OPEN ACCESS

# Interdisciplinary Life Sciences

**(A Triannual, Refereed, & Peer Reviewed Journal of Life Sciences)**
Published by: AASTEMER, Mumbai, (MS) India
Journal homepage: https://jls.aastemer.org/
ISSN (Online): xxxx-xxxx
Volume 1, Issue 1, April 2026; Pages 19 – 28
DOI: 10.5281/zenodo.18765048

# Mycobacterial membrane vesicles in host pathogen interactions and pathogenesis - The detailed overview

**Chaitali Banerjee**

*Department of Zoology, Vidyasagar College for Women (Affiliated to University of Calcutta) 39 Sankar Ghosh Lane, Kolkata 700006 West Bengal, India*

**Article history:**

Received 21 July 2025
Received in revised form
26 September 2025
Accepted 30 September 2025
Available online 24 April 2026

*Corresponding author Email:*
chaitali.banerjeeibl@gmail.com



**Abstract**

*Mycobacterium* species poses threat globally and are the leading infectious agent world-wide. It's success in establishing the infection owes to its highly developed strategies in invading the cell and evasion of host immune system, thus increasing the incidence and prevalence of disease. A classical and well conserved phenomenon is the generation of extra cellular vesicles in different bacterial species. In the context of *Mycobacterium* sp. pathogenesis these generated membrane vesicles have been extensively studied and are associated with various mechanisms that can modulate host immune responses. Their characterization has revealed that these membrane vesicles are released by them itself as well as triggered to be released by the bacilli-infected cells. Largely they are involved in intercellular communication and cargo transportation of different kinds. The pathways and signalling molecules typically modulated inside the host cells are the prime targets behind the success of different *Mycobacterium* sp in establishing the infection. This review is an attempt to elucidate the signalling pathway triggered leading to the generation of different kinds of membrane vesicles, their biochemical characterization, it's implications to probable phenomena and conditions integral to pathogenesis. Several research studies have established these membrane vesicles as important candidate for vaccines and biomarkers. Collectively herein is focused the potential roles of the bacilli in effectively modulating the host immune machineries and being successful in surviving intracellularly with primarily emphasizing on membrane vesicles.

**Keywords:** *Mycobacterium*, Membrane, Vesicles, Immune, Pathogenesis, Survival

## Introduction

Mycobacteria are categorised under the order Actinomycetales, and are the only genus belonging to family Mycobacteriaceae [1]. The genus *Mycobacterium* includes acid-fast rod shaped bacteria and are predominantly pathogenic. These bacilli are characterized for possessing complex lipids in their cell wall like mycolic acids, hence rendering hydrophobicity. This hydrophobic nature let them grow as fungus-like pellicles on their culture media (broth): hence the nomenclature *Mycobacterium* – 'fungus bacterium'. These bacteria are referred to as acid-alcohol-fast. This typically means that after staining with Acid-Fast Stain (Ziehl-Neelsen stain) they resist decolorization with acidified alcohol as well as strong mineral acids and retain the primary red colour imparted by Carbol-fuchsin. There is no impact of the counter stain, Methylene Blue on the acid fast bacteria used at the later steps of staining procedure [2].

The characteristics species of *Mycobacterium* include *M. tuberculosis, M. avium, M. paratuberculosis, M. kansasii, M. marinum, M. fortuitum, M. scrofulaceum, M. xenopi, M. chelonei, M. lepraemirium, M. leprae, M. africanum, M. bovis* and many [3]. Overall there exists around 187 species of Mycobacterium, though the exact number is debatable. The Mycobacterial cell wall has an unique combination and are critically involved in the several important phenomena. The main components of the cell wall are the peptidoglycan (PG), different lipopolysaccharides (arabinogalactan, lipomannan and lipoarabinomannan) and the outer membrane rich in mycolic acids, phthiocerol dimycocerosates and several other glycolipids. Precisely, the mycolic acids comprises approximately 60 % of the bacterial cell wall. Their thin layer of inner peptidoglycan is in turn linked to arabinogalactan (D-arabinose and D-galactose). The arabinogalactan is further linked to an outer membrane that possess high-molecular weight mycolic acids. This arabinogalactan/mycolic acid layer is further overlaid by polypeptides and mycolic acids consisting of free lipids, glycolipids and peptidoglycolipids. Other important glycolipids include lipoarabinomannan and phosphatidyinositol mannosides (PIM). Similar to the outer membrane of the Gram-negative cell wall, porins are essential for Acid-Fast bacteria that facilitates transport of small hydrophilic molecules through their outer membrane [4]. This typically characterized membrane of Mycobacterial species are implicated in diverse biological processes like immunomodulation, iron acquisition, host defence factors, prevention against antibiotics, cell detoxification and bacterial communication, transfer of DNA and transportation of virulence factors [5].

Origin of vesicles from membrane has been initially reported for Gram negative bacteria. Though researchers in next few years conclusively demonstrated the formation of membrane vesicles in Gram positive bacteria and particularly in Mycobacterial sp. as well [6]. These vesicles are membrane bound structures that may range several nm in size. Their journey of nomenclature is also long and researchers gradually reached to the terminology of "extracellular vesicles", as a generic term for different secreted vesicles. The mechanism of formation of vesicles has always been a fascinating field of research. Several ways of formation has been proposed like membrane blebbing, explosive cell lysis involving the enzyme "endolysin" that works on peptidoglycan, bubbling cell death involving endolysin though through a different distinctive process. Howsoever, the exact mechanism of vesicles' formation in Mycobacterial species is poorly understood owing to the complicated structure of their cell wall as explained earlier. The Figure 1 incorporated in the latter part of the review is a pictorial presentation of biogenesis of these vesicles in different types of bacterial population [7]. The membrane vesicles particularly in case of Mycobacterial species have been reported to be of different shapes – sometimes tube like or spherical and at times it could have a shrunken appearance. These different shapes have been well evidenced by electron microscopic studies [8].

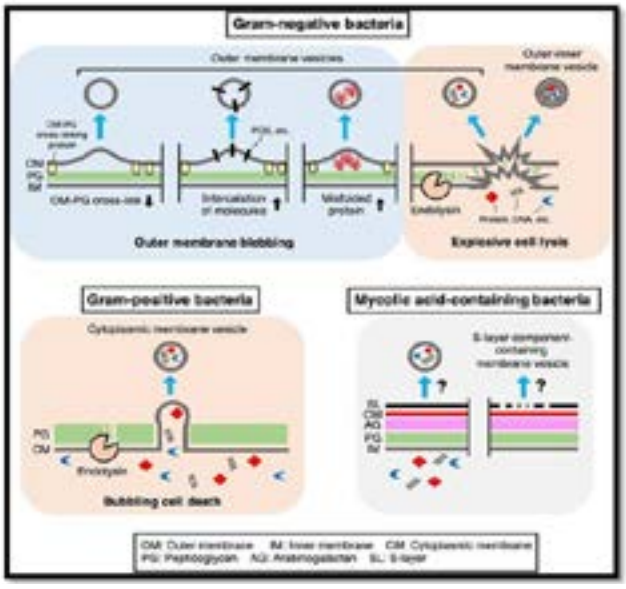


**Figure 1:** Mechanisms of EV biogenesis (Source: Nagakubo T, Nomura N, Toyofuku M. Cracking Open Bacterial Membrane Vesicles. Front Microbiol. 2020 Jan 17;10:3026. doi: 10.3389/fmicb.2019.03026. PMID: 32038523; PMCID: PMC6988826.)

The membrane vesicles are implicated in different

biological processes. It is reported that the membrane vesicles initiate pathways involving both innate and acquired immune system [9]. The vesicles have been proved to be involved in transfer of virulence effectors, bacterial communication and maintenance of the bacilli's communities. Another important phenomena in bacterial pathogenesis is iron acquisition. Several reports have conclusively demonstrated that the membrane vesicles originating from *Mycobacterium* sp. are typically involved in this. These are the probable reasons that explains the strategies they employ in their intracellular survival successfully to establish the infection [10].

**Membrane Vesicles - The elaborative illustration**

The formation and release of membrane vesicles are one of the most conserved cellular processes. These vesicles are involved in the cargo delivery of various kinds across and within cells. According to Gould and Raposo, 2013 these vesicles have been variously named and categorised [11]. Few readily used nomenclature being - extracellular membrane vesicles, membrane vesicles, microvesicles, exo/ecto somes (specially in case of Eukaryotes), microparticles and others. "Outer Membrane Vesicles (OMV)" are commonly referred in relation to Gram negative bacteria whereas "Membrane Vesicles (MV) or Extracellular Vesicles (EV)" are referred in the context of Gram positive bacteria. In few Gram negative bacteria like *Shewanella vesiculosa, Pseudomonas aeruginosa, Neisseria gonorrhoeae* it has been revealed that vesicles often pull cytoplasmic content involving protrusion of both outer and plasma membranes and these are referred as outer-inner membrane vesicles (O-IMV). In different bacterial species the formation of intracellular vesicles have been reported pertaining to certain conditions like overproduction of some membrane proteins [12]. The membrane vesicles are diversely implicated into different biological functions. In general the vesicles generated from Gram-positive bacteria are reported to contain nucleic acids, proteins, lipids and other metabolites whereas in Gram positive bacteria, they are suggested to be involved in biofilm formation, stress responses, immune regulation and others [13].

In respect of Mycobacterial species, it is worth mentioning that membrane vesicles of approximately 40 – 250 nm has been reported collectively from virulent, avirulent, fast and slow growing strains [14]. Extracellular vesicles in Mycobacteria were reported later than its initial report in Gram negative bacteria. However this Mycobacterial vesicles are important and involved in various aspects. The molecular characterization of *Mycobacterium*-specific extracellular vesicles have been done by analysing the (glyco)lipid content and involving techniques like 2D-TKC and MALDI-TOF. The results have indicated prevalence of phospholipids like PhosphatidylinositolMannoside–PIM2Ac1,PIM2Ac2; Phosphatidylglycerol, Phosphatidylethanolamine, Phosphatidylinositol, cardiolipin whereas phenolic glycolipids, polyacylated trehalose were detected in lower quantity. The proteomic analysis of the membrane vesicles have indicated the presence of different Mycobacterial proteins involved in virulence and immunogenic antigens like culture filtrate protein 10, Superoxide Dismutase, Catalase, the antigen 85 complex amongst others. The various lipoproteins and Toll like Receptor 2 agonists like LprG, LpqH, LprA, PstS1, and LppX are reported from these vesicles as well. The composition to a large extent indicates that the origin of these vesicles are from the inner Mycobacterial membrane [15].

These vesicles serves as important mechanisms for modulating immune responses, it's pathogenicity and subsequent interaction with the host, disease development, preventing measures and different diagnostic and therapeutic applications [9]. The next section of the review would focus on different aspects and mechanisms through which the Mycobacterial vesicles influence the diverse processes and shapes the outcome.

**Membrane vesicles in host pathogen interaction**

Host and microbes interaction is integral to understanding of infection, disease prevention and treatment. The typical candidates influencing host-pathogen interactions like pathogen associated molecular patterns, chromosomal and extra-chromosomal virulence factors, other toxins keep getting shuttled by Mycobacterial Extracellular Vesicles [16]. These and other such secreted candidates facilitates pathogen survival and evasion of immune responses, induces cytotoxicity and often mediates tissue damage of the host. Amongst several steps of pathogenesis few like proper contact or exposure with the potential disease-causing agent, attachment to the entry portal, invasive spread to the nearby tissues are critical to the process. This is usually followed by replication of the pathogen to establish the infection. In case of Mycobacteria induced pathogenesis, the initial contact and subsequent attachment to the site attracts phagocytic cells like neutrophils, macrophages, dendritic cells and often instigates inflammatory responses. The prime reason of Mycobacterial success relies on their classic ability to evade innate immunity and delay the initiation of adaptive immunity.

Basically the sentinel cells are manipulated by the bacillus to get transformed into permissive and safe cellular niche for multiplication and survival. This multipronged effect is remarkably achieved by the extensive players they harbour and mainly aims by targeting lysosomal pathways, blocking phagosomal maturation, manipulating inflammasome, evading and impairing autophagy and apoptotic mode of cell death, compromising the production and recruitment of antimicrobial peptides at the site and detaining the adaptive immune responses of the host [17].

These membrane vesicles harbour specific Mycobacterial proteins like lipoarabinomannan, Ag85 complex, important TLR2 ligands (lipoproteins – LpqH & LprG) etc. These vesicles in turn trigger inflammatory responses following antigen presentation inducing further migration of professional phagocytes like macrophages, neutrophils and other important cellular machineries of adaptive system like lymphocytes to the site. Experimental evidences at both *in vivo* and *in vitro* conditions have proved that EVs can induce the secretion of inflammatory cytokines (like tumor necrosis factor-α (TNF-α), Interleukins – 1β, 6, 12p70) and chemokines (like CXCL1, CCL3) amongst others. These differential gradient of chemokines and cytokines thus generated recruits various cellular machineries of immune system viz., Natural Killer cells, different subsets of T cells thus amplifying the signalling cascade. The 19kda lipoprotein (important cell wall component of Mycobacterium sp.) in association with mycolyl-arabinogalactan-peptidoglycan complex (mAGP complex) subvert the macrophages' activation by Interferon-γ. This in turn compromises with the host's ability of pathogen clearance. Lipoprotein19 kDa is reported to trigger generation of IL-1, IL-12p40 and TNF-α through TLR-2 ligation in various in vitro models of Mycobacterial infection. Research have conclusively shown that the mutant strain of Mycobacterium sp. lacking this mature 19 kDa lipoprotein failed to induce the secretion of TNF-α and interfered with the inducible Nitric Oxide Synthase (iNOS) expression and thereby Nitric Oxide production. Additionally these studies confirmed that the EVs generated from different Mycobacterial species follows typical TLR and myeloid differentiation factor 88-dependent (Myd88) signalling cascade. The production of cytokines like IL-6, IL-8, IL-10, TNF-α, IFN-γ by the phagocytes in response to Mycobacterial EVs is an evidence of involvement of pro-inflammatory pathway in the pathogenesis [15].

The adaptive immune responses play important role in the EVs mediated pathogenesis of the host. Literature studies have shown the expression of various cell surface molecules related to acquired immunity such as HLA-DR, G protein CD195, co-stimulatory protein CD40 and other important markers like CD80/86, CD81 in response to EVs triggered infections. The chief source of IFN-γ is TH1 and as discussed earlier, IFN-γ has been established to be an important player of EVs induced pathogenesis. This clearly concludes the involvement of TH1 mediated immune responses in disease pathogenesis. These EVs are reported to transport specific antigens to T cells which often trigger the expression of GRAIL. GRAIL/ RNF128, a E3 ubiquitin-protein ligase and type I transmembrane protein is characterized in the negative regulation of T cell responsiveness and related cytokine production. A downregulation of TH1 cell responses leads to immune evasion, facilitates pathogen multiplication, interferes with antigen presentation and compromises with other mechanisms of T cell mediated effector functions [18].

**Membrane Vesicles in Iron Acquisition**

Iron exists either as Fe2+ and Fe3+ (often referred as Fe(II) and Fe(III) or ferrous and ferric, respectively) in all biological systems. Ferric is the oxidized form and are prevalent in the earth's crust. However the ferrous form is favoured at lower pH and oxygen concentrations [19] and are particularly important from the pathogen perspective. The interconversions between these two states of iron are particularly important as they are the basis of many redox reactions occurring inside the cells. Inspite of their abundance within the living organisms, it is mainly sequestered in different intracellular compartments by different proteins like transferrin, hemoglobin, ferritin, lactoferrin and are not freely available. Iron also are complexed and identified as integral component of Haem cofactor (cytochromes, haem type catalases), in iron-sulphur forms (rubredoxins, ferredoxins, nitrogenase, different iron-sulphur proteins, mono- or dinuclear non-haem iron) or in enzymes like class Ia ribonucleotide reductases superoxide dismutase amongst others [20].

Iron is an essential requisite for various biological processes and pathogens' need for iron is not an exception. Limited iron availability in the host niche is a prime host strategy as iron-restricted environment interferes with intracellular survival and multiplication of invading pathogens. Bacteria uses several strategies for acquisition of iron; chelation and reduction being the prime strategies for the same. This is primarily attained through ways like – increasing solubility of ferric iron by lowering the external pH, reduction

of ferric iron to the ferrous form (more soluble) and involving chelators and solubilizers for ferric iron form [21]. The high affinity extracellular chelators or carriers known as Siderophores plays important role in this. Siderophores are the low molecular weight carriers that have high affinity and specificity for ferric iron form. Iron is basically solubilized in aqueous solution by the formation of complexes with siderophores. As mentioned earlier, ferric-siderophore complexes shows higher affinity than the desferri-siderophore form and hence gets transported across the cell envelope. Once intracellular, the iron is released from the carrier and becomes readily available to become metabolically functional. There are other ways of acquiring iron apart from involving siderophores like binding of iron to other cellular components like lipopolysaccharides for Gram negative bacteria.

The Mycobacterial species also respond to iron restricted condition and essentially shows the involvement of siderophores; referred as mycobactins. Iron deficiency as it arises due to Mycobacterial pathogenesis has shown to stimulate EVs release as evident from radioactive and electron microscopic studies. Analysis of lipid content were done by ultra performance liquid chromatography in the EVs grown in low and high iron medium. The results confirmed higher iron loaded mycobactin content in the lipid profiles of EVs grown in low iron medium. This conclusively indicated maturation of mycobactins in limited iron condition. The EVs acts as important iron delivering vehicles and this was made evident from studies with ST142 (mbtB mutant). This mutant doesn't have siderophores and hence fails to multiply in iron deficit conditions unless supplemented properly from exogenous source. Addition of EVs from H37Rv (grown in low iron minimal medium) restored growth of ST142 mutant in iron-restricted medium. Contrary to that, EVs recovered from minimal medium, H37Rv cultures failed to re-store the growth of ST142 strain [22]. These different studies unveiled the importance of iron availability on the production and lipid content of EVs and critically indicated their involvement in the transport of iron and hence influencing pathogenesis.

**Involvement of Host Extracellular Vesicles (hEV) in Mycobacterial pathogenesis**

Along with bacteria-mediated vesicles, host generated vesicles pertaining to pathogens' sensing and interaction is crucial to understanding the underlying mechanisms of pathogenesis and host immunity. In the context of host originating vesicles, exosomes and microvesicles have derived the major focus. Both of these have overlapping sizes and display often common markers; hence are co-purified together. Nevertheless, they are differently originated and are different in their content and functioning. The microvesicles generally develop as outward budding of the plasma membrane and are then gradually released. Their formation hence relies on cytoskeletal reorganization and contractility. The Rho, Rac and Cdc42 GTPases chiefly regulate cytoskeletal remodeling and are considered to be the primary factor in the process. Rho proteins are phosphorylated to activate downstream target proteins that controls actin polymerization. Microtubule organizing centres helps in assembling the asymmetric network of microtubules and plays important role in the cell architecture and remodeling. Actin and it's related specialized structures like tunneling nanotubes are primarily regulated by Rho GTPases and these structures have been severely implicated in the disease pathogenesis [23]. Exosomes emerge as intraluminal vesicles inside the multivesicular bodies (MVBs). They are gradually released via the fusion of these MVBs with the plasma membrane. Exosome biogenesis is accomplished either by endosomal sorting complexes required for transport (ESCRT) dependent and ESCRT-independent pathways [24].

In the former pathway, the multiprotein complex of ESCRT works sequentially that leads to membrane remodeling. This results in bending or budding of membrane away from the cytosol leading to ILV and MVB generation. In the later pathway designated as ESCRT independent- ceramides, sphingomyelinase and tetraspanins (molecular scaffolds) are largely implicated. They assists in membrane curvature and thereby in the biogenesis of ILV and MVB. However the events of trafficking and fusion is executed by different GTPases like Rab11, Rab27, Rab35 to mention primarily. These hEV are involved in intercellular transfer of functional proteins, lipids and RNAs and often generates systemic responses as they are reported to carry MHC molecules, cytokines, chemokines and diverse pathogenic antigens. Hence their involvement as transport vesicles lead them to play effective role in cellular communication and immune modulation. During Mycobacterial pathogenesis, immune cells have been reported to release MHCI- peptide complexes that trigger other antigen presenting cells, hence priming CD8+ T lymphocytes. Often the macrophages releases vesicles containing distinct pathogen associated molecular patterns and present them to APCs to induce signalling pathway involving TLRs, MAPKs, NF-κB, inducible NOS, cGMP etc. Reports suggest that these vesicles are involved in the induction of anti-MTB immunity by killing the

intracellular bacilli and priming the uninfected cells for upcoming infection. Vesicles generated exclusively from bacilli-infected macrophages have been characterized to contain important mycobacterial proteins - Antigen 85-C, ESAT-6 and others [25]. Thus the regulatory role of hEV as transport medium and immuno-modulators are well evidenced in shaping the pathogenicity in general and precisely in different Mycobacterium species.

**Membrane vesicles as vaccine**

Membrane vesicles of varied origin are conserved phenomena across species and are noteworthy for playing the central role in modulating diverse biological processes [26]. The carried cargo usually are biological molecules like proteins of metabolic/cytoskeletal/ biochemical pathways, lipids, nucleic acids, pathogen determinants/antigens, membrane fragments, host immune machineries etc. As detailed earlier, these vesicles plays essential role in cargo transportation, pathogen spread, immuno-modulation and disease pathogenesis.

The property of self-adjuvation, immunogenicity and their prompt uptake by immune cells makes them attractive candidate for vaccine. Literature studies suggest that they can be readily modified and lipopolysaccharide reactogenicity being a classical example. The carried antigens can be overexpressed or modified for expressing multiple antigenic variants. These features have facilitated their usage in a range of applications [27].

A classical vaccine has few important properties like instigating the immune system to prevent from the deadly diseases that could happen in future, evoke effective and long lasting immune responses encompassing both the important arms of immune system. Additionally, the vaccine product should be within proper size range and must be inclusive of PAMPs and specific pathogen-associated antigens [28]. These vesicles have relatively permissible size (30-300 nm) that allows them to enter the lymph vessels and facilitates uptake by APCs. The inside package containing surface antigens stimulates humoral and cell-mediated immune responses of the host [29].

OMVs have a proper size (20 - 200 nm) to enable their entry into lymph vessels and uptake by antigen presenting cells. They naturally contain components resembling the antigenic surface of the pathogen; hence stimulating the humoral and cell mediated immune responses. Howsoever the naturally derived vesicles come with some limitations like high reactogenicity of associated molecules, lower expressing antigens, interference of relatively more dominant and non-related antigens that could result into misdirected immune responses and might also carry molecules which are immunosuppressive or could interfere with protective immune responses. To deal with these shortcomings and hurdles, the technology of genetic engineering is often employed to produce safe and high yielding products with desired alterations. The field of vaccine development is advancing from the whole attenuated/inactivated strains to subunit vaccine form. These genetically engineered nano sized vesicles are high yielding, sterile, stable, efficiently delivered and can be coated, loaded and formulated as desired. It renders EVs as a promising and potent vaccine platform [30]. Extracellular vesicles are tremendously used against severe disease causing pathogens like *N. gonorrhoeae*, extraintestinal pathogenic *E. coli* (EXPEC), *V. cholerae, Shigella* sp, *Salmonella* sp., Influenza virus, Corona virus and many others.

In the context of *Mycobacterium* sp. as well, these novel strategies of nanosized vaccine platform is in practice and successfully administered. Nanoparticles (NPs) are well established delivery vehicles. The vaccine candidate (antigen of interest) is either encapsulated or are dodged onto the surface of the NP. Various types of NPs are used like Virus Like Particles (VLPs), liposomes, non-degradable NPs, polymeric NPs, saponins with varied mode of administration are used [31]. In the case of M. *tuberculosis* EVs, proteins like Ag85B, LprA and LprG are well characterized for being immunodominant. Hence, mesoporous silica nanoparticles (MSNs) was used as a platform using these immuno-modulators containing EVs and exposed to macrophages. The results confirmed a significant secretion of TNF-α (pro-inflammatory cytokine) and IL-10 (anti-inflammatory cytokine) than MSNs without these additional proteins. Thus, it is evident that incorporation of different components to the originally prevailing EVs can further increase the feasibility of nanosystems and convert them into effective vaccine delivering tools [32].

**Membrane vesicle based diagnostic biomarkers**

The effective treatment of *Mycobacterium* sp. induced diseases relies on quick and firm diagnostic analysis of the bacilli. Though techniques of imaging like chest radiography, immunological aanalysis of blood for interferon gamma release assay (IGRA) that measures cellular immune response to the TB infection similar to the Mantoux traditional tuberculin skin test (TST), microbiological detection like sputum culture, many modern diagnostic techniques like non-invasive whole body imaging with Positron emission tomography

(PET) integrated with computer tomography (CT) are in practice. Many of these comes with several drawbacks and hence consistent research is going on for determining the ideal diagnostic marker. As evidenced from myriad of studies that majority of the nucleic acids are packaged as cargo in the small extracellular vesicles and hence could serve as ideal diagnostic biomarkers. Gradually several studies were undertaken to establish this novel approach of diagnosis using EV encoded sRNA (sRNA) by real-time qRT-PCR [33].

Bacterial small regulatory RNA (sRNA) work through a wide variety of regulatory mechanisms like binding to the protein targets and thus modifying the function of the bound protein or interacting with messengerRNA targets and hence regulating gene expression. The latter is usually accomplished by regulating the gene expression following binding to complementary mRNA and thereby blocking translation or ribosome docking site [34]. Thus EV carrying sRNA based diagnosis are minimally invasive yet potent diagnostic screening tool for *Mycobacterium* sp.

**Membrane vesicle and antibiotic resistance**

In the history of medicines, antibiotics are the most successful discovery. They aid in controlling pathogens' mediated infections and regulate the health status of wide range of medical procedures. However in the lieu of injudicious use of clinically approved antibiotics and their unnecessary usage, there is increased evolution of multi-drug and extremely drug resistant strains of the pathogens hence compromising the health care in several aspects [35]. Antibiotics often triggers membrane blebbing as an initiation for vesicle formation. Aminoglycoside like gentamicin do so by interfering with membrane lipid packaging thus creating bilayer instability. Polymyxins creates stress of bacterial envelope, DNA damaging quinolones induces SOS response by triggering the inactivation of LexA blocker protein, hence activating SOS gene. β-lactam antibiotics interferes with bacterial cell wall synthesis by creating pores in the peptidoglycan layer. As briefed, antibiotics has various ways of affecting the bacterial population; but they often lead to common outcome of creating stress and hence generation of membrane vesicles [36].

An important concerning EVs-related phenomenon is antibiotic resistance. The vesicles' basic structure comprises of bacterial envelope and it's different associated components has affinity to bind the antibiotics. Many reports have confirmed that the EVs generating from the bacteria are rich in antibiotics-binding protein and decoy receptors, thus facilitating bacterial survival [37]. At times the EVs throw the antimicrobial compounds outside using efflux pumps they harbour thus leaving hardly any traces of these available for affecting the bacteria. Often the EVs are reported to carry enzymes that can degrade the antibiotics or employ the strategy of gene transfer to confer antibiotics' resistance [38]. In the pathogenesis of *Mycobacterium* sp., the involvement of enzymes, genes specific for resistance being carried by chromosome and plasmid and inter-bacterial gene transfer of similar mechanisms have been implicated [9]. The phenomenon primarily relies on the horizontal gene transfer by mediating phage infection. The genes for virulence and antibiotic resistance are particularly transferred by the Mycobacteriophages and this makes them efficient genomic engineers. The classical studies by Cheng and Schorey 2019 have shown that when macrophages were infected with drug resistant *M. tuberculosis* strain in the presence of antibiotics; EVs synergized with the antibiotics to expedite bacterial clearance and improved lungs'conditions as evident from investigated parameters [39]. These observations further shows that EVs can can be utilised as an immunotherapeutic platform and be an effective measure to treat drug-resistant Mycobacterial strains.

**Membrane vesicles as molecule transfer vector**

Extracellular vesicles have been established as a potential vehicle of communication as they could reach everywhere through circulation, could efficiently get lodged into biological fluids without degradation, get readily endocytosed by APCs and adhere to the tissues. They exhibit heterogeneity in carrying the cargo inside.

They are proved to carry diverse proteins and enzymes such as proteases and ureases, toxins namely cholera toxin of *Vibrio cholerae*, cytolysin A of enterotoxic *Escherichia coli*, cytolethal distending toxin (CDT) from *Campylobacter jejuni*, vacuolating cytotoxin (VacA) of *Helicobacter pylori*, siderophores: carboxymycobactin and mycobactin of *Mycobacterium tuberculosis* and many others. Hence, the EVs are efficient transfer vector carrying virulence factors, nucleic acids – DNA & RNA, adhesins, toxins, communication compounds, immunomodulatory factors, nutrient-scavenging factors and others [40]. These characteristics also make EVs excellent candidates as vaccination agents and potential biomarkers in the diagnosis of different disease conditions like progression of cancer, neurodegenerative and autoimmune disorders and during infections prevailing in the body.

**Conclusion and future perspectives**

The membrane bound nano vesicles, here referred throughout in the review as Extracellular Vesicles (EV)

are naturally released by archae bacteria, prokaryotes and eukaryotes; thus showing conserved evolutionary significances. However, in the context of biological science and it's applied technology, it is of immense importance and have been implicated in several processes, mechanisms and phenomena. Extracellular vesicles influence communication between the cells and hence are involved in transferring a myriad of biological molecules. The exact mechanisms of extracellular vesicles' biogenesis was not studied and the notion was strongly believed that release of EVs was impossible amongst them. The mycolic acid containing bacteria are known for their complex lipid-enriched envelope and hence in posing several additional challenges. But research in past few years using biochemical analysis and imaging techniques have conclusively detected EVs and implicated their fundamental involvement in several processes. Different studies have extrapolated various inducers of EV generation like DNA damage, inhibition of cell wall synthesis, fatty acid biosynthesis inhibition, endolysin mediated generation being some of the foremost. Accumulating research evidences have also highlighted various routes of EV formation, namely membrane blebbing, alterations in cell envelope and explosive cell lysis to be the prime ways. In Mycobacteria-induced pathogenesis, the EVs have been well-studied and detailed studies regarding their architecture, functioning and indulgence have been done. These EVs are enriched with lipoproteins, TLR-2 agonists and immunogenic determinants. The molecular composition of EVs clearly implicates their strong potential to influence host immune responses and generate protective immune response. Additionally, their capacity to deliver cargo (molecules of varied origin) tremendously supports their involvement in cell to cell communication and hence further influencing the host cell responses to the pathogen.

The experimental evidences have greatly indicated the involvement of EVs in different functioning like iron acquisition, shaping the virulence of the bacilli, immunomodulation, influencing important signalling pathways like antigen presentation, humoral and cellular immune responses and several others. Precisely to conclude, it is important to mention that the diverse potential of EVs have harnessed them as a candidate for vaccine development. Already the prevailing reports have confirmed the usage of these nano sized vesicles as effective vaccine platform for their uniqueness of size, high yield, sterile nature, stability and ease of delivery with formulations as desired. There are however some major limitations relating to vesicle production and their regulations including some questions that remains unanswered like: exact mechanisms through which the microbial components gets circumsphered inside the vesicles, exact mechanisms that the bacilli adopt/ modify to incorporate few selected molecules into the vesicles, does the variation in composition of vesicles accordingly bring out any changes in the virulence pattern of the bacilli, how other uninfected neighbouring cells are influencing the process. Nevertheless, there are convincing and quite interesting reports justifying the pivotal role of EVs in infection models including *Mycobacterium* species induced pathogenesis. Rigorous and continued research in this direction will definitely pave the way for better understanding of underlying mechanisms and it's implicated immune responses. The EVs are already considered important diagnostic markers and candidates for vaccine. A little more intricate work could definitely revolutionize this field of biology and would benefit mankind with novel findings to fight the disease and neutralize the damages of body initiated by the bacillus.

**Conflict of interest**

The authors declare no conflict of interest.

**References**

1. Delghandi, M.R.; El-Matbouli, M.; Menanteau-Ledouble, S. Mycobacteriosis and Infections with Non-tuberculous Mycobacteria in Aquatic Organisms: A Review Microorganisms 2020, 8, 1368. https://doi.org/10.3390/microorganisms8091368.
2. Forrellad, M. A.; Klepp, L. I.; Gioffré, A.; Sabio y García, J.; Morbidoni, H. R.; de la Paz Santangelo, M.; Cataldi, A. A.; Bigi, F. Virulence factors of the *Mycobacterium tuberculosis* complex. Virulence 2013, 4, 3-66. Doi: 10.4161/viru.22329.
3. Riello, F.N.; Brígido, R.T. S.; Araújo, S. et al. Diagnosis of mycobacterial infections based on acid-fast bacilli test and bacterial growth time and implications on treatment and disease outcome. BMC Infectious Diseases. 2016, 16, 142. https://doi.org/10.1186/s12879-016-1474-6.
4. Hernandez, S.; M.; Morlock, G. P.; Butler, W. R.; Crawford, J. T.; Cooksey, R. C. Identification of Mycobacterium species by PCR-restriction fragment length polymorphism analyses using fluorescence capillary electrophoresis. Journal of Clinical Microbiology. 1999, 37, 3688-3692. Doi: 10.1128/JCM.37.11.3688-3692.
5. Daffé, M.; Reyrat, J.M. The mycobacterial cell envelope. American Society for Microbiology, Washington

(DC). 2008, DOI: 10.1128/9781555815783.

6. Brennan, P. J. Structure, function, and biogenesis of the cell wall of *Mycobacterium tuberculosis*. Tuberculosis (Edinb). 2003, 83, 91-97. Doi: 10.1016/s1472-9792(02)00089-6.
7. Toyofuku, M.; Nomura, N.; Eberl, L. Types and origins of bacterial membrane vesicles. Nature Reviews Microbiology. 2019, 17, 13-24. https://doi.org/10.1038/s41579-018-0112-2.
8. Nagakubo, T.; Tahara, Y. O.; Miyata, M.; Nomura, N.; Toyofuku, M. Mycolic acid-containing bacteria trigger distinct types of membrane vesicles through different routes. iScience. 2021, 24, 102015. Doi: 10.1016/j.isci.2020.102015.
9. Chiplunkar, S. S.; Silva, C. A.; Bermudez, L. E., Danelishvili, L. Characterization of membrane vesicles released by *Mycobacterium avium* in response to environment mimicking the macrophage phagosome. Future Microbiology, 2019, 14, 293-313. Doi: 10.2217/fmb-2018-0249.
10. Gupta, S.; Rodriguez, G. M. Mycobacterial extracellular vesicles and host pathogen interactions. Pathogens and Diseases. 2018, 76, fty031. Doi: 10.1093/femspd/fty031.
11. Rodriguez, G. M.; Prados-Rosales, R. Functions and importance of mycobacterial extracellular vesicles. Applied Microbiology and Biotechnology. 2016, 100, 3887-3892. Doi: 10.1007/s00253-016-7484-x.
12. Gould, S. J.; Raposo, G. As we wait: coping with an imperfect nomenclature for extracellular vesicles. Journal of Extracellular Vesicles. 2013, 15, 2. Doi: 10.3402/jev.v2i0.20389.
13. Deimantaviciute, G., 2022. Producing intracellular and extracellular lipid vesicles in E. coli for vaccine development (Doctoral dissertation, University of Kent).
14. Cao, Y.; Lin, H. Characterization and function of membrane vesicles in Gram-positive bacteria. Applied Microbiology and Biotechnology. 2021, 5, 1795-1801. Doi: 10.1007/s00253-021-11140-1.
15. Prados-Rosales, R., Baena, A., Martinez, L. R.; Luque-Garcia, J.; Kalscheuer, R.; Veeraraghavan, U.; Camara, C.; Nosanchuk, J. D.; Besra, G. S.; Chen, B.; Jimenez, J.; Glatman-Freedman, A.; Jacobs, W. R Jr.; Porcelli, S. A.; Casadevall,. A. Mycobacteria release active membrane vesicles that modulate immune responses in a TLR2-dependent manner in mice. Journal of Clinical Investigation. 2011, 4, 1471-83. Doi: 10.1172/JCI44261.
16. Mehaffy, C.; Ryan, J. M.; Kruh-Garcia, N. A.; Dobos, K. M. Extracellular vesicles in Mycobacteria and tuberculosis. Frontiers in Cellular and Infection Microbiology. 2022, 12, 912831. Doi: 10.3389/fcimb.2022.912831.
17. Gioseffi, A.; Edelmann, M. J.; Kima, P. E. Intravacuolar pathogens hijack host extracellular vesicle biogenesis to secrete virulence factors. Frontiers in Immunology. 2021, 12:662944. doi: 10.3389/fimmu.2021.662944.
18. Miggiano, R.; Rizzi, M.; Ferraris, D. M. *Mycobacterium tuberculosis* pathogenesis, infection prevention and treatment. Pathogens 2020, 9, 385. https://doi.org/10.3390/pathogens9050385.
19. Athman, J. J.; Sande, O. J.; Groft, S. G.; Reba, S. M.; Nagy, N.; Wearsch, P. A.; Richardson, E. T.; Rojas, R.; Boom, W.; H.; Shukla, S.; Harding, C. V. *Mycobacterium tuberculosis* membrane vesicles inhibit T cell activation. Journal of Immunology. 2017, 198, 2028-2037. Doi: 10.4049/jimmunol.1601199.
20. Sanchez, K. K.; Chen, G. Y.; Schieber, A. M. P.; Redford, S. E.; Shokhirev, M. N.; Leblanc, M.; Lee, Y. M.; Ayres, J. S. Cooperative metabolic adaptations in the host can favor asymptomatic infection and select for attenuated virulence in an enteric pathogen. Cell. 2018, 175, 146-158.e15. doi: 10.1016/j.cell.2018.07.016.
21. Caza, M.; Kronstad, J. W. Shared and distinct mechanisms of iron acquisition by bacterial and fungal pathogens of humans. Frontiers in Cellular and Infection Microbiology. 2013, 3:80. Doi: 10.3389/fcimb.2013.00080.
22. Gupta, S.; Marcela Rodriguez, G. Isolation and characterization of extracellular vesicles produced by iron-limited Mycobacteria. Journal of Visualized Experiments. 2019, 152, Doi: 10.3791/60359.
23. Prados-Rosales, R.; Weinrick, B. C.; Piqué, D. G.; Jacobs, W. R Jr.; Casadevall, A.; Rodriguez, G. M. Role for *Mycobacterium tuberculosis* membrane vesicles in iron acquisition. Journal of Bacteriology. 2014, 196, 1250-1256. Doi: 10.1128/JB.01090-13.
24. Zhang, S.; Kazanietz, M. G.; Cooke, M.; Rho GTPases and the emerging role of tunneling nanotubes in physiology and disease. American Journal of Physiology - Cell Physiology. 2020, 319, 877-884. Doi: 10.1152/ajpcell.00351.2020.
25. Tu, C.; Ortega-Cava, C. F.; Winograd, P.; Stanton, M. J.; Reddi, A. L.; Dodge, I.; Arya, R.; Dimri, M.; Clubb, R. J.; Naramura, M.; et al. Endosomal-sorting complexes required for transport (ESCRT) pathway-dependent endosomal traffic regulates

the localization of active Src at focal adhesions. Proceedings of the National Academy of Sciences USA. 2010, 107, 16107-16112. Doi: 10.1073/pnas.1009471107.

26. Schorey, J. S.; Cheng, Y.; McManus, W. R. Bacteria- and host-derived extracellular vesicles-two sides of the same coin? Journal of Cell Sciences. 2021, 134, 256628.
27. van der Pol, L.; Stork, M.; van der Ley, P. Outer membrane vesicles as platform vaccine technology. Biotechnology Journal. 2015, 11, 1689-1706. Doi: 10.1002/biot.201400395.
28. Mohammadzadeh, R.; Ghazvini, K.; Farsiani, H.; Soleimanpour, S. *Mycobacterium tuberculosis* extracellular vesicles: exploitation for vaccine technology and diagnostic methods. Critical Reviews in Microbiology. 2021 47, 13-33. Doi: 10.1080/1040841X.2020.1830749.
29. Pollard, A.J.; Bijker, E.M. A guide to vaccinology: from basic principles to new developments. Nature Reviews Immunology. 2021, 21, 83-100. https://doi.org/10.1038/s41577-020-00479-7.
30. Bachmann, M.; Jennings, G. Vaccine delivery: a matter of size, geometry, kinetics and molecular patterns. Nature Reviews Immunology. 2010, 10, 787-796. https://doi.org/10.1038/nri2868.
31. Herrmann, I. K.; Wood, M. J. A.; Fuhrmann, G. Extracellular vesicles as a next-generation drug delivery platform. Nature Nanotechnology. 2021, 16, 748-759. https://doi.org/10.1038/s41565-021-00931-2.
32. Gregory, A.; EE; Titball, R.; Williamson, D. Vaccine delivery using nanoparticles. Frontiers in Cellular Infection Microbiology. 2013, 3:13. Doi: 10.3389/fcimb.2013.00013.
33. Montalvo-Quirós, S.; Gómez-Graña, S.; Vallet-Regí, M.; Prados-Rosales, R. C.; González, B.; Luque-Garcia, J. L. Mesoporous silica nanoparticles containing silver as novel antimycobacterial agents against *Mycobacterium tuberculosis*. Colloids and Surfaces B: Biointerfaces. 2021, 197:11405. Doi: 10.1016/j.colsurfb.2020.111405.
34. Lu, GG; Jiang, X.; Wu, A.; Zhou, J.; Liu, H.; He, F.; Zhang, Q.; Zen, K.; Gu, S.; Wang, J. Two small extracellular vesicle sRNAs derived from *Mycobacterium tuberculosis* serve as diagnostic biomarkers for active pulmonary tuberculosis. Frontiers in Microbiology. 2021 12:642559. Doi: 10.3389/fmicb.2021.642559.
35. Gottesman, S.; Storz, G. Bacterial small RNA regulators: versatile roles and rapidly evolving variations. Cold Spring Harbor Perspectives in Biology. 2011, 3:a003798. Doi: 10.1101/cshperspect.a003798.
36. Aminov, R. I. A brief history of the antibiotic era: lessons learned and challenges for the future. Frontiers in Microbiology. 2010, 1:134. Doi: 10.3389/fmicb.2010.00134.
37. Mozaheb, N.; Mingeot-Leclercq, M. P. Membrane vesicle production as a bacterial defense against stress. Frontiers in Microbiology. 2020, 11:600221. Doi: 10.3389/fmicb.2020.600221.
38. Andreoni, F.; Toyofuku, M.; Menzi, C.; Kalawong, R.; Mairpady Shambat, S.; François, P.; Zinkernagel, A. S.; Eberl, L. Antibiotics stimulate formation of vesicles in *Staphylococcus aureus* in both phage-dependent and -independent fashions and via different routes. Antimicrobial Agents and Chemotherapy. 2019, 63:e01439-18. Doi: 10.1128/AAC.01439-18.
39. Bose, S.; Aggarwal, S.; Singh, D. V.; Acharya, N. Extracellular vesicles: An emerging platform in Gram-positive bacteria. Microbial Cell 2020, 7, 312-322.
40. Cheng, Y., Schorey, J. S. Extracellular vesicles deliver Mycobacterium RNA to promote host immunity and bacterial killing. EMBO Reports 2019, 20:e46613. Doi: 10.15252.
41. Mir, B., Goettsch, C. Extracellular vesicles as delivery vehicles of specific cellular cargo. Cells 2020, 9, 1601. Doi: 10.3390/cells9071601.
42. Kayagaki, N.; Warming, S.; Lamkanfi, M.; Walle, L.V.; Louie, S.; Dong, J.; Newton, K.; Qu, Y.; Liu, J.; Heldens, S.; et al. Non-canonical inflammasome activation targets caspase-11. Nature 2011, 479, 117–121