

MOLECULAR PATHOGENESIS OF *VIBRIO VULNIFICUS*: VIRULENCE MECHANISMS AND HOST–PATHOGEN INTERACTIONS DURING INFECTION

Joseph Miguel A. Abringe^[1], Ma. Paula V. Bergado^[1], Slyven Dela Cruz^[1],
Mandrake Verula^[1], Mary Claire A. Viola^[1] and Gecelene C. Estorico^{[1] [2]}

Technological University of the Philippines - Taguig^[1]

Civil and Allied Department; Bachelor of Science in Environmental Science

De La Salle University - Dasmarias Cavite Philippines^[2]

ABSTRACT

Vibrio vulnificus is a highly lethal zoonotic marine pathogen that causes life-threatening primary septicemia and rapid necrotizing wound infections. Pathogenesis is primarily driven by the multifunctional autoprocessing repeats-in-toxin (MARTX) toxin, whose translocated effector domains—including DUF1, RID, MCF, and ACD—synergistically disrupt host NAD⁺ homeostasis, degrade Rab GTPases, and collapse the actin cytoskeleton. MARTX pore-forming activity further cooperates sequentially with phospholipase PlpA to execute rapid necrotic cell lysis and triggers red blood cell phosphatidylserine exposure that promotes venous thrombosis. Population genomics demonstrates that severe clinical strains are genetically diverse across phylogenetic lineages and ecological groups, underscoring the necessity for rapid molecular diagnostics and antivirulence therapies.

Keywords:

Vibrio vulnificus, MARTX toxin, exotoxin synergy, primary septicemia, necrotizing fasciitis, population genomics.

INTRODUCTION

Vibrio vulnificus is a highly pathogenic, halophilic Gram-negative bacterium that naturally inhabits warm marine and estuarine environments worldwide (G. Choi, 2022; C. Li et al., 2025). As an opportunistic zoonotic pathogen, it poses a severe threat to human health through the consumption of raw or undercooked seafood—particularly oysters—or through the exposure of open skin wounds to contaminated seawater (G. Choi, 2022; J. Chen et al., 2025; K. Kling & K. Satchell, 2022). Foodborne acquisition typically initiates gastroenteritis but can rapidly progress to primary septicemia, which carries a mortality rate exceeding 50% in medically fragile individuals, particularly those with chronic liver diseases, hemochromatosis, or immunocompromised conditions (G. Choi, 2022; C. Li et al., 2025; K. Ishida et al., 2023). Direct wound inoculation frequently leads to necrotizing soft-tissue infections and necrotizing fasciitis that progress within 24 hours, often requiring surgical debridement or limb amputation (C. Li et al., 2025; K. Kling & K. Satchell, 2022). Driven by global climate change, rising sea surface temperatures, and extreme weather events, the geographic distribution and seasonal infection window of *V. vulnificus* are expanding into higher-latitude temperate regions, escalating its status as an emerging global public health crisis (G. Choi, 2022; C. Li et al., 2025; J. Zhang et al., 2023).

The rapid onset and fulminant nature of *V. vulnificus* infections stem from a complex array of virulence factors, among which the **multifunctional autoprocessing repeats-in-toxin (MARTX)** toxin, encoded by *rtxA1*, serves as the primary driver of systemic cytotoxicity and lethality (G. Choi, 2022; J. Chen et al., 2025; A. Herrera et al., 2024). Secreted as a large single polypeptide, the MARTX toxin initially anchors to host plasma membranes via its N-terminal **Aknot domain**, which selectively recognizes internal obligate N-acetylglucosamine (GlcNAc) residues on host surface biantennary N-glycans, predominantly targeting the cell adhesion glycoprotein L1CAM (J. Chen et al., 2025). Following cell attachment and pore formation, the central effector region is translocated into the host cytoplasm, where host-specific signals stimulate autoproteolytic processing to liberate discrete

effector domains (G. Choi, 2022; S. Choi et al., 2024). These delivered effectors operate synergistically to paralyze host defenses: the **DUF1–RID effector duet** hijacks calmodulin to degrade intracellular NAD(P)⁺ while inactivating Rac1 to silence macrophage reactive oxygen species (ROS) generation (S. Choi et al., 2024); the **Makes Caterpillars Floppy (MCF)** cysteine protease cleaves host Rab GTPases to trigger Golgi collapse and intrinsic apoptosis (A. Herrera et al., 2024); and the **Actin Cross-linking Domain (ACD)** catalyzes covalent actin oligomerization to drive cytoskeletal collapse and breakdown of epithelial barrier integrity (P. Woida & K. Satchell, 2022).

Pathogenesis is further amplified through multi-toxin cooperation, host metabolic manipulation, and genomic adaptation. The pore-forming MARTX toxin acts in sequential synergy with the phospholipase PlpA, wherein MARTX-induced cell membrane blebs are subsequently lysed by PlpA to execute rapid, complete necrotic host cell death (C. Cho et al., 2022). At sub-hemolytic concentrations, MARTX pore-forming activity triggers intracellular calcium influx in red blood cells, activating scramblase and phosphatidylserine exposure to shed procoagulant microvesicles that promote deep venous thrombosis (H. Chung et al., 2022). Locally, MARTX-facilitated bacterial proliferation in skeletal muscle induces severe tissue catabolism, depleting creatine reserves and altering host tricarboxylic acid (TCA) cycle intermediate pools (K. Ishida et al., 2023). Concurrently, population genomics reveals that clinical strains capable of severe human disease are genetically diverse across phylogenetic lineages and ecological groups rather than restricted to a single genotype, complicating surveillance and control strategies (K. Kling & K. Satchell, 2022; J. Zhang et al., 2023).

OBJECTIVES

The objective of this systematic review is to evaluate the factors driving virulence, host-pathogen interactions, and clinical disease progression in *Vibrio vulnificus* infections by synthesizing experimental and epidemiological evidence across animal models and human clinical cohorts. Specifically, the review aims to characterize the diverse clinical manifestations observed across primary septicemia, necrotizing fasciitis, and infection-associated deep vein thrombosis, contrasting in vivo animal kinetics with human cell line models. Furthermore, it seeks to investigate the roles of toxicological, environmental, and host modifiers—such as MARTX effector domain synergies (DUF1, RID, MCF, ACD, and Aknot), exotoxin PlpA cooperation, climate warming, and iron overload—in driving cell death and tissue necrosis. Additionally, the review aims to analyze how population genomic diversity across phylogenetic lineages and ecological groups influences clinical outcome severity, while assessing the efficacy of emerging rapid molecular diagnostics (such as CRISPR-Cas12a/RPA assays and serum CPK biomarkers) and small-molecule antivirulence therapeutic strategies. Collectively, these objectives support an integrated clinical and public health framework intended to refine diagnostic speed, disarm pathogenic mechanisms, and optimize therapeutic outcomes for patients affected by this lethal marine pathogen.

METHODOLOGY

The study used a systematic methodology structured into three main components: Input, Process, and Output (IPO).

This study used a systematic methodology structured around the **Input-Process-Output (IPO)** framework and conducted in strict adherence to the **PRISMA 2020** (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines. The review specifically synthesizes evidence across experimental animal infection models, human cell lines, and clinical isolated cohorts to characterize the molecular, genomic, and phenotypic variability driving virulence and disease severity.

INPUT		PROCESS		OUTPUT
Literature Search Parameters - Relevant databases (PubMed, ScienceDirect, Web of Science, Scopus, ASM Journals, Wiley Online Library). - Keywords and Boolean search strings related to <i>Vibrio vulnificus</i>, MARTX toxin, virulence factors, and clinical outcomes.	→	Database Searching Electronic searches of the selected databases for relevant studies published through 2025.	→	Compiled Literature Collection of potentially relevant studies on the molecular pathogenesis and clinical effects of <i>Vibrio vulnificus</i>.
Eligibility Criteria Inclusion and exclusion criteria covering relevant pathogens, experimental models, human cell lines, clinical isolates, and molecular or clinical data.	→	Study Screening Screening of titles and abstracts, followed by full-text assessment according to the study's eligibility criteria.	→	Selected Studies A curated set of 13 sources, as reported in the research paper.
Data Extraction Matrix Variables covering host models, virulence genes, MARTX effector domains, clinical manifestations, environmental factors, diagnostics, and therapies.	→	Structured Data Extraction Systematic collection and organization of molecular, genomic, experimental, and clinical findings from the selected studies.	→	Organized Research Database Structured evidence on bacterial virulence, host-pathogen interactions, and disease progression.
Quality Assessment Criteria Standards for evaluating molecular techniques, diagnostic precision, phenotypic characterization, and study quality.	→	Quality Appraisal Evaluation of the methodological quality and strength of the evidence reported in the included studies.	→	Quality-Assessed Evidence Evidence organized according to the methodological rigor and depth of the available findings.
Analytical Framework Molecular mechanisms, toxin interactions, genomic diversity, clinical outcomes, diagnostic methods, and therapeutic strategies.	→	Thematic and Comparative Synthesis Integration of findings on toxin synergy, host-cell damage, clinical severity, genomic diversity, and potential interventions.	→	Integrated Research Synthesis A comprehensive understanding of <i>Vibrio vulnificus</i> pathogenesis, disease outcomes, and potential diagnostic, therapeutic, and public health strategies.

Figure 1: IPO Diagram of the Study

Data Collection

The data collection phase involved a comprehensive electronic search for primary research and review articles focusing on the clinical and molecular dynamics of *Vibrio vulnificus*. Information was retrieved from major biomedical databases, including **PubMed, ScienceDirect, Web of Science, Scopus, ASM Journals, and the Wiley Online Library**. The search strategy employed targeted keywords and Boolean operators: ("Vibrio vulnificus" OR "V. vulnificus") AND ("MARTX toxin" OR "rtxA1" OR "exotoxin" OR "effector domain" OR "population genomics" OR "necrotizing fasciitis" OR "primary septicemia"). This phase focused on capturing a wide range of studies, from in vivo animal models and structural biology to population genomics and clinical epidemiological cohort evaluations published through 2025.

Study Selection

Following the identification of records, a two-stage screening process was conducted to ensure eligibility. Initially, titles and abstracts were screened for relevance to *V. vulnificus* virulence mechanisms and clinical outcomes. Subsequently, a full-text assessment was performed on candidate studies. A total of **13 sources** were selected based on specific inclusion criteria: (a) a primary focus on *Vibrio vulnificus*; (b) the inclusion of animal models (e.g., mice, rats, tilapia, oysters, brine shrimp), human cell lines, or clinical isolate cohorts; and (c) the availability of detailed molecular, genomic, or clinical manifestation data. Studies that investigated shared toxin domains or regulators using distinct methodologies—such as structural X-ray crystallography versus in vivo murine challenge models—were included as distinct sources to provide a multifaceted perspective.

Data Extraction

Data were systematically extracted from the 13 included studies into a standardized extraction matrix to ensure consistency and accuracy. The following variables were captured for each source:

- 1) Host & Model Information: Experimental model species (e.g., ICR mice, Sprague-Dawley rats, farmed tilapia, brine shrimp, oysters), human cell lines (e.g., HeLa, HEK293T, T84, human RBCs), or human clinical isolate cohort specifications (e.g., COVIS database).
- 2) Virulence & Genetic Data: Specific exotoxin gene targets (rtxA1, vvhA, plpA), MARTX effector domains (DUF1, RID, MCF, ACD, Aknot), transcription factors (e.g., HlyU, IscR, NagC, MetJ, Tet2), and population genomic designations (MLST lineages 1–6, ecological groups EG1/EG2, vcg alleles).
- 3) Clinical & Pathological Manifestations: Presenting physical symptoms (e.g., sudden fever, localized edema, bullae, severe wound pain) and systemic pathological effects (e.g., primary septicemia, necrotizing fasciitis, deep vein thrombosis, intestinal epithelial disruption, organ dissemination).
- 4) Modifiers & Diagnostic/Therapeutic Strategies: Environmental drivers (climate warming, salinity, microplastic colonization), host risk factors (iron overload, liver cirrhosis), emerging diagnostics (CRISPR-Cas12a/RPA, serum CPK metabolomic biomarkers), and antivirulence therapies (small-molecule transcription factor inhibitors).

Quality Assessment

The methodological quality of each study was appraised based on the depth of phenotypic characterization and the precision of the diagnostic and laboratory techniques reported. Preclinical and cell-based studies were evaluated for their use of definitive molecular methods, such as site-directed mutagenesis (e.g., deletion mutants, catalytic point mutations), Next-Generation Sequencing (NGS), X-ray crystallography, mass spectrometry, and quantitative real-time PCR (qPCR). Review articles and genomic studies were assessed based on the breadth of the literature synthesis and the inclusion of international isolate cohorts.

Data Synthesis

A thematic and comparative synthesis was performed to analyze the evidence regarding *Vibrio vulnificus* pathogenesis and clinical heterogeneity. The synthesis focused on identifying mechanistic synergies among exotoxins, such as the sequential cooperation between MARTX pore formation and PlpA phospholipase lysis or the DUF1–RID effector duet hijacking host calmodulin to silence macrophage ROS. Furthermore, the synthesis evaluated the clinical continuum across foodborne septicemia and necrotizing wound infections by contrasting in vivo animal tissue dissemination with human cell line cytotoxicity and patient outcomes. Data from global genomic cohorts were synthesized to characterize the impact of ecological groups (EG1 vs. EG2) and phylogenetic lineages on environmental adaptation and human disease severity. The resulting synthesis provides a framework for an integrated clinical and public health approach to *V. vulnificus* management.

RESULTS AND DISCUSSION

Synthesizing the 13 selected studies reveals that *Vibrio vulnificus* functions as a complex pathogen characterized by marked clinical and genomic heterogeneity. By integrating experimental, genomic, and epidemiological data, this section evaluates how specific exotoxin regulators and MARTX effector domains drive a spectrum of outcomes—ranging from localized cutaneous lesions to fatal primary septicemia and necrotizing fasciitis.

Table 1. *Vibrio vulnificus* Pathogenesis in Human Cell Lines and Clinical Cohorts: Toxin Mechanisms, Disease Severity, and Diagnostics

Title	Study Type / Model	Mechanism	Molecular Functions	Host-Pathogen Interactions	Symptoms/effects	Diagnostics	Findings	Authors
Complex regulatory networks of virulence factors in <i>Vibrio vulnificus</i>	Review / Human Clinical & Cell Context	Spatiotemporal gene regulation controlling exotoxins (<i>rtxA</i> , <i>vvhA</i> , <i>plpA</i> , <i>vvpE</i>) during human infection	Coordinates CPS, EPS, and exotoxin synthesis to evade host immune responses	Evasion of human mucosal immunity ; MARTX and VvhA disrupt epithelial barrier integrity	Sudden high fever, chills , severe local pain, swelling, hemorrhagic bullae, vomiting, diarrhea.	Transcriptional profiling of virulence regulators and evaluation of small-molecule TF inhibitors (QStatin, CM14)	<i>V. vulnificus</i> accounts for 95% of all seafood-related deaths in the USA with a case-fatality rate >50% in primary septicemia . MARTX and VvhA cause enterocyte paracellular permeabilization, apoptosis, and autophagic cell death, while small-molecule inhibitors (CM14, QStatin) block <i>rtxA</i>, <i>vvhA</i>, and <i>plpA</i> transcription to prevent cell lysis.	Garam Choi & Sang Ho Choi (2022)

It's time to act: Understanding and combating Vibrio vulnificus	Review / Human Epidemiology & Clinical Management	Multi-toxin pathogenesis combined with climate-driven geographic expansion and iron acquisition	MARTX and VvhA toxins cause direct host cell membrane damage and immune suppression	Iron overload (hemochromatosis, liver cirrhosis) dramatically enhances bacterial proliferation and human mortality	Necrotizing fasciitis (within 24 h), primary septicemia (70% mortality in cirrhosis), septic shock, 14% amputation rate	CRISPR-Cas12a / RPA-CRISPR (30 min, 1–10 CFU/mL), real-time qPCR (<i>vvhA/rpoS</i>), mNGS , TCBS/C HROMagar culture, MALDI-TOF MS	Primary septicemia in cirrhosis patients carries up to 70% mortality due to elevated transferrin saturation (>60%, p=0.003) and derepressed ferroportin. Wound infections progress to necrotizing fasciitis within 24 h with a 14% amputation rate and median time to death of 4 days. CRISPR-Cas12a/RPA assays detect 1–10 CFU/mL within 30 min.	Chao Li, Gang Li & Ming Li (2025)
Dissemination of pathogenic bacteria is reinforced	Human Cell Lines & Primary Cells (HEK293T, HeLa,	DUF1–RID effector duet: RID _{CBD} hijacks host	RDTND degrades intracellular NAD ⁺ /NADP ⁺ ; RID	Silences human macrophage ROS generation, inhibiting	Depletion of host cellular NAD(P)⁺, early innate immune	PBMC intracellular NAD⁺ depletion assays, flow	DUF1 (RDTND) hijacks host CaM (K _d = 8.6 nM) to	Sanghyeon Choi et al. (2024)

d by a MARTX toxin effector duet	RAW 264.7, Human PBMCs)	calmodulin (CaM) to convert DUF1 into an NAD(P) ⁺ hydrolase	modifies Rac1 via fatty acylation to block NOX2 complex assembly	pro-inflammatory cytokines (IL-6, TNF- α , MCP-1)	paralysis, cytokine storm, acute kidney injury, and fatal septic shock	cytometric ROS measurement, and ELISA for serum cytokines (IL-6, MCP-1)	convert into an NAD(P) ⁺ hydrolase, rapidly degrading host cell NAD ⁺ /NADP ⁺ into nicotinamide and ADPR. In human macrophages, RID modifies Rac1 to block NOX2 NADPH oxidase assembly, abolishing ROS generation within 1.5 h and suppressing IL-6, TNF- α , and MCP-1.	
Vibrio MARTX toxin binding of biantennary N-glycans at host cell surfaces	Human Cell Lines (HEK293, CHO, HeLa L1CAM ^{-/-} , T84 human intestinal epithelial cells)	Aknot domain binds internal obligate GlcNAc residues on complex biantennary N-glycans	Selective high-affinity binding to spatial presentations of biantennary N-glycans on host surface glycoproteins, predominantly L1CAM	Anchors MARTX toxin to human epithelial and endothelial cell surfaces for pore formation and effector delivery	Foodborne gastroenteritis , intestinal epithelial cell lysis, and rapid progression to fatal septic shock (50–100% mortality without antibiotics)	Flow cytometry & immunofluorescence surface binding assays , AP-MS target identification, and LDH cytotoxicity release assays	Aknot domain specifically binds internal obligate GlcNAc residues on complex biantennary N-glycans, predominantly targeting human surface glycoprotein	Jiexi Chen et al. (2025)

IJETRM

INTERNATIONAL JOURNAL OF ENGINEERING TECHNOLOGY RESEARCH & MANAGEMENT (IJETRM)

<https://ijetrm.com/>

							L1CAM. Aknot mutants abolish MARTX pore formation and LDH cytotoxicity in HEK293, HeLa, CHO, and T84 cells, explaining broad human tissue tropism during foodborne sepsis.	
Vibrio MARTX toxin processing and degradation of cellular Rab GTPases by the cytotoxic effector Makes Caterpillars Floppy	Human Cell Lines (HEK293 T, Cos7)	Host ARF1/A RF3 GTPases stimulate autoproce ssing of the MCF cysteine protease domain	Cysteine protease activity broadly cleaves and degrades ~50% of host Rab GTPases (Rab1B, Rab23, Rab4B, Rab11A)	Cleavage strips Rab C-terminal tails, causing Rab1B dispersal away from perinuclear regions	Golgi apparatus collapse, organelle destruction, loss of cellular transport, and intrinsic apoptosis	Mass spectrometry peptide profiling of 20–30 kDa proteins, Western blotting for Rab degradation, and immunofluorescence confocal microscopy	Host ARF1/A RF3 GTPases stimulate autoproce ssing of MCF cysteine protease, which cleaves and degrades ~50% of host Rab GTPase family members (Rab1B, Rab23, Rab4B, Rab11A) at their flexible C-terminal tails in HEK293 T cells.	Alfa Herrera et al. (2024)

							This strips membrane localization, driving Golgi collapse and intrinsic apoptosis.	
MARTX toxin of <i>Vibrio vulnificus</i> induces RBC phosphatidylserine exposure that can contribute to thrombosis	Human Blood Cells & Endothelial Cells (Human RBCs & HUVECs)	Sub-hemolytic MARTX toxin pore formation triggers intracellular Ca^{2+} influx in human red blood cells	Elevated $[Ca^{2+}]_i$ activates caspase-3 and scramblase, driving phosphatidylserine (PS) externalization and microvesicle (MV) shedding	PS-exposing human RBCs and MVs assemble prothrombinase complexes (factors Xa/Va) to accelerate thrombin generation	Hemolytic anemia, RBC self-aggregation, RBC-endothelial adhesion, procoagulant MV shedding, and deep venous thrombosis	Flow cytometry (PS exposure, MV generation, Fluo-4 AM Ca^{2+} measurement, caspase-3 activity), prothrombinase assays, and SEM/TEM	Sub-hemolytic infection (MOI 1–10, 1 h) upregulates <i>rtxA</i> expression and Ca^{2+} influx in human RBCs. Elevated $[Ca^{2+}]_i$ activates caspase-3 and scramblase, driving phosphatidylserine (PS) externalization and microvesicle (MV) shedding. PS-exposing RBCs and MVs assemble prothrombinase complexes, increasing	Han Young Chung et al. (2022)

							g thrombi n generatio n, RBC self- aggregati on, and adhesion to human endotheli al cells (HUVEC s).	
Actin Cross-Linking Effector Domain of the Vibrio vulnificus F-Type MARTX Toxin Dominates Disease Progression During Intestinal Infection	Human Intestinal Epithelial Cells (T84 IECs)	ACD domain catalyzes covalent actin cross-linking; RRSP cleaves Ras/Rap1 GTPases	ACD drives actin oligomerization and DAMP signaling; RRSP inhibits ERK phosphorylation to suppress IL-8	ACD triggers early pro-inflammatory gene expression , while codelivered RRSP temporarily mutes early gut inflammation	Rapid human intestinal epithelial cell rounding/lysis , actin collapse, loss of barrier integrity, and mucosal tissue necrosis	Western blotting for actin cross-linking/RAS cleavage/phospho-ERK , RNA-seq transcriptomics, NanoString nCounter panel, and ELISA for IL-8	In T84 human intestinal epithelial cells, ACD catalyzes covalent actin cross-linking into oligomers within 2 h , driving rapid cell rounding, cytoskeletal collapse, and barrier breakdown. ACD acts as a DAMP upregulating IER2/3, FOS, JUNB, and CXCL8/IL-8 , while codelivered RRSP cleaves	Patrick J. Woida & Karla J. F. Satchell (2022)

							Ras/Rap 1 to inhibit ERK phosphorylation and temporarily mute IL-8 secretion	
Reduced virulence of the MARTX toxin increases the persistence of outbreak - associated Vibrio vulnificus in host reservoirs	Human Outbreak Cohort & Cell Lines (Israeli human outbreak cases & HeLa cells)	Biotype 3 MARTX toxin contains an atypical cysteine protease domain (CPD) with a VLE autocleavage site	Autocleavage at VLE alters C-terminal β -flap orientation to sterically block the catalytic active site	Dampened autophagy reduces release of downstream effectors (DUF1, RID, ABH, ExoY)	Human wound infections and bacteremia with marked reduction in cell rounding cytotoxicity and lower acute lethality	Edman degradation sequencing of CPD cleavage sites , X-ray crystallography (PDB 7D5Y), and HeLa cell rounding cytotoxicity assays	Biotype 3 MARTX CPD (CPD_BAA87) undergoes autocleavage at VLE, altering β-flap orientation to sterically block its catalytic active site and disable release of downstream effectors (DUF1, RID, ABH, ExoY) . This significantly reduces HeLa cell rounding cytotoxicity (0–15% vs 90–100% for biotype 1 at 120	Sanghyeon Choi et al. (2021)

							min, $p < 0.0001$), explaining lower acute human toxicity in Israeli outbreaks.	
Vibrio vulnificus PlpA facilitates necrotic host cell death induced by the pore forming MARTX toxin	Human Epithelial Cell Lines (HeLa cells & 3T3-L1 cells)	Sequential cooperation between pore-forming MARTX toxin and phospholipase A ₂ (PlpA)	MARTX toxin forms membrane pores to induce cell blebbing; PlpA hydrolyzes bleb phosphatidylcholine to burst blebs	Synergistic toxin action causes rapid cytosolic protein (LDH) release and cytosolic contents leakage	Rapid, complete necrotic host cell death , acute tissue necrosis, necrotizing skin lesions, and septicemia	Live-cell microscopic monitoring of bleb disruption , LDH cytotoxicity release assays, and deletion mutant profiling	In HeLa cells and 3T3-L1 fibroblasts (MOI 10), pore-forming MARTX toxin forms membrane pores that induce ballooned cell blebs in ~80% of cells within 1–2 h . Subsequent expression of PlpA (phosphatidylcholine-hydrolyzing phospholipase A ₂) lyses these blebs, causing complete cytosolic LDH release (100% cytotoxicity) and	Changyi Cho et al. (2022)

							rapid necrotic cell death.	
Genetic divergence of Vibrio vulnificus clinical isolates with mild to severe outcomes	Human Clinical Isolate Cohort (109 U.S. clinical isolates from COVIS database 2001– 2019)	Genomic diversity analysis across MLSA Lineages 1 and 2 and MARTX toxino- types (C, E, F, M, D)	Evaluate s correlati on between core- genome MLSA lineages, vcg alleles (<i>vcgE</i> vs <i>vcgC</i>), and disease outcomes	Clinical isolates from gastroent eritis and wound infection s invade host tissues across diverse genetic backgrou nds	Primary septicemi a (30% death), severe wound infection (14% amputatio n, 11% long hospital stays >14d), and gastroent eritis (39%)	TCBS & CHROM agar Vibrio culture, vcg allele PCR, 10- locus MLSA sequencin g, ribosomal MLST, and COVIS clinical database matching	Whole- genome sequencin g of 109 U.S. clinical isolates paired with COVIS patient outcomes (2001– 2019) reveals equal distributi on between Lineage 1 (51%) and Lineage 2 (49%). Clinical outcomes include 30% mortality , 14% amputati on, 11% hospital stay >14d, 34% liver disease, and 27% alcoholis m. Lineage, vcg allele (<i>vcgC</i> / <i>vcgE</i>), and MARTX toxino- type do not predict	Kendall Kling et al. / Karla J. F. Satchell (2022)

							human infection severity.	
Genome-wide phenotypic profiling of transcription factors and identification of novel targets to control the virulence of <i>Vibrio vulnificus</i>	Human Intestinal & Blood Cell Lines (INT-407, Caco-2, and Human RBCs)	Systematic mutagenesis of 285 TFs identifies 89 active TFs governing human host infectivity	TFs NagC, MetJ, and Tet2 regulate transcription of major exotoxin genes (<i>rtxA</i> , <i>vvhA</i> , <i>plpA</i>)	Exotoxin transcription drives human enterocyte damage, cytotoxicity, and red blood cell lysis	Primary foodborne septicemia, severe wound tissue damage, gastroenteritis, and fatal septic shock	Signature-tagged mutagenesis (STM) with qPCR, INT-407/Caco-2 LDH release assays, and hRBC hemolysis assays	Screening 285 TF mutants identifies 49 TFs regulating human enterocyte cytotoxicity (INT-407/Caco-2 LDH release) and hRBC hemolysis. Discovers 3 novel growth-independent TFs—NagC, MetJ, and Tet2—that specifically control transcription of major exotoxins (<i>rtxA</i> , <i>vvhA</i> , <i>plpA</i>), serving as ideal antivirulence drug targets.	Dayoung Sung et al. (2024)
Identification of potential pathogenic targets and	Human Clinical Isolates & Population	6 key co-selected genes (<i>purH</i> , <i>gmr</i> , <i>yiaV</i> ,	EG2 isolates exhibit high clinical represent	EG2 isolates exhibit high clinical represent	Foodborne primary septicemia (>50% mortality	Whole-Genome Sequencing (WGS), GWAS	GWAS and GWES across 518 global	Jia-Xin Zhang et al. (2023)

survival strategies of <i>Vibrio vulnificus</i> through population genomics	Genomics (58 human clinical isolates out of 518 global strains)	<i>dsbD</i> , <i>ramA</i> , <i>wbpA</i>) drive purine synthesis, c-di-GMP, and LPS biogenesis	ation (86/459 vs 1/59 in EG1) and superior human host colonization	ation (86/459 vs 1/59 in EG1) and superior human host colonization	in liver disease), necrotizing wound infection (17% mortality), and gastroenteritis	(Pyseer, DBGWA S), GWES (SpydrPic k), VITEK-2 automated identification, and MIC testing	isolates (87 clinical, 420 environmental) identify 6 core co-selected genes (<i>purH</i> , <i>gmr</i> , <i>viaV</i> , <i>dsbD</i> , <i>ramA</i> , <i>wbpA</i>) associated with clinical pathogenicity ($p < 1.74 \times 10^{-9}$). EG2 accounts for 86/459 clinical cases compared to only 1/59 in EG1, associated with primary septicemia (>50% mortality).	
Characteristic Metabolic Changes in Skeletal Muscle Due to <i>Vibrio vulnificus</i> Infection in a	Human Clinical Patients Context & Biomarker Analysis	V. <i>vulnificus</i> necrotizing wound infection alters skeletal muscle energy metabolism	MARTX toxin drives local bacterial proliferation, resulting in muscle tissue catabolism and TCA cycle	Bacterial muscle invasion induces severe muscle destruction and systemic inflammatory response	Rapid necrotizing fasciitis, severe skeletal muscle tissue necrosis, high serum CPK levels, and fatal	CE-TOFMS metabolomic profiling and serum creatine phosphokinase (CPK) clinical biomarker tracking	Connects muscle tissue degradation during necrotizing fasciitis with specific TCA cycle alterations (succinate	Kai Ishida et al. (2023)

Wound Infection Model			perturbation		septic shock		e accumulation and creatinine depletion). Muscle catabolites leak into the bloodstream, elevating serum creatinine and creatinine levels, identifying serum creatinine phosphokinase (CPK) as a key clinical blood biochemistry biomarker for early diagnosis and prognosis.	
-----------------------	--	--	--------------	--	--------------	--	--	--

The following section presents the thematic and comparative synthesis of the evidence compiled in Table 1, detailing the molecular mechanisms, cellular functions, host–pathogen interactions, and clinical outcomes of *Vibrio vulnificus* infections across four structured dimensions:

a. Mechanism

Vibrio vulnificus exports its primary virulence factor, the multifunctional autoprocessing repeats-in-toxin (MARTX) toxin encoded by *rtxA1*, through a Type I Secretion System (T1SS; RtxB/D/E–TolC complex). Initial host cell surface anchoring is mediated by the N-terminal Aknot domain, a 273-amino acid insertion with tandem immunoglobulin-like folds that specifically binds internal N-acetylglucosamine (GlcNAc) residues on complex biantennary N-glycans, targeting cell surface glycoproteins such as L1CAM. Following membrane insertion, the N- and C-terminal repeat regions assemble a pore in the host plasma membrane, translocating the central effector domains and the cysteine protease domain (CPD) into the host cytosol. Cytosolic eukaryotic inositol hexakisphosphate (InsP6) binds and activates CPD autoprocessing, releasing discrete effector domains into host cell compartments. In biotype 3 clinical outbreak strains, an atypical CPD featuring a unique VLE autocleavage site and C-terminal b-flap sterically dampens autoproteolytic effector processing, attenuating acute cellular cytotoxicity. Live-cell imaging demonstrates a two-step exotoxin synergy wherein MARTX pore formation

induces host cell blebbing, followed by phospholipase (PlpA) hydrolysis of membrane phosphatidylcholine, which disrupts the blebs and executes rapid necrotic cell death with complete LDH release

b. Molecular functions

The DUF1–RID effector duet functions in a mutually reinforcing manner. DUF1 acts as a RID-dependent transforming NADase domain (RDTND) that binds host calcium-free calmodulin to hydrolyze intracellular and into nicotinamide and ADPR. RID functions as an -fatty acyltransferase that modifies C-terminal polybasic lysine residues of Rac1 at the plasma membrane. The Makes Caterpillars Floppy-like (MCF) effector is activated by host ADP-ribosylation factor (ARF1/ARF3) GTPases to undergo autoprocessing and N-terminal acetylation, operating as a broad-spectrum cysteine protease that cleaves approximately 50% of host Rab GTPases (such as Rab1B) within their flexible C-terminal tails. The Actin Cross-linking Domain (ACD) catalyzes covalent isopeptide bonding between Lys-50 and Glu-270 of G-actin monomers to drive actin cross-linking and cytoskeletal collapse. Codelivered Ras/Rap1-specific endopeptidase (RRSP) functions as a site-specific endopeptidase that cleaves host Ras and Rap1 GTPases. At sub-hemolytic concentrations, the pore-forming activity of the MARTX toxin elevates intracellular iin red blood cells (RBCs), activating caspase-3 and scramblase to induce phosphatidylserine (PS) externalization and shedding of procoagulant microvesicles

c. Host-Pathogen Interactions

The DUF1–RID duet completely abolishes NOX2-mediated reactive oxygen species (ROS) generation in macrophages within 1.5 hours by depleting and competitively hijacking and acylating Rac1 to block NOX2 complex assembly, thereby silencing pro-inflammatory cytokines (IL-6, TNF, MCP-1) and suppressing early innate immune clearance. MCF-mediated cleavage of host Rab GTPases strips Rab1B from cellular membranes, resulting in Golgi collapse, organelle destruction, and intrinsic mitochondrion-mediated apoptosis. ACD-mediated actin cross-linking dismantles the host cytoskeletal scaffold and breaks down mucosal epithelial barrier integrity. Although actin cross-linking acts as a DAMP to upregulate early response genes, codelivered RRSP cleaves Ras/Rap1 GTPases to suppress ERK phosphorylation and temporarily mute early IL-8 secretion. PS-exposing human RBCs and shed microvesicles assemble prothrombinase complexes that accelerate thrombin generation, promote RBC self-aggregation, and enhance RBC adherence to endothelial cells, providing a molecular driver for deep vein thrombosis. Host iron overload in conditions like liver cirrhosis elevates non-transferrin-bound serum iron, upregulating bacterial iron uptake systems and fueling explosive bacterial growth. In skeletal muscle wound infections, MARTX-promoted bacterial proliferation triggers protein catabolism and disrupts the TCA cycle through succinate accumulation

d. Symptoms and effects

Ingestion of contaminated seafood or marine wound exposure initially presents with localized physical symptoms including high fever, chills, gastroenteritis, localized erythema, severe wound pain, and bullae formation. These symptoms rapidly progress within 24 to 72 hours to life-threatening systemic outcomes, including primary septicemia with septic shock, necrotizing fasciitis requiring surgical debridement or limb amputation, acute kidney injury, and multi-organ failure, carrying a mortality rate exceeding 50% in septicemia cases. Genomic analysis of 109 U.S. clinical isolates paired with CDC COVIS patient records demonstrates that human clinical cases are equally distributed between Lineage 1 and Lineage 2, with severe outcomes such as 14% limb amputation and 11% prolonged hospitalization (>14 days) occurring across both lineages. Genome-wide association studies across 518 global isolates show that Ecological Group 2 (EG2) strains dominate clinical cases due to superior thermal (growth), osmotic, and biofilm adaptations. Skeletal muscle degeneration leaks catabolites into circulation, establishing serum creatine phosphokinase (CPK) as a key diagnostic biochemistry biomarker. Point-of-care CRISPR-Cas12a / Recombinase Polymerase Amplification (RPA) visual assays targeting vvhA achieve visual detection limits of 1–10 CFU/mL within 30 minutes. Antivirulence strategies targeting master exotoxin transcription factors such as HlyU, NagC, MetJ, and Tet2 with small-molecule inhibitors (like CM14) effectively disarm toxin expression and prevent systemic tissue damage

Table 2. *Vibrio vulnificus* Pathogenesis in Animal Models and Aquatic Reservoirs: Toxin Mechanisms, Tissue Tropism, and Environmental Adaptations

Title	Study Type / Model	Mechanism	Molecular Functions	Host-Pathogen Interactions	Symptoms/effects	Diagnoses	Findings	Authors
Complex regulatory networks of virulence factors in <i>Vibrio vulnificus</i>	Review / Animal Models (Oysters, marine fish, mouse models)	Spatiotemporal gene regulation by TFs (BrpR, SmcR, IscR, HlyU, Fur) responding to Ca^{2+} , AI-2, Fe-S, and ROS/RNS	Transcriptional coordination of exotoxins (<i>rtxA</i> , <i>vvhA</i> , <i>plpA</i> , <i>vvpE</i>), CPS, EPS, and matrix proteins	c-di-GMP/BrpR drives oyster tissue colonization; SmcR-driven biofilm dispersal enables planktonic spread	Hemorrhagic septicemia in marine fish; internal organ tissue damage and septic death in mice	In vivo bacterial load tracking in oyster tissues and mouse organs; transcriptomic profiling	In oysters, environmental Ca^{2+} elevates c-di-GMP to upregulate BrpR, driving tissue biofilm colonization. In mice, IscR senses Fe-S status and ROS/RNS to activate gbpA and vvhA , while treating infected mice with the small-molecule HlyU inhibitor CM14 reduces systemic inflammation and significantly improves mouse survival rates.	Garam Choi & Sang Ho Choi (2022)

IJETRM

INTERNATIONAL JOURNAL OF ENGINEERING TECHNOLOGY RESEARCH & MANAGEMENT (IJETRM)

<https://ijetrm.com/>

It's time to act: Understanding and combating Vibrio vulnificus	Review / Aquaculture & Animal Models (Oysters, blue crabs, tilapia, tongue sole)	Type IV pili (pilA) & LuxS/AI-2 quorum sensing drive environmental biofilm formation	Multi-toxin expression (MARTX, VvhA) and iron-acquisition systems in aquatic hosts	Microplastics and algal blooms increase bacterial colonization in shellfish by up to 100-fold	High mortality and severe tissue damage in aquaculture species (tilapia, tongue sole)	Aquaculture bacterial surveillance using qPCR and colorimetric assays	Seawater temperatures >15°C double bacterial reproduction rates every 20 min. Co-exposure to microplastics and algal blooms increases bacterial detection in oyster farming areas 2.3-fold compared to natural waters , while tongue sole aquaculture vaccines deliver high protective immunity.	Chao Li, Gang Li & Ming Li (2025)
Dissemination of pathogenic bacteria is reinforced by a MARTX toxin effector duet	In Vivo Mouse Model (Female ICR mice, 5–7 weeks) & Murine BMDMs	DUF1–RID effector duet synergy: RID _{CBD} hijacks calmodulin (CaM) to turn DUF1 into an NAD(P)⁺ hydrolase	DUF1 degrades intracellular NAD ⁺ /NADP ⁺ ; RID modifies Rac1 to block NOX2 complex assembly	Silences macrophage ROS generation , blocking pro-inflammatory signals (IL-6, TNF-α, MCP-1) during early infection	Local muscle myofibril disintegration , hypothermia, rapid bacterial spread to spleen/liver, and 100% mortality within 20 h	Tissue bacterial load counts (CFU in blood, spleen, liver) , liver H&E histology, and flow cytometric ROS measurement	Subcutaneous challenge (5 × 10 ⁶ CFU) with wild-type strains causes 100% mouse mortality within 20 hpi with severe hypothermia	Sanghyeon Choi et al. (2024)

							mia. Bacterial loads appear in blood, spleen, and liver at 10 hpi , whereas RDTND catalytic (E/Q) or CaM-binding (4mt) mutants result in 100% mouse survival.	
Vibrio MARTX toxin binding of biantennary N-glycans at host cell surfaces	In Vivo Mouse Model (Female ICR mice)	Aknot domain (273 aa Ig-like folds) binds internal obligate GlcNAc residues on complex biantennary N-glycans	Selective host surface glycan binding to anchor MARTX toxin for pore formation and effector translocation	Mediates MARTX toxin surface attachment across intestinal epithelial cells during oral infection	Severe intestinal epithelial destruction, mucosal breakdown, and rapid mouse mortality	Intestinal tissue histological staining, in vivo lethality assays, and oral gavage infection tracking	Oral gavage challenge ($3.5\text{--}4.0 \times 10^7$ CFU/mL) with wild-type strains causes severe intestinal epithelial collapse and 100% mouse mortality within 36 h. Deleting the Aknot domain (ΔAknot) completely abolishes intestinal tissue damage and results in 100% mouse	Jiexi Chen et al. (2025)

							survival (0% mortality).	
Vibrio MARTX toxin processing and degradation of cellular Rab GTPases by the cytotoxic effector Makes Caterpillars Floppy	In Vivo Mouse Model (ICR mice, oral gavage challenge)	Host ARF1/ARF3 GTPases stimulate autophagy of the MCF cysteine protease domain	Cysteine protease activity broadly cleaves and degrades ~50% of host Rab GTPases at their C-terminal tails	Cleavage strips Rab GTPases from membranes , causing Rab1B dispersal and Golgi apparatus collapse	Loss of small intestinal Rab1B density , extensive intestinal organelle damage, and accelerated vibriosis mortality	Immunohistochemical tissue signal quantification , intestinal histology, and Western blot of tissue lysates	Translocated MCF cysteine protease causes loss of endogenous Rab1B protein signal in mouse small intestinal tissue , driving extensive organelle destruction, mucosal apoptosis, and significantly accelerated mouse mortality during oral infection.	Alfa Herrera et al. (2024)
MARTX toxin of Vibrio vulnificus induces RBC phosphatidylserine exposure that can contribute to thrombosis	In Vivo Rat Model (Male Sprague-Dawley rats, inferior vena cava ligation)	MARTX toxin pore-forming region triggers intracellular Ca^{2+} influx in red blood cells	Elevated $[Ca^{2+}]_i$ activates caspase-3 and scramblase, driving phosphatidylserine (PS) externalization	PS-exposing RBCs and microvesicles (MVs) assemble prothrombinase complexes to promote thrombin generation	RBC discocyte-to-echinocyte/spherocyte transition , procoagulant MV shedding, and deep venous thrombosis	Rat IVC ligation model thrombus weight measurement , SEM/TEM microscopy, and flow cytometry	In a rat inferior vena cava (IVC) thrombosis model (10^8 – 10^9 CFU IV + IVC ligation), intravenous wild-type infection significantly increases thrombus	Han Young Chung et al. (2022)

							s weight (up to ~25 mg) at 1 h post-infection, whereas the ΔrtxA mutant fails to induce thrombosis (thrombus weight equal to uninfected controls, $p < 0.05$).	
Actin Cross-Linking Effector Domain of the <i>Vibrio vulnificus</i> F-Type MARTX Toxin Dominates Disease Progression During Intestinal Infection	In Vivo Mouse Model (Female ICR mice, intragastric oral gavage)	ACD domain catalyzes covalent actin cross-linking into oligomers, causing cytoskeletal collapse	ACD acts as a DAMP triggering early pro-inflammatory genes (IER2/3, CXCL8); codelivered RRSP cleaves Ras/Rap1	ACD-driven mucosal damage enables rapid bacterial escape across the intestinal epithelial barrier	Intestinal epithelial cell rounding/lysis, severe gut mucosal inflammation, bacterial spread to liver/spleen, and 90% mortality within 12h	Organ bacterial load counts (CFU in blood, liver, spleen), NanoString inflammation panel, and tissue transcriptomics	Intragastric infection (1×10^9 CFU/mL) with active ACD strains causes 90–100% mouse mortality within 12–24 hpi with rapid bacterial dissemination of 10^4–10^6 CFU to liver and spleen. Inactivation of ACD results in significantly delayed/reduced mouse	Patrick J. Woida & Karla J. F. Satchell (2022)

							mortality and suppressed organ dissemination.	
Reduced virulence of the MARTX toxin increases the persistence of outbreak - associated <i>Vibrio vulnificus</i> in host reservoirs	In Vivo Tilapia Reservoir & Mouse Models (Farmed tilapia & female ICR mice)	Biotype 3 MARTX toxin harbors an atypical cysteine protease domain (CPD) with a unique VLE autocleavage site	Autocleavage at VLE alters C-terminal β -flap orientation, sterically blocking the catalytic site and dampening effector release	Attenuated host cytotoxicity allows bacteria to colonize tilapia host reservoirs without causing rapid fatal disease	Delayed/reduced mouse mortality and minimal HeLa cell rounding compared to biotype 1 progenitor strains	X-ray crystallography (PDB 7D5Y) , in vitro cleavage assays, CD spectroscopy, and mouse survival tracking	Biotype 3 MARTX (MO6-24/O/MARTX_BA A87) shows attenuated virulence to persist longer in farmed tilapia (<i>Oreochromis</i> spp.) reservoirs . In mice (5×10^7 CFU/mL subcutaneous), biotype 1 strains cause 100% mortality within 15 hpi , whereas biotype 3 MARTX yields 50% mortality at 48 hpi .	Sanghyeon Choi et al. (2021)
<i>Vibrio vulnificus</i> PlpA facilitates necrotic host cell death induced by the	In Vivo Mouse Infection Models (Intradermal skin & orogastric mouse challenge)	Sequential cooperation between pore-forming MARTX toxin and phospholipase	MARTX pore formation induces host membrane blebbing; PlpA hydrolyze	Synergistic toxin action accelerates tissue necrosis and enables rapid bacterial	Rapid necrotizing skin lesion formation , extensive tissue destruction, and	In vivo skin lesion area measurement , LDH release assays, and	In mouse intradermal skin and orogastric infection models, multi-toxin deletion	Changyi Cho et al. (2022)

pore forming MARTX toxin	)	pase A ₂ (PlpA)	s phosphatidylcholine to lyse blebs	invasion	severe systemic infection	multi-exotoxin mutant infection tracking	mutant analysis demonstrates that pore-forming MARTX toxin and PlpA phospholipase act sequentially in vivo to drive rapid necrotic tissue disruption and accelerated lesion expansion.	
Genetic divergence of <i>Vibrio vulnificus</i> clinical isolates with mild to severe outcomes	In Vivo Mouse Virulence Assays (Subcutaneous injection in iron dextran-treated mice)	Core-genome MLSA lineages (Lineage 1 vs 2) and MARTX toxinotype diversity	Evaluate s exotoxin expression and iron acquisition across diverse genetic lineages in vivo	Bacterial proliferation in iron-overloaded mice tests in vivo lethality across different isolate strains	Variable mouse mortality and skin lesion severity depending on bacterial dose and strain type	In vivo LD ₅₀ determination in iron-dextran mice, <i>vcg</i> allele PCR, and MLSA 10-locus typing	Subcutaneous challenge in iron dextran-pretreated mice confirms that while host iron overload promotes bacterial proliferation, in vivo strain lethality varies widely across individual isolates regardless of core-genome MLSA lineage	Kendall Kling et al. / Karla J. F. Satchell (2022)

							assignme nt.	
Genome-wide phenotypic profiling of transcription factors and identification of novel targets to control the virulence of <i>Vibrio vulnificus</i>	In Vivo Animal Models (Axenic brine shrimp <i>Artemia franciscana</i> & female ICR mice)	Systematic mutagenesis of 285 TFs identifies 89 active TFs governing survival and infectivity	TFs NagC, MetJ, and Tet2 regulate transcription of major exotoxin genes (<i>rtxA</i> , <i>vvhA</i> , <i>plpA</i>)	NagC, MetJ, and Tet2 drive exotoxin-mediated host damage during live animal infection	Fatal septicemia and lethality in both brine shrimp bath challenge and mouse infection models	Signature-tagged mutagenesis (STM) library screening with qPCR , brine shrimp & mouse lethality assays	Screening 285 TF mutants in brine shrimp (10^4 CFU) and mice (10^9 CFU intragastric / 10^6 CFU IP) identifies 33 TFs affecting in vivo infectivity . 10 TFs (including HlyU, CRP, Tet2, CysB, GacA, SeqA, IscR, NagC, AphA, Lrp) significantly reduce animal mortality when mutated ($p < 0.0001$).	Dayoung Sung et al. (2024)
Identification of potential pathogenic targets and survival strategies of <i>Vibrio vulnificus</i> through	Environmental & Aquatic Isolates Analysis (518 global isolates)	Core-genome SNP phylogeny divides species into 6 lineages and 2 ecological groups (EG1 and EG2)	6 key co-selected genes (<i>purH</i> , <i>gmr</i> , <i>yiaV</i> , <i>dsbD</i> , <i>ramA</i> , <i>wbpA</i>) control purine synthesis, c-di-	EG2 isolates exhibit enhanced stress tolerance (salinity 5–25 ppt, 37°C) and superior biofilm colonization	Enhanced persistence in aquatic environments and higher colonization efficiency in aquatic	Whole-Genome Sequencing (WGS) , GWAS (Pyseer, DBGWAS), GWES (SpydrPic) co-selection	Population genomics of 518 global isolates divides the species into 6 MLST lineages and 2	Jia-Xin Zhang et al. (2023)

populati on genomics			GMP, and LPS biogenesi s	on	hosts	networks) , and MIC testing	Ecologica l Groups. EG2 isolates demonst rate superior environ mental adaptati on, surviving salinity of 5–25 ppt, pH 6–8, 37°C growth, and displayin g significa ntly higher biofilm formatio n (A590 ~0.8 vs ~0.3 in EG1).	
Characte ristic Metaboli c Changes in Skeletal Muscle Due to Vibrio vulnificu s Infection in a Wound Infection Model	In Vivo Mouse Wound Model (Male Crl:CD1 mice & neutropen ic CPA- treated mice)	V. vulnificu s wound infection triggers site- specific muscle tissue metabolic alteration s	MARTX toxin (rtxA1) facilitates local bacterial multiplic ation, altering the TCA cycle and muscle catabolis m	MARTX toxin (rtxA1) facilitates local bacterial multiplic ation, altering the TCA cycle and muscle catabolis m	Severe skeletal muscle protein catabolis m, creatine depletion, succinate accumula tion, and local tissue necrosis	CE- TOFMS metabolo mic profiling of tissue extracts, tissue CFU burden, RT-qPCR for cytokines , and muscle histology	In a mouse caudal thigh wound model (10 ⁶ CFU), infection causes muscle creatine depletion (250,000 to <80,000 nmol/g) and TCA cycle shifts (succinat e accumul ation	Kai Ishida et al. (2023)

							from ~1000 to >10,000 nmol/g). MARTX deletion (Δ rtxA1) abolishes metaboli c changes, but these are restored in neutrope nic (CPA- treated) mice.	
--	--	--	--	--	--	--	--	--

The following section presents the thematic and comparative synthesis of the empirical evidence compiled in Table 2, evaluating the molecular mechanisms, cellular functions, host–pathogen interactions, and pathological outcomes of *Vibrio vulnificus* across animal host models and environmental reservoirs across four structured dimensions

a. Mechanism

Vibrio vulnificus exports its primary exotoxin, the multifunctional autoprocessing repeats-in-toxin (MARTX) toxin encoded by rtxA1, into the extracellular environment through a Type I Secretion System composed of the RtxB/D/E–TolC complex. Surface anchoring to host tissues is initiated by the N-terminal Aknot domain, a 273–amino acid insertion with tandem immunoglobulin-like folds that specifically binds internal obligate N-acetylglucosamine (GlcNAc) residues on complex biantennary N-glycans, targeting cell surface glycoproteins such as LICAM. Following membrane insertion, the conserved N- and C-terminal repeat regions form a pore in the host plasma membrane, translocating the central effector domains and the cysteine protease domain (CPD) into the host cytosol. Eukaryotic cytosolic inositol hexakisphosphate binds and activates CPD autoprocessing, releasing discrete effector domains into subcellular compartments. In biotype 3 clinical outbreak strains, an atypical CPD featuring a unique VLE autocleavage site and C-terminal b-flap sterically blocks the catalytic active site after initial cleavage, dampening effector release and attenuating acute cellular cytotoxicity. Live-cell imaging demonstrates a two-step exotoxin synergy wherein MARTX pore formation induces host plasma membrane ballooning and cell blebbing, followed by phospholipase (PlpA) hydrolysis of membrane phosphatidylcholine, which disrupts the blebs and executes rapid necrotic cell death with complete cytosolic LDH release

b. Molecular Functions

The DUF1–RID effector duet operates in a mutually reinforcing manner within host cells. DUF1 functions as a RID-dependent transforming NADase domain (RDTND) that binds host calcium-free calmodulin to rapidly hydrolyze intracellular and into nicotinamide and ADPR. Simultaneously, RID acts as an b-fatty acyltransferase that modifies C-terminal polybasic lysine residues of the host small GTPase Rac1 at the plasma membrane. The Makes Caterpillars Floppy-like (MCF) effector is activated by host ADP-ribosylation factor GTPases to undergo autoprocessing and N-terminal acetylation, operating as a broad-spectrum cysteine protease that cleaves approximately 50% of host Rab GTPases (such as Rab1B) within their flexible C-terminal tails. The Actin Cross-linking Domain (ACD) catalyzes covalent isopeptide bonding between Lys-50 and Glu-270 of G-actin monomers to drive rapid actin cross-linking and cytoskeletal collapse. Codelivered Ras/Rap1-specific endopeptidase (RRSP) functions as a site-specific endopeptidase that cleaves host Ras and Rap1 GTPases. At sub-hemolytic concentrations, the pore-forming region of the MARTX toxin induces an influx of ab1B cleavage dismantles intestinal epithelial barrier integrity, driving bacterial translocation across the gut mucosal wall into the bloodstream and disseminating to distal organs, including the liver and spleen. The DUF1–RID duet completely

abolishes NOX2-mediated reactive oxygen species (ROS) generation in macrophages within 1.5 hours by depleting and acylating Rac1, silencing pro-inflammatory cytokines (IL-6, TNF, MCP-1) and preventing early innate immune clearance. This immune evasion enables wild-type strains to cause severe hypothermia, systemic cytokine storms, and 100% lethality in mice within 20 hours post-subcutaneous infection. In rat inferior vena cava models, sub-hemolytic MARTX pore activity in red blood cells promotes prothrombinase assembly, accelerating thrombin generation, red blood cell self-aggregation, and endothelial adherence to trigger deep vein thrombosis. In skeletal muscle wound infections, MARTX-promoted bacterial proliferation induces extensive protein catabolism, depleting muscle creatine reserves and disrupting the host TCA cycle through succinate accumulation. In estuarine environments, seawater temperatures exceeding, microplastic biofilms (achieving 100-fold higher density via Type IV pili and LuxS/AI-2 quorum sensing), and algal blooms enhance bacterial colonization in shellfish and aquaculture species. Master transcription factors (including HlyU, IscR, NagC, MetJ, Tet2, BrpR, and SmcR) coordinate c-di-GMP signaling, biofilm formation, and exotoxin gene expression (rtxA1, vvhA, plpA) in brine shrimp (*Artemia franciscana*) and murine models.

c. Symptoms and effects

In subcutaneous and intragastric mouse infection models, wild-type bacterial challenge induces severe hypothermia, acute muscle necrosis, inflammatory cytokine storms, rapid systemic bacterial dissemination to internal organs (liver and spleen), and 90% to 100% mortality within 12 to 24 hours. Outbreak strains of biotype 3 associated with farmed tilapia (*Oreochromis* spp.) exhibit attenuated CPD autoproteolytic activity, reducing mouse lethality (50% mortality at 48 hours compared to 100% mortality at 15 hours for biotype 1) to trade acute toxicity for prolonged environmental persistence in host reservoirs. Rising sea surface temperatures and estuarine microplastic pollution expand pathogen geographic range and density, causing severe loss in the aquaculture industry due to hemorrhagic septicemia and fish mortality. Genome-wide mutant profiling in brine shrimp and mouse models identifies non-essential transcription factors (HlyU, NagC, MetJ, Tet2) as prime targets for small-molecule antivirulence inhibitors (such as CM14) that disarm exotoxin expression without imposing selective pressure for antibiotic resistance, while muscle catabolite leakage into the bloodstream establishes serum creatine phosphokinase (CPK) as a key biochemistry biomarker for diagnosing severe necrotizing fasciitis

CONCLUSION

This systematic review demonstrates that *Vibrio vulnificus* is a fulminant marine pathogen whose high mortality rate (>50% in primary septicemia) is driven by complex exotoxin synergies, host metabolic vulnerabilities, and environmental adaptations. The primary engine of pathogenicity is the MARTX toxin, which uses its N-terminal Aknot domain to bind internal GlcNAc residues on host biantennary N-glycans (predominantly L1CAM), translocating effector domains that act in concert: the DUF1–RID duet depletes host NAD(P)⁺ and silences macrophage ROS generation, the MCF cysteine protease cleaves host Rab GTPases to induce Golgi collapse, and the ACD domain catalyzes covalent actin cross-linking to break down epithelial barriers. Pathogenesis is further amplified through multi-toxin cooperation—where MARTX pore formation and phospholipase PlpA act sequentially to lyse cell blebs and execute necrotic cell death—and sub-hemolytic MARTX pore activity that elevates red blood cell calcium to drive phosphatidylserine exposure and venous thrombosis. In wound infections, bacterial proliferation induces muscle catabolism and TCA cycle disruption, highlighting serum CPK as an essential diagnostic biomarker. Importantly, genomic analysis of clinical cohorts disproves the assumption that severe disease is restricted to a single genotype, demonstrating that clinical cases are equally distributed across Lineages 1 and 2, while Ecological Group 2 (EG2) strains dominate clinical prevalence due to superior thermal and osmotic adaptations. Countering this escalating public health crisis requires combining rapid 30-minute CRISPR-Cas12a/RPA point-of-care diagnostics with non-antibiotic antivirulence therapies targeting master exotoxin transcription factors such as HlyU, NagC, MetJ, and Tet2.

ACKNOWLEDGEMENT

We would like to express our sincere gratitude to Professor Gecele C. Estorico for her invaluable guidance and for sharing her expertise throughout the conduct of this systematic review. We are also deeply thankful to our groupmates for their collaborative dedication to the data collection, screening, and synthesis of the 13 peer-reviewed studies analyzed.

REFERENCES

- 1) Chen, J., Goerdeler, F., Jaroentomeechai, T., Hernandez, F. X. S., Wang, X., Clausen, H., Narimatsu, Y., & Satchell, K. J. F. (2025). *Vibrio* MARTX toxin binding of biantennary N-glycans at host cell surfaces. *Science Advances*, 11(15), Article eadt0063. <https://doi.org/10.1126/sciadv.adt0063>

- 2) Cho, C., Choi, S., Kim, M. H., & Kim, B. S. (2022). *Vibrio vulnificus* PlpA facilitates necrotic host cell death induced by the pore forming MARTX toxin. *Journal of Microbiology*, 60(2), 224–233. <https://doi.org/10.1007/s12275-022-1582-7>
- 3) Choi, G., & Choi, S. H. (2022). Complex regulatory networks of virulence factors in *Vibrio vulnificus*. *Trends in Microbiology*, 30(12), 1205–1216. <https://doi.org/10.1016/j.tim.2022.06.006>
- 4) Choi, S., Kim, B. S., Hwang, J., & Kim, M. H. (2021). Reduced virulence of the MARTX toxin increases the persistence of outbreak-associated *Vibrio vulnificus* in host reservoirs. *Journal of Biological Chemistry*, 296, Article 100777. <https://doi.org/10.1016/j.jbc.2021.100777>
- 5) Choi, S., Lee, Y., Park, S., Jang, S. Y., Park, J., Oh, D. W., Kim, S.-M., Kim, T.-H., Lee, G. S., Cho, C., Kim, B. S., Lee, D., Kim, E.-H., Cheong, H.-K., Moon, J. H., Song, J.-J., Hwang, J., & Kim, M. H. (2024). Dissemination of pathogenic bacteria is reinforced by a MARTX toxin effector duet. *Nature Communications*, 15, Article 6218. <https://doi.org/10.1038/s41467-024-50650-0>
- 6) Chung, H. Y., Bian, Y., Lim, K.-M., Kim, B. S., & Choi, S. H. (2022). MARTX toxin of *Vibrio vulnificus* induces RBC phosphatidylserine exposure that can contribute to thrombosis. *Nature Communications*, 13, Article 4846. <https://doi.org/10.1038/s41467-022-32599-0>
- 7) Herrera, A., Packer, M. M., Rosas-Lemus, M., Minasov, G., Chen, J., Brumell, J. H., & Satchell, K. J. F. (2024). *Vibrio* MARTX toxin processing and degradation of cellular Rab GTPases by the cytotoxic effector Makes Caterpillars Floppy. *Proceedings of the National Academy of Sciences*, 121(25), Article e2316143121. <https://doi.org/10.1073/pnas.2316143121>
- 8) Ishida, K., Shimohata, T., Kanda, Y., Nguyen, A. Q., Masuda, R., Yamazaki, K., Uebanso, T., Mawatari, K., Kashimoto, T., & Takahashi, A. (2023). Characteristic metabolic changes in skeletal muscle due to *Vibrio vulnificus* infection in a wound infection model. *mSystems*, 8(3), Article e01290-22. <https://doi.org/10.1128/msystems.01290-22>
- 9) Kling, K., & Satchell, K. J. F. (2022). Genetic divergence of *Vibrio vulnificus* clinical isolates with mild to severe outcomes. *mBio*, 13(4), Article e01500-22. <https://doi.org/10.1128/mbio.01500-22>
- 10) Li, C., Li, G., & Li, M. (2025). It's time to act: Understanding and combating *Vibrio vulnificus*. *Virulence*, 16(1), Article 2569998. <https://doi.org/10.1080/21505594.2025.2569998>
- 11) Sung, D., Choi, G., Ahn, M., Byun, H., Kim, T. Y., Lee, H., Lee, Z.-W., Park, J. Y., Jung, Y. H., Han, H. J., & Choi, S. H. (2024). Genome-wide phenotypic profiling of transcription factors and identification of novel targets to control the virulence of *Vibrio vulnificus*. *Nucleic Acids Research*, 53(3), Article gkae1238. <https://doi.org/10.1093/nar/gkae1238>
- 12) Woida, P. J., Kitts, G., Shee, S., Godzik, A., & Satchell, K. J. F. (2022). Actin cross-linking effector domain of the *Vibrio vulnificus* F-type MARTX toxin dominates disease progression during intestinal infection. *Infection and Immunity*, 90(4), Article e00627-21. <https://doi.org/10.1128/iai.00627-21>
- 13) Zhang, J.-X., Yuan, Y., Hu, Q.-H., Jin, D.-Z., Bai, Y., Xin, W.-W., Kang, L., & Wang, J.-L. (2023). Identification of potential pathogenic targets and survival strategies of *Vibrio vulnificus* through population genomics. *Frontiers in Cellular and Infection Microbiology*, 13, Article 1254379. <https://doi.org/10.3389/fcimb.2023.1254379>