. Future research should focus on the systemic roles of BEVs, exploring their potential as biomarkers and correlating their taxonomy with gut microbiota metabolic activity [7].

In non-alcoholic fatty liver disease (NAFLD), a multidisciplinary approach is essential for effective management, with new therapies targeting the gut-liver axis and metabolic pathways showing promise [19]. BEVs could play a role in these therapies by modulating gut microbiota composition and function, thereby influencing liver health. Additionally, the biophysical characterization of OMVs, including their membrane phase behavior, could have diagnostic applications in microbiological diagnostics and oncology, differentiating vesicles from healthy and diseased cells [10].

The therapeutic potential of BEVs is vast, encompassing applications from infectious disease management to cancer therapy and liver disease treatment. Continued research into the mechanisms of BEVs and their complex interactions with host systems is essential for fully realizing their therapeutic potential, particularly in areas like cancer diagnostics and treatment, where BEVs may facilitate intercellular communication, modulate immune responses, and deliver bioactive molecules [4, 5, 6].

6.4 Future Research Directions

Future research on bacterial extracellular vesicles (BEVs) and their roles in liver diseases should prioritize several key areas to enhance scientific understanding and therapeutic applications. Large-scale randomized controlled trials are essential to validate the therapeutic potential of gut microbiota (GM) modulation, investigating the safety profiles of these interventions, and exploring personalized approaches based on individual microbiota compositions [1]. Such studies are crucial for establishing the efficacy and safety of microbiome-targeted therapies in liver disease management.

Optimizing OMV isolation techniques is another critical area for future research, facilitating the exploration of the functional significance of different OMV characteristics in relation to liver diseases [14]. This optimization is essential for developing engineered BEVs for targeted therapeutic applications, particularly in modulating gut microbiota and metabolic interventions aimed at liver disease.

Longitudinal studies tracking microbiome changes over time are necessary to understand the dynamic interactions between the microbiome and liver health, aiding in developing targeted therapies based on individual microbiome profiles. Investigating the mechanisms of OMV entry across various bacterial species, alongside the influence of OMV size and composition on their immunogenic properties, is crucial for understanding their role in immune modulation. Such research will elucidate how distinct bacterial cell wall structures, particularly specific components like lipopolysaccharide O antigen, affect the kinetics and efficiency of OMV uptake by host cells. Insights from this research could advance understanding of OMVs in disease pathogenesis and their potential applications in vaccine development and antibiotic delivery strategies [13, 15, 3, 17, 6].

Developing reliable methods for tracing EVs to their bacterial origins will enhance understanding of their diverse functions and potential therapeutic applications. In cancer contexts, future research should focus on elucidating BEV action mechanisms, exploring their therapeutic potential, and developing standardized methods for their analysis in clinical settings. The utilization of nanovesicles (NVs) derived from mesenchymal stromal cells (MSCs) in inflammatory diseases like sepsis demonstrates their potential to modulate immune responses and reduce cytokine storms, suggesting NVs as a promising therapeutic avenue for liver diseases and other inflammatory conditions, thereby broadening the application of vesicle-based therapies and addressing challenges associated with traditional extracellular vesicle (EV) isolation methods [13, 16].

The research directions highlighted in recent studies underscore the critical need for a multidisciplinary approach to effectively leverage the potential of biologically engineered vehicles (BEVs) in clinical settings. This approach is particularly promising for preventing and treating liver diseases, such as non-alcoholic fatty liver disease (NAFLD), characterized by complex pathophysiological mechanisms, including oxidative stress and gut microbiota interactions. By integrating insights from various fields, including microbiome research and innovative therapeutic strategies—such as the use of PPAR agonists and GLP-1 agonists—clinicians can enhance patient outcomes and address systemic conditions more comprehensively [20, 19].

7 Conclusion

Bacterial extracellular vesicles (BEVs), particularly Outer Membrane Vesicles (OMVs), have emerged as pivotal elements in the pathogenesis of liver diseases, highlighting their potential as both biomarkers and therapeutic targets. The intricate processes of BEV biogenesis facilitate the encapsulation and transport of diverse biomolecules, crucial for microbial communication and pathogenic mechanisms. OMVs, primarily derived from Gram-negative bacteria, significantly influence liver disease progression by modulating immune responses and enhancing pathogen virulence through the delivery of bioactive molecules. These vesicles play a vital role in the microbiome-liver axis, impacting liver pathology by altering immune responses and facilitating intercellular communication. The potential therapeutic applications of BEVs, such as in vaccine development and disease diagnostics, underscore their significance in advancing liver disease management. Continued research into the mechanisms of BEVs and their interactions with host systems is essential for harnessing their full therapeutic potential, offering promising avenues for innovative prevention and treatment strategies in liver diseases.

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