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REVIEW

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Precision medicine for personalized cholecystitis care: integrating molecular diagnostics and biotherapeutics

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Abstract

Background Acute cholecystitis, inflammation of the gallbladder, can lead to serious complications if not promptly diagnosed and managed. Conventional therapies have limitations, necessitating newer personalized approaches.

Main body of the abstract This review examines recent advances transforming cholecystitis care. Diagnostically, molecular techniques like next-generation sequencing rapidly identify causative microbes from gallbladder specimens, enabling targeted antimicrobial therapy. Regarding treatment, phage therapy uses viruses to lyse pathogenic bacteria. RNA interference and CRISPR-Cas9 gene editing silence microbial virulence factors. Probiotics competitively exclude pathogens. Robotics and fluorescence imaging refine surgical techniques. Additional emerging modalities include biosensors detecting inflammatory mediators, regenerative gallbladder tissue engineering using stem cells, and artificial intelligence for real-time decision support. However, the optimal integration of novel technologies with current best practices remains unknown. Further research is needed to validate and optimize personalized diagnostics and therapeutics for cholecystitis.

Short conclusions Advances in next-generation sequencing, CRISPR gene editing, robotics, and other biotechnologies promise to transform the precision and personalized management of cholecystitis when thoughtfully implemented. However, controlled trials are still required to define optimal integration with conventional supportive care and antibiotics.

Keywords Cholecystitis, Next-generation sequencing, CRISPR, Gene editing, Phage therapy, Regenerative medicine, Robotic surgery

Background

Cholecystitis involves inflammation of the gallbladder, a small organ under the liver that stores and concentrates bile (Sakamoto et al. 2021). It develops when the cystic duct, transporting bile from gallbladder to intestine, is blocked (Costanzo et al. 2023). This obstruction frequently arises from gallstones lodged in the duct, termed calculous cholecystitis. Gallstones form from cholesterol saturation, imbalance in bile salts, and insufficient contractions allowing stone growth. Cystic duct blockage by stones prevents bile drainage, increasing gallbladder pressure and irritation (Kuhlenschmidt et al. 2021). Infection is another cause, called acalculous cholecystitis. Bacteria like *Escherichia coli* and *Klebsiella*, or parasites including *Ascaris* and *Clonorchis*, directly infect the gallbladder (Hamid et al. 2021). Both obstruction and infection lead to gallbladder distension, epithelial irritation, swelling, and eventual

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necrosis. Understanding the causal factors facilitates preventative strategies and guides diagnostic testing and treatment approaches (Costanzo et al. 2023). Characteristic right upper quadrant abdominal pain is the most common cholecystitis symptom. Pain results from inflammation and irritation of the gallbladder located under the liver. Patients also exhibit low-grade fever from associated infection, along with nausea and vomiting from abdominal distress. As cholecystitis progresses, worsening pain, high fever, dehydration and intractable vomiting can occur. Lack of treatment risks complications like ruptured, perforated or gangrenous gallbladder requiring urgent surgery. Recognizing the typical pain, fever and nausea enables prompt diagnosis to manage cholecystitis before progression to dire outcomes. Diagnostic testing like ultrasound or HIDA biliary scans are then initiated. Fast diagnosis of this acute abdominal pain cause improves morbidity and mortality by facilitating timely care and possible cholecystectomy (Gallaher and Charles 2022). Potential complications include gallbladder perforation, empyema, jaundice, ascending cholangitis, fistulas, abscesses, acute pancreatitis and rarely gallbladder cancer. Perforation from severe inflammation leaks infected bile, inciting peritonitis, sepsis and higher mortality. Empyema arises when pus and necrotic debris accumulate, potentially seeding pathogens into the bloodstream. Biliary obstruction can cause jaundice as bilirubin accumulates. Gallstones blocking the common bile duct also risk cholangitis from bacterial spread. Gangrenous cholecystitis with severe tissue death is especially dangerous, increasing morbidity and mortality (Auda et al. 2022).

Microbiology of cholecystitis

Common microorganisms involved

Bacteria

The development of acute cholecystitis often involves infection by various microorganisms. Bacteria are the most frequent infectious agents, with *Escherichia coli*, *Klebsiella* species, and Enterococcus species being the predominant bacterial species cultured in patients with acute cholecystitis (Karasawa et al. 2021). *E. coli* is a gram-negative rod-shaped bacterium normally found in the large intestine. However, *E. coli* can migrate through the papilla of Vater into the bile ducts and establish infection of the gallbladder. Specific virulent strains of *E. coli* contain genes that allow them to adhere to and colonize the gallbladder epithelium. *Klebsiella* bacteria are also gram-negative and normally found in the gastrointestinal tract. Like *E. coli*, *Klebsiella* has virulence factors that enable it to spread to the biliary system (Hadano and Hijikata 2023). *Klebsiella* is more frequently associated with severe necrotizing cholecystitis compared to *E. coli*. Enterococcus is a gram-positive cocci species that is a common cause of hospital-acquired infections. Biliary instrumentation and procedures can introduce Enterococcus into the gallbladder and cause infectious cholecystitis, especially in immunocompromised patients (Hara et al. 2021). Culture of these bacterial organisms from bile or gallbladder tissue confirms the diagnosis of acute bacterial cholecystitis. Molecular techniques like PCR allow for rapid identification of cholecystitis-causing bacteria. Understanding the key bacterial players in infectious cholecystitis guides appropriate antibiotic therapy when medical management is undertaken. Further research into novel vaccines or probiotic approaches may help prevent bacterial colonization and subsequent infection of the gallbladder.

Parasites

In addition to bacteria, parasitic organisms can also cause infectious cholecystitis. Intestinal helminth infections are the main parasitic cause of acute cholecystitis globally. The roundworm *Ascaris lumbricoides* and the liver fluke *Clonorchis sinensis* are the two most common parasites implicated in parasitic cholecystitis (Addissouky et al. 2024a). *Ascaris lumbricoides* is one of the largest nematode parasites that infect humans. After ingestion, larvae migrate through the gastrointestinal tract and into bile ducts. Their large size can lead to obstruction of the cystic duct and subsequent cholecystitis. Eggs released by mature Ascaris worms can also incite inflammation and scarring. Ascaris is prevalent in tropical developing regions and is estimated to infect over 1 billion people worldwide (Canes et al. 2022). *Clonorchis sinensis* is a small trematode parasite that inhabits the bile ducts of the liver. Chronic infection causes progressive inflammation and fibrosis of the biliary tree. *Clonorchis* infections are acquired by ingesting undercooked freshwater fish. Regions with highest prevalence include Southeast Asia and Korea (Wang et al. 2023). Both *Ascaris* and *Clonorchis* cholecystitis result from direct obstruction and irritation of the biliary system by migrating parasites. Anti-parasitic drugs like albendazole, praziquantel, and nitazoxanide may help resolve parasite-induced cholecystitis. However, cholecystectomy is sometimes still required for definitive treatment. Further public health initiatives promoting mass deworming, improved sanitation, and meal education can help reduce global burdens of parasitic cholecystitis.

Fungi

While less common than bacteria and parasites, fungal infections can also contribute to some cases of acute cholecystitis. Of the medically relevant fungi, *Candida*

species are the most frequently isolated from patients with acalculous cholecystitis (Dyrhovden et al. 2020). *Candida albicans* is the predominant cause of fungal cholecystitis. Candida is a commensal organism that can transform into an opportunistic pathogen in susceptible individuals. Those at highest risk include patients undergoing cancer chemotherapy, organ transplant recipients on immunosuppressive medications, and severely ill patients in intensive care units. Additional risk factors like diabetes, parenteral nutrition, and broad-spectrum antibiotics further increase susceptibility to invasive *Candida* infections (Sharma and Chakrabarti 2023). Disseminated candidiasis allows seeding of yeast into the gallbladder wall and bile. Adherence factors like biofilms and secreted aspartyl proteinases enable Candida to colonize and invade the gallbladder epithelium. The resulting cellular damage, inflammation, and bile stasis culminate in acute acalculous cholecystitis. Patients often display fever, right upper quadrant pain, and may have concurrent candidemia or hepatosplenic candidiasis (Eshima et al. 2022). Early detection of fungal cholecystitis through blood cultures and radiographic imaging helps guide antifungal therapy. The azole class of antifungals, particularly fluconazole, is first-line for treatment. However, severe cases may ultimately require cholecystectomy. Further research into novel antifungal medications and preventative vaccination continues in order to improve outcomes for this serious form of infectious cholecystitis (Addissouky, et al. 2024).

Virulence factors of microbes

Adhesion proteins, lipid polysaccharides-attachment to epithelium

Critical to the ability of bacteria, parasites, and fungi to establish infection of the gallbladder is their expression of specific virulence factors that enable adhesion to epithelial cells lining the gallbladder mucosa. These infectious microbes produce various surface proteins and polysaccharides that mediate binding to the host epithelium, representing an essential first step in pathogenesis (Abril et al. 2022). Bacteria such as Escherichia coli and *Klebsiella* express hair-like projections on their cell surface called pili or fimbriae. The tips of these pili contain adhesins that bind to complementary receptors on epithelial cells as depicted in Table 1. Examples include P fimbriae of uropathogenic *E. coli* strains and Type 1 fimbriae of *Klebsiella*. In addition, bacterial lipopolysaccharides facilitate adhesion and anchor other proteins involved in invasion (Geurtsen et al. 2022). Parasites like *Clonorchis* release secretory glycoproteins that attach to host cells. The fluke also relies on oral and ventral suckers to maintain adhesion. For *Candida* fungal species, secreted hydrolase enzymes and yeast cell wall glycoproteins known as adhesins allow stable attachment to gallbladder epithelial cells resistant to clearance (Fröhlich 2022).

Toxins-damage/disrupt cell membranes

In addition to adhesion, many pathogens produce toxins that directly damage and disrupt the epithelial cell membranes of the gallbladder. These pore-forming toxins represent another key virulence factor contributing to acute cholecystitis. Recent studies show cholecystectomy may promote colorectal cancer by altering bile acid metabolism and gut microbiota. More research is needed on the carcinogenic effects of secondary bile acids and microbes post-cholecystectomy. Targeted therapies regulating bile acids and microbiota may represent promising strategies for preventing colorectal cancer after gallbladder removal (Jiang et al. 2022). Bacteria release exotoxins that target and permeabilize host cell membranes. For example, *E. coli* produces a toxin called hemolysin that forms large pores in cell membranes, leading to cytolysis. Other pore-forming toxins released by cholecystitis-causing bacteria include streptolysin O and pneumolysin. The Gram-positive bacterium *Enterococcus faecalis* generates a toxin called cytolysin with membrane-disrupting activity

Table 1 Major microbial pathogens implicated in infectious cholecystitis

Pathogen	Key virulence factors	Antibiotic susceptibility
Escherichia coli	Adhesins (P fimbriae), α-hemolysin toxin, lipopolysaccharide	Ampicillin, cephalosporins, fluoroquinolones, aminoglycosides
Klebsiella pneumoniae	Adhesins (Type 1 fimbriae), polysaccharide capsule, lipopolysaccharide	Cephalosporins, carbapenems, fluoroquinolones, tigecycline
Enterococcus faecalis	Cytolysin toxin, aggregation substance adhesin, extracellular superoxide	Ampicillin, vancomycin, linezolid
Candida albicans	Adhesins (ALS family), hydrolase enzymes, secreted aspartyl proteases	Fluconazole, amphotericin B, echinocandins
Ascaris lumbricoides	Proteinases, hemolysin, collagenase	Albendazole, ivermectin, nitazoxanide
Clonorchis sinensis	Proteases, lysophospholipase, cathepsin B	Praziquantel, albendazole, nitazoxanide

(González et al. 2022). Parasites also induce cell lyses through various secretory molecules. *Ascaris* releases proteinases that degrade cell membranes. *Clonorchis* produces a lysophospholipase that disrupts membrane integrity. *Candida* fungal species secrete phospholipases and membrane-damaging peptides like candidalysin (Qiao et al. 2023). The pore-forming toxins liberated by these pathogens allow spillage of cell contents, calcium influx, loss of membrane potential, and ultimately host cell death. This direct cytotoxicity coupled with the inflammatory response to the microbial toxins leads to the tissue damage, edema, and necrosis observed in acute cholecystitis (Addissouky et al. 2024b). Further characterization of the specific toxin families will elucidate their mechanisms of membrane disruption. This understanding may help identify therapeutic inhibitors and stimulate development of toxoid vaccines. Additional research into microbial toxins and their role in acute cholecystitis pathogenesis remains an impactful area of study (Addissouky et al. 2024c).

Enzymes-breakdown cell structures/extracellular matrix

Pathogenic microbes involved in acute cholecystitis produce a variety of extracellular enzymes that directly breakdown and destroy host cell structural components and the surrounding extracellular matrix. These degradative enzymes facilitate tissue invasion and damage during infection (Xu et al. 2022). Bacteria such as *Klebsiella* pneumoniae secrete proteases like collagenase that cleave collagen, a major structural protein in connective tissue. Collagen degradation weakens the integrity of the gallbladder wall. Pseudomonas aeruginosa releases elastase and alkaline protease enzymes that break down elastin and other proteins comprising the matrix. Bacterial hyaluronidases degrade hyaluronic acid to spread through tissue planes (Ghosh et al. 2022). Helminth parasites also express degradative enzymes. *Ascaris lumbricoides* secretes a collagenase that digests collagen fibers to permit tissue migration. *Clonorchis sinensis* produces proteases like cathepsin B that dissolves extracellular matrix proteins (Sun et al. 2022). For *Candida* fungal species, secreted aspartic proteases (Sap) and phospholipases facilitate tissue invasion and damage during disseminated candidiasis infections. The Saps degrade structural proteins like collagen and mucin. The phospholipases damage cell membranes and aid in nutrient acquisition (Travail et al. 2023).

Microbial processes within cells

Transcription, translation of virulence factors

The production of virulence factors by bacteria, parasites, and fungi causing cholecystitis involves coordinated transcription and translation of the genes encoding these pathogenicity proteins. Understanding the molecular mechanisms regulating virulence factor expression provides insight into microbial triggers for initiating cholecystitis. Pathogens sense specific signals in the biliary tract, such as bile salts, pH, or lipid composition, indicating their presence within the gallbladder. These environmental cues activate bacterial transcription factors that bind promoter regions on virulence genes to upregulate mRNA synthesis. For example, the ToxT regulator of Vibrio cholerae drives transcription of genes encoding cholera toxin and the TCP pilus during small intestine colonization. Following transcription, the host-induced microbial mRNAs are translated by ribosomes into the amino acid sequences comprising functional virulence factors like adhesins, toxins, and proteases. The coordinated increase in virulence protein production equips the pathogens to attach to gallbladder epithelial cells, degrade tissue barriers, evade immune clearance, and establish infection (Addissouky et al. 2024d).

Metabolism to obtain nutrients from host cells

For microorganisms to proliferate and thrive within the nutrient-limited environment of the gallbladder, they must adapt their metabolism to obtain essential nutrients from host cells. Bacteria, parasites, and fungi infecting the gallbladder epithelium scavenge various nutrients to support their growth and survival. Bacteria upregulate transport systems to import sugars, amino acids, nucleotides, and other metabolites from the cytoplasmic contents of lysed host cells. *Klebsiella* pneumoniae induces transporters for myo-inositol when growing in epithelial environments. *Salmonella enterica* serovar Typhimurium produces permeases to import amino acids liberated from host proteins degraded by secreted proteases (Portincasa et al. 2023). Helminths like *Clonorchis sinensis* metabolize host lipids as an energy source through beta oxidation and oxidative phosphorylation pathways. The parasite also derives glucose from host glycogen stores to fuel glycolysis. For *Candida albicans*, secreted aspartyl proteases generate amino acids that are transported into the fungal cell for protein biosynthesis (Kim et al. 2022).

Secretion of toxins/enzymes

The secretion of toxins and enzymes is a key virulence strategy used by diverse microbes to establish infection and cause damage to the gallbladder epithelium. Advances in cell biology and molecular techniques have provided greater understanding into the complex protein trafficking pathways used by bacteria, parasites, and fungi to release these pathogenic factors (Sato et al. 2003). Following translation, bacterial toxins and enzymes are exported across the inner and outer membrane in

a highly orchestrated process. Two major secretion systems utilized are type II, which directly transports proteins from the cytoplasm to the extracellular space, and type III, which injects toxins directly into host cells (Jouault et al. 2022). For example, *Pseudomonas aeruginosa* uses a type III system to inject the cytotoxin ExoU into epithelial cells where it disrupts phospholipid membranes (Silistre et al. 2021). Parasitic helminths often rely on exocytosis from gland cells to release proteins. The parasitic trematode *Schistosoma mansoni* secretes over 300 proteins involved in immune evasion, tissue penetration, and host cell modulation. *Clonorchis* liver flukes release excretory/secretory products like proteases through exocytic mechanisms (Hambrook and Hanington 2021). In fungal *Candida* species, the secreted aspartyl proteinase (Sap) virulence factors are transported out of the cell via secretory vesicles. Saps are released during the colonization of host tissues like the gallbladder. Understanding secretion mechanisms allows for development of inhibitors. For example, miltefosine is an anti-parasitic that impairs exocytosis (Knuplez et al. 2021).

Host response in cholecystitis

Figure 1 illustrates the key roles of neutrophils and T cells in the pathogenesis of cholesterol gallstone formation. Cholesterol or calcium crystals present in gallbladder bile are taken up by neutrophils, causing release of lytic enzymes and extracellular chromatin that forms web-like neutrophil extracellular traps (NETs). These NETs aggregate and bind cholesterol crystals together, acting like a "glue" that promotes gallstone growth. Additionally, the joint effects of T cells and cholesterol crystals induce mucin gene expression and accumulation of mucin gel in the gallbladder epithelium, further facilitating cholesterol gallstone formation. T cells and cholesterol crystals also stimulate release of pro-inflammatory cytokines like IL-1β, IFNγ, and TNFα, resulting in gallbladder inflammation, tissue damage, and dysfunction—changes that predispose to cholesterol gallstone development (Jiao et al. 2022). Overall, the figure summarizes how neutrophils and T cells, through release of NETs, cytokines, and inducing mucin production, play integral roles in the pathogenic processes leading to cholesterol gallstone formation.

Inflammation and immune mechanisms

The host responds to acute cholecystitis through a coordinated innate and adaptive immune response aimed at clearing the infectious pathogens and limiting tissue damage. Advances in immunology have elucidated the intricate molecular signaling, recruitment of immune cells, and secretion of inflammatory mediators that comprise the host reaction (Javed et al. 2022). Infection and obstruction of the gallbladder activate resident macrophages and epithelial cells to secrete pro-inflammatory cytokines including IL-1, IL-6, and TNF-alpha. These cytokines initiate a localized inflammatory response and recruit additional leukocytes. Neutrophils are the first responders, followed by monocytes that differentiate into macrophages at the site of infection (Pyfrom et al. 2023). Macrophages and neutrophils

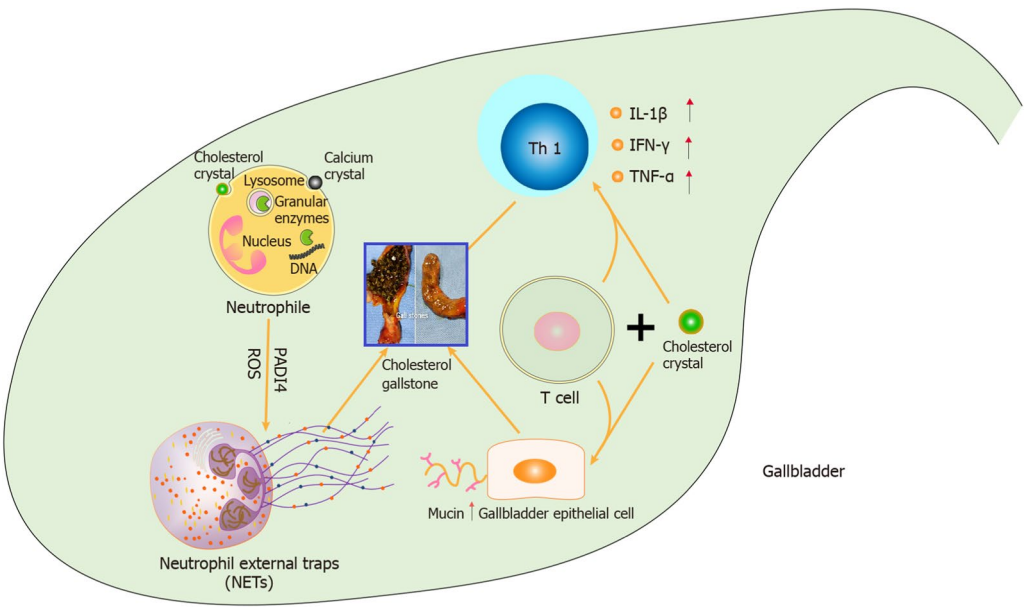

Fig. 1 The role of immune cells in gallstone formation

phagocytose bacteria, while releasing reactive oxygen species and antimicrobial peptides to kill pathogens. Dendritic cells process microbial antigens and present them to T-cells, triggering adaptive immunity (Panni et al. 2022). Activated T-cells secrete cytokines like IFN-gamma that further coordinate the immune response. B-cells are stimulated to produce opsonizing antibodies targeting the invading pathogens (Cao et al. 2023). While necessary to clear infection, excessive inflammation can damage host tissues. Therapies that modulate specific cytokine pathways or block particular immune cell subsets may help reduce inflammation during severe or chronic cholecystitis. Further research into optimizing the host immune response continues, with the goal of efficaciously battling infection while minimizing collateral damage (Addissouky et al. 2023a).

Release of cytokines

Cytokines are key signaling proteins that orchestrate the host inflammatory and immune response to microbial infections causing acute cholecystitis. Advances in cytokine research have defined the specific mediators produced during gallbladder infection and their myriad downstream effects (Ozdemir 2023). Among the first cytokines released by resident macrophages and epithelial cells are pro-inflammatory cytokines like interleukin-1beta (IL-1β), IL-6, and tumor necrosis factor-alpha (TNF-α). These cytokines help initiate and amplify the local inflammatory response. IL-1β and TNF-α act on endothelial cells to increase expression of cellular adhesion molecules that recruit neutrophils and monocytes to the site of infection (Akbaraliev et al. 2022). As inflammation progresses, anti-inflammatory cytokines like IL-10 are produced to counterregulate the pro-inflammatory mediators. T-helper 1 (Th1) cytokines, including interferon-gamma, interleukin-12, and TNF-α predominate during acute cholecystitis, activating cellular immunity against pathogens (Hu et al. 2023). Excessive or prolonged cytokine responses can damage host tissues through sustained inflammation and immunopathology (Feng et al. 2023). Identifying key cytokine targets for immunomodulatory drugs may help temper the damaging effects of acute inflammation. Biologic anti-cytokine therapies have proven effective for some chronic inflammatory conditions like inflammatory bowel disease. Similar approaches may improve outcomes for complicated cases of acute infectious cholecystitis.

Migration of neutrophils/macrophages

The directed migration of neutrophils and macrophages to the site of infection is a crucial component of the innate immune response to acute cholecystitis. Advances in cellular immunology have elucidated the tightly coordinated mechanisms facilitating this rapid leukocyte movement. Release of cytokine signals like interleukin-1 and chemokines such as CCL2 and CXCL8 establishes a chemical gradient that guides neutrophil migration from the bloodstream into infected tissues. Additional cytokines trigger a conformational change in integrins on the neutrophil surface that increases their affinity for cellular adhesion molecules displayed on activated endothelial cells. This multi-step cascade allows neutrophils to adhere to vascular endothelium and migrate into the interstitial space (Akhtar et al. 2023). Once in infected tissues, neutrophils follow cytokine and chemokine signals toward the invading microbes. Strategies to block leukocyte integrins or inhibit cytokine/chemokine activity can slow this migration process (Lindsberg et al. 1996). Circulating monocytes migrate into infected tissues by similar mechanistic pathways before differentiating into macrophages. Migratory macrophages can then fuse to form multinucleated giant cells that engulf and destroy larger pathogens (Addissouky et al. 2023b).

Phagocytosis of microbes

Phagocytosis of invading pathogens by neutrophils and macrophages represents a key defense mechanism activated during acute cholecystitis. Advances in cell biology have revealed intricate details of the receptor-mediated steps facilitating microbial engulfment and ensuing destruction within the phagolysosome. Phagocytes express various surface receptors that recognize and bind to conserved molecular patterns on microbes, initiating the ingestion process. Opsonins like antibodies and complement proteins coat the microbial surface and engage Fc and complement receptors to stimulate phagocytosis (Sajjanar and Vagha 2023). Once bound, actin cytoskeletal rearrangements generate the forces needed to internalize the pathogen within a vesicle termed a phagosome. The phagosome then fuses with lysosomes loaded with degradative enzymes, reactive oxygen species, and antimicrobial peptides to destroy the ingested microbe (Pietrzak et al. 2023).

Effects on gallbladder

Swelling, epithelial damage

The inflammation and obstruction accompanying acute cholecystitis induces significant effects on the gallbladder anatomy and function. Imaging techniques and histological studies have detailed the structural changes occurring in the gallbladder wall and mucosal epithelium (Mossaab et al. 2022). Edema caused by the released inflammatory mediators, coupled with increased pressure from bile stasis, leads to marked swelling and distension

of the gallbladder. This is evident on ultrasound exams, which show gallbladder wall thickening of over 3–4mm during acute cholecystitis (Miller et al. 2022). The normally functioning columnar epithelium lining the mucosa becomes damaged from the retention of concentrated bile salts, pressure necrosis, and direct cytotoxicity from microbial toxins. Epithelial sloughing, ulceration, and hemorrhage are visible microscopically. Severe cases cause full-thickness gallbladder necrosis (Arnott et al. 2023). Improved understanding of the timeline of histopathological changes during acute cholecystitis can aid diagnosis. Identifying factors that prevent excessive edema and epithelial damage could help maintain gallbladder function in early or mild cases.

Gallstones formation

The pathogenesis of acute cholecystitis is intimately linked to the development of gallstones, which obstruct bile drainage and initiate gallbladder inflammation. Advanced imaging and analytical techniques have provided greater insight into the specific mechanisms of gallstone formation and growth. Gallstones typically form when bile becomes supersaturated with cholesterol, bilirubin, or calcium salts. Hypersecretion of cholesterol from the liver and impaired gallbladder motility contribute to cholesterol stone development. Bilirubin stones form during hemolytic diseases when excessive bilirubin is excreted into bile (Fujita et al. 2021). Once nucleated, stones enlarge over months to years through deposition of crystalline material from bile. Calcium bilirubinate, palmitate, and carbonate facilitate calcium salt crystal growth. The presence of mucin gel and biofilms on the gallstone surface also enhance stone enlargement (Zdanowicz et al. 2022). As stones grow larger, they can occlude the cystic duct and prevent bile emptying, leading to gallbladder distension, inflammation, and acute cholecystitis as depicted in Table 2.

Bile stasis, jaundice

The obstruction of bile drainage from the gallbladder that occurs in acute cholecystitis has two major detrimental effects-bile stasis within the gallbladder and jaundice due to the backup of bile constituents. Advanced imaging and hepatic function testing allows for precise definition of these secondary effects. Bile stasis leads to distension and inflammation of the gallbladder, causing right upper quadrant pain. Ultrasound findings of a distended gallbladder filled with sludge or stones confirms blocked bile flow. Positron Emission Tomography (PET) scans also show impaired gallbladder emptying function during cholecystitis (Liu et al. 2023). Obstruction of the larger bile ducts draining from the liver causes back up of bilirubin, leading to hyperbilirubinemia and the clinical manifestation of jaundice, or yellowing of the skin and sclera. Elevated conjugated bilirubin on liver function tests accompanies this finding (Nve et al. 2023). If prolonged, bile duct obstruction and jaundice can cause liver damage. Early relief of blockages is important to prevent sequalae. The mechanisms underlying both stasis and jaundice must be considered when tailoring therapeutic approaches aiming to restore normal bile flow in cholecystitis patients (Han et al. 2022).

Biotechnology applications

The advancement of biotechnology and molecular techniques has provided powerful new tools to enhance the diagnosis, characterization, and treatment of acute cholecystitis as illustrated in Table 3. Ongoing research continues to uncover novel biotechnology-driven approaches aimed at combating this disease. Gene therapy techniques using CRISPR/Cas9 systems or RNAi may selectively edit microbial genomes to disable virulence mechanisms. Blocking pathogen toxin secretion pathways is another avenue under exploration. Further advances in biotechnology hold enormous potential for improving cholecystitis prevention, diagnosis, and treatment. Biosensor technology shows promise for point-of-care diagnostic applications. Implantable microchips containing sensors for inflammatory mediators may enable real-time monitoring of infection (Zheng et al. 2023). Nanoparticle platforms can deliver targeted antimicrobials and anti-inflammatories as illustrated in Fig. 2.

Table 2 Outlining the typical timeline of histopathological changes in the gallbladder during acute cholecystitis progression

Timepoint	Histopathological changes
< 24 h	Edema, dilated blood vessels in mucosa and submucosa
24–48 h	Epithelial sloughing, subepithelial hemorrhage, neutrophil infiltration
2–4 days	Full thickness inflammation, ulceration, abscess formation
5–7 days	Transmural necrosis, vascular thrombosis, mucosal ulceration
2 + weeks	Chronic inflammation, fibrosis, gallbladder wall thickening

Table 3 Enumerating emerging biotechnology tools for cholecystitis diagnosis, treatment, and prevention

Category	Technology	Applications
Diagnostics	PCR, NGS sequencing	Rapid pathogen identification, antimicrobial resistance profiling
Diagnostics	Microarrays, multiplex PCR	High-throughput microbial detection
Diagnostics	LAMP, microfluidic PCR	Point-of-care rapid molecular diagnostics
Diagnostics	Biosensors, microchips	Real-time monitoring of infection
Therapeutics	Phage therapy	Targeted antimicrobial therapy
Therapeutics	RNAi, CRISPR	Silencing microbial virulence genes
Therapeutics	Probiotics	Competitive exclusion of pathogens
Therapeutics	Nanoparticles	Targeted delivery of antimicrobials
Prevention	Vaccines	Blocking adhesion, neutralizing toxins
Prevention	Microbiome modulation	Restoring homeostatic balance
Monitoring	Digital health apps	Patient symptom tracking and care coordination

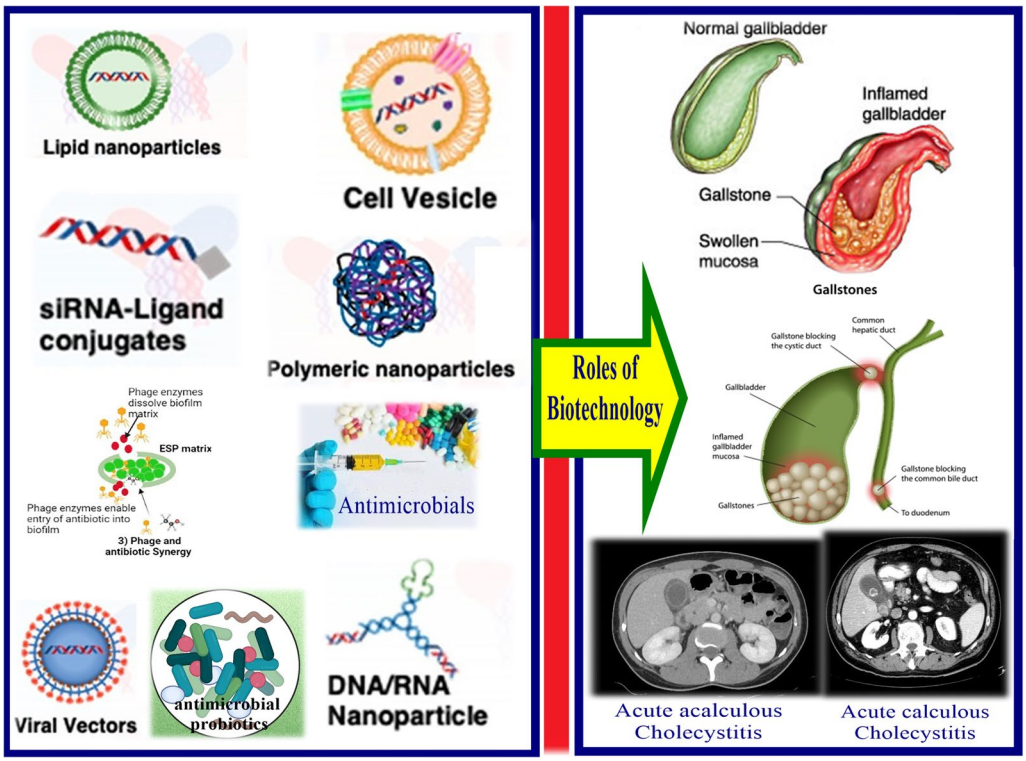

Fig. 2 Biotechnology applications versus cholecystitis

Molecular techniques

Molecular detection methods like PCR allow rapid, sensitive identification of bacterial, parasitic, and fungal pathogens in gallbladder tissues. Genetic sequencing characterizes virulence factors and drug resistance profiles. Fluorescent in situ hybridization (FISH) visually localizes pathogens within tissues.

PCR detection of microbial DNA

Polymerase chain reaction (PCR) allows rapid, sensitive detection of microbial DNA from clinical specimens like gallbladder tissue or bile. This aids diagnosis of the infectious agents causing cholecystitis. Broad-range PCR utilizes universal primers targeting conserved genes like 16S rRNA present across bacterial, fungal, or

viral genomes. This amplifies DNA from any pathogen without needing to specify the organism. Multiplex PCR combines multiple primer sets in one reaction to test for different microbes simultaneously (Kakizaki et al. 2023). Real-time quantitative PCR (qPCR) also measures the amount of microbial DNA. This provides a quantitative measure of bacterial, fungal or viral load. High pathogen loads correlate with disease severity. Specific PCR primers can also be designed to identify individual species. For example, qPCR primers targeting the Invasion Plasmid Antigen H (ipaH gene) accurately identify Shigella. This degree of specificity aids in implicating disease etiology. PCR greatly enhances sensitivity of detection compared to culture methods. Even fastidious, difficult to culture organisms are identifiable by PCR. It also detects non-viable or dead microbes (Dan et al. 2023). Rapid molecular techniques like loop-mediated isothermal amplification are being explored to further reduce PCR assay times to less than an hour. Point-of-care PCR diagnostics enable actionable results at the bedside.

Sequencing for pathogen identification

Sequencing of microbial DNA extracted from gallbladder tissues or bile can definitively identify pathogens involved in cholecystitis to the strain level. Broad-range PCR first amplifies conserved bacterial, fungal, viral, or parasitic genomic regions. Amplicons are then sequenced using platforms like Sanger, pyrosequencing, or Illumina. Sanger sequencing provides reads of 400–1000 base pairs from individual DNA fragments. Pyrosequencing generates hundreds of thousands of short reads (~ 200 bp) in parallel. Illumina produces enormous datasets of billions of reads enabling high resolution. Sequences are compared to large genomic databases to match organisms (Poudel et al. 2023). Next-generation sequencing allows detection of multiple microbes in a sample. It identifies difficult-to-culture and fastidious organisms missed by conventional cultures. Analysis of whole microbial communities (microbiome) is possible with metagenomic sequencing. This reveals compositional dysbiosis associated with cholecystitis. Bioinformatics tools annotate sequences, assemble genomes, and analyze phylogenetic relationships. In-depth strain characterization is achievable. Sequencing also detects antimicrobial resistance and virulence genes aiding treatment (Tohya et al. 2022). Portable nanopore sequencers are emerging for rapid near-patient testing. These can diagnose cholecystitis microbes within hours. However, challenges include complex sample preparation, sequencing errors, and data analysis.

Antimicrobials

Antibiotics tailored to microbial profiles

Selecting optimal antibiotic therapy for cholecystitis requires accurately identifying the causative pathogens using advanced diagnostic techniques. Culture-independent methods like PCR and DNA sequencing can rapidly detect microbial species and strains from gallbladder tissues and bile. This guides selection of first-line antibiotics that specifically target the organisms involved. Susceptibility testing via automated platforms like VITEK 2 and Phoenix provides antibiotic resistance profiles of isolated pathogens within hours. Molecular techniques like microarrays and PCR can identify antibiotic resistance genes. Whole genome sequencing fully elucidates the resistome. These approaches facilitate selecting antibiotics to which the pathogens are susceptible. Narrow spectrum antibiotics are preferred when the etiology is defined to avoid gut dysbiosis (Lee et al. 2023). For empirical therapy before diagnostic results, combination broad-spectrum antibiotics may be used to provide adequate coverage. Carbapenems, piperacillin-tazobactam, and extended spectrum penicillins target gram negatives like *E. coli* and *Klebsiella*. Vancomycin is added for Enterococcus coverage. Adjustments are made once culture results become available. Dosing regimens are optimized based on pharmacokinetic-pharmacodynamic principles to maximize antibiotic efficacy. Therapeutic drug monitoring helps ensure adequate antibiotic concentrations. Duration of therapy is tailored to clinical response but generally 7–10 days. Follow-up cultures assess microbial eradication (Yoon et al. 2022).

Phage therapy

Phage therapy utilizes lytic bacteriophages, viruses that infect and lyse bacterial cells, to treat bacterial infections like cholecystitis. Each phage strain is specific for certain bacterial species or strains. To develop phage therapy for cholecystitis, individual phages or cocktails targeting the major pathogens like *E. coli*, *Klebsiella*, and Enterococcus can be created. Candidate phages are isolated from environmental sources and screened to select those with desired bacterial host range and potent lytic activity. Genome sequencing and bioinformatics of the phage isolate determines its bacterial targets (Lin et al. 2017). Phages can be administered by oral, intravenous, or site-directed routes to access the gallbladder. A single dose is often sufficient as phages amplify themselves at the infection site. Phages penetrate biofilms and lyse bacteria using lysins and holins to degrade cell walls and membranes (Doss et al. 2017). This rapid lytic action provides a high-level bactericidal effect.

Additionally, phages are self-replicating with exponential amplification until the bacterial target is eliminated. This overcomes limitations of antibiotic concentration decline (Wei et al. 2020). Compared to antibiotics, phages are highly specific without disruption of commensal microbiota. Phages show synergy with antibiotics and can overcome antibiotic-resistant pathogens. They have minimal toxicity since human cells lack receptors for bacteriophage attachment and entry. No serious adverse effects have been reported in clinical trials. However, anti-phage immune responses can affect efficacy requiring careful pharmacokinetic studies. High-titer phage preparations and engineered phages are being developed to improve efficacy (Rogovski et al. 2021).

Gene silencing

siRNA to block virulence factor expression

Small interfering RNAs (siRNAs) show promise for treating cholecystitis by silencing the expression of key microbial virulence factors. siRNAs are short double-stranded RNAs that induce sequence-specific degradation of complementary mRNAs. This inhibits translation of target proteins. siRNAs can be designed to specifically match and degrade mRNAs encoding virulence factors critical for cholecystitis pathogenesis including bacterial adhesins, toxins, secreted proteases, and lipopolysaccharides. Knocking down these factors impairs microbial adherence, toxicity, tissue invasion, and inflammation (Wołowiec et al. 2023). Chemically modified siRNAs have been developed to optimize stability, potency, and prolong activity when administered. Cationic lipids or nanoparticles are used as carriers for intracellular delivery of siRNAs into target cells. Gallbladder epithelium cells can take up formulated siRNAs to block pathogen gene expression occurring internally (Kola et al. 2024).

High-throughput screening enables rapid in vitro identification of optimal siRNA sequences. Genome-scale siRNA libraries targeting all microbial genes are used to find key targets. Lead candidates are then tested in vivo to confirm virulence attenuation without resistance developing (Quintero-Ruiz et al. 2024). Challenges for therapeutic use include off-target effects, variability in delivery/uptake, and adequate dosing. Combining siRNAs against multiple virulence genes improves overall efficacy. Further research into targeted in vivo delivery methods may help translate this approach into viable clinical treatments.

CRISPR disruption of toxin secretion

The CRISPR-Cas system provides a promising approach to block bacterial toxin secretion and pathogenesis in cholecystitis. CRISPR allows precise gene editing and disruption in microorganisms. The type II CRISPR-Cas9 method uses a guide RNA to target the Cas9 nuclease to specific genomic sites for cleavage. This can be used to knock out toxin secretion genes in pathogens like *E. coli* and *Klebsiella*. Disrupting these key virulence factors inhibits their ability to damage gallbladder epithelial cells (Liu et al. 2024). For example, deleting a toxin transporter gene in *E. coli* prevents secretion of Shiga toxin which induces gallbladder injury. CRISPR interference by dCas9 can also repress toxin gene transcription without cutting DNA. This avoids potential for resistance. Phage-delivered CRISPR systems have been engineered that infect and directly eliminate toxin gene expression in pathogens present in the gallbladder. Theseprogrammable "smart" phages act as antimicrobials (Gliźniewicz et al. 2024). Challenges include efficient delivery of CRISPR components into target microbes in vivo. Advanced nanocarriers are being developed that protect CRISPR elements from degradation and ferry them into cells. Combining CRISPR with select antibiotics may augment overall impact.

Probiotics

Probiotic administration shows promise as a therapeutic strategy for cholecystitis by promoting growth of beneficial microbes that can outcompete pathogens.

Competition with pathogens

Probiotic strains like *Lactobacillus* and *Bifidobacterium* are selected based on their ability to directly inhibit gastrointestinal pathogens like *E. coli* and *Klebsiella*. Mechanisms include production of antimicrobial compounds, lowering pH, competing for nutrients and adhesion sites, and modulating host immunity (Kim et al. 2023). Introducing high doses of probiotic bacteria can shift the microbial composition of the gut and gallbladder away from a dysbiotic state back toward homeostasis. The "good" bacteria crowd out opportunities for pathogen overgrowth and biofilm formation on the gallbladder epithelium. Pre-operative probiotic treatment in cholecystitis patients has been shown to significantly reduce numbers of pathogenic Enterobacteriaceae adhered to the gallbladder wall compared to controls. Less viable pathogens leads to reduced risk of acute inflammation and postoperative infections (Kullar et al. 2023). However, clinical outcomes depend on using well-characterized probiotic strains with proven efficacy. *Lactobacillus* GG and *Saccharomyces boulardii* supplementations have shown particular promise. Further research is still needed to optimize probiotic protocols and assess long-term impacts.

Production of antimicrobial compounds

Many probiotic bacteria generate antimicrobial substances that inhibit pathogens implicated in cholecystitis such as *E. coli* and *Klebsiella*. *Lactobacillus* probiotics secrete a range of antimicrobials including organic acids, hydrogen peroxide, and bacteriocins. The lactic and acetic acid produced lower local pH, disrupting pathogen membrane function and protein activity. Bacteriocins like nisin, plantaricin, and acidolin act as toxins that form pores in bacterial membranes, deplete ATP, and block cell wall synthesis. Genomic analysis has revealed numerous bacteriocin biosynthetic gene clusters in *Lactobacillus* (Karimi et al. 2018). *Bifidobacterium* species also generate organic acids and bacteriocins with broad antimicrobial efficacy. Postbiotic supernatants from *Bifidobacterium* longum cultivation demonstrate potent anti-*E. coli* effects (Vincenzo et al. 2023). The antimicrobial arsenal produced by probiotics varies widely by strain. Combining multiple complementary probiotic strains can provide greater pathogen inhibition through synergistic mechanisms. Timed-release encapsulation technologies help deliver viable probiotics to the gallbladder (Tang et al. 2023). Probiotic antimicrobial compounds likely contribute to reductions in acute and postoperative cholecystitis infections. However, quantification of local probiotic metabolite production and impact on pathogen load in vivo is needed.

Innovative tools for managing cholecystitis

As listed in Table 4, Laparoscopic cholecystectomy is the current gold standard surgery for cholecystitis. Emerging tools like robotics, NOTES, and 3D imaging aim to make surgery even less invasive. Fluorescence aids visualization while new energy devices enhance safety. AI and data science technologies provide real-time augmented intelligence. Ongoing advances continue to minimize morbidity and improve outcomes.

Herbal and traditional medicine

Various herbal medicines and traditional remedies have been used to treat cholecystitis symptoms for centuries. Traditional Chinese Medicine utilizes herbal formulations containing plants such as dandelion, chamomile, chicory, and milk thistle to help relieve inflammation and pain associated with cholecystitis. These herbs contain bioactive compounds that exert anti-inflammatory, antispasmodic, and analgesic effects on the gallbladder (Addissouky et al. 2024e). In Ayurvedic medicine, herbs like ginger, turmeric, triphala, and aloe vera are commonly used to improve gallbladder function and reduce bile stagnation in cholecystitis. Triphala has exhibited antibacterial effects against enteric pathogens in vitro (Naseri et al. 2022). Homeopathy uses highly diluted natural substances to stimulate self-healing mechanisms. Homeopathic remedies like *Carduus mar*, *Chelidonium*, and *Lycopodium* are sometimes prescribed to alleviate cholecystitis symptoms. However, clinical evidence for homeopathy remains limited. While some herbs and traditional formulations show promising anti-inflammatory, antispasmodic, and antimicrobial effects relevant to cholecystitis in preliminary studies, high-quality randomized controlled trials are still needed to validate efficacy and safety in humans (Chen et al. 2019). Potential concerns with herbal remedies include variable quality control, lack of standardization in active compounds, and risks of toxicity or herb-drug interactions. Consultation with a knowledgeable practitioner is advised.

Stem cells

Stem cell therapy is an emerging approach undergoing investigation for reducing inflammation and restoring gallbladder function in cholecystitis. Several preclinical studies have assessed mesenchymal stem cells (MSCs) derived from bone marrow, adipose tissue, or umbilical cord blood for their immunomodulatory effects in animal models of cholecystitis. MSCs appear to home to

Table 4 Current surgical techniques and novel innovations for treating cholecystitis

Category	Technique	Description
Current standard	Laparoscopic cholecystectomy	Minimally invasive gallbladder removal using laparoscopic tools and cameras
Robotic surgery	Robotic cholecystectomy	Increased dexterity and vision with wristed robotic instruments like da Vinci system
Natural orifice transluminal endoscopic surgery (NOTES)	Transgastric gallbladder removal	Incisionless removal through natural orifices like the mouth or vagina
Visualization aids	Fluorescence cholangiography	Enhanced bile duct imaging using near-infrared fluorescent dyes
Energy devices	Ultrasonic shears, bipolar sealers	Precision dissection and hemostasis with less bile/blood leakage
3D imaging	3D laparoscopy	Depth perception and spatial orientation improved with 3D displays
AI integration	Smart robotics, image analysis	Augmented intelligence to enhance surgical judgment and performance
Analytics	Surgical data science	Patient risk modeling and prediction of optimal surgical approaches

injured gallbladder tissues and secrete anti-inflammatory cytokines that suppress gallbladder damage (Wang et al. 2022a). In addition to modulating host immune responses, MSCs may help repair and regenerate the damaged gallbladder epithelium based on their capacity to differentiate into tissue-specific cell types. However, definitive evidence for reconstitution of functional gallbladder tissue by transplantation of exogenous MSCs remains limited (Rahim et al. 2023). Challenges for clinical translation include optimizing delivery methods, engraftment, and survival of MSCs in the inflamed gallbladder. Use of biomaterials like hydrogels to provide a supportive scaffold for MSC implantation is being explored. Tracking of transplanted cells and their long-term fate requires further study (Lucocq et al. 2022). Endogenous stem cells resident in the gallbladder, such as self-renewing epithelial progenitor cells, also hold regenerative potential. Stimulating the proliferation and differentiation of these intrinsic stem cells pharmacologically may aid mucosal healing (Wang et al. 2022b).

Novel surgery tools

Advances in minimally invasive surgery are providing new state-of-the-art tools for treating cholecystitis. Robot-assisted laparoscopic cholecystectomy using the da Vinci system is an emerging technique (Gantschnigg et al. 2023). The wristed robotic instruments offer greater dexterity and visualization compared to conventional laparoscopy. Early data indicates reduced pain and hospital stay in cholecystitis patients (Chandhok et al. 2022). Natural orifice transluminal endoscopic surgery (NOTES) accesses the peritoneal cavity through natural orifices like the mouth or vagina. NOTES is being explored in clinical trials for transgastric gallbladder removal to further minimize invasive incisions (Ullah et al. 2022). Fluorescence imaging uses near-infrared dyes to enhance visualization of the extrahepatic bile ducts during laparoscopic cholecystectomy. This helps prevent bile duct injuries that can complicate gallbladder removal (Givvimani and Thota 2022). Energy devices like ultrasonic shears and bipolar vessel sealers enable more precise dissection and hemostasis during laparoscopic cholecystectomy. This reduces bleeding and bile leakage risks. Virtual reality and three-dimensional visualization systems are being incorporated into laparoscopic surgery workstations. These augmented display technologies improve depth perception and spatial orientation (Koutlas et al. 2023). Surgical data science applies machine learning algorithms to large databases to predict outcomes, optimize procedures, and enhance surgical performance. This includes tailoring personalized approaches for cholecystitis patients (Sachs et al. 2023).

Artificial intelligence (AI)

Artificial intelligence (AI) and machine learning have significant potential to transform the management of cholecystitis through enhanced diagnosis, treatment planning, and care delivery. AI algorithms can rapidly analyze ultrasound, CT, and MRI imaging data to identify characteristic signs of cholecystitis. Automated image interpretation can aid clinicians in rapid diagnosis and evaluation of disease severity (Obaid et al. 2023). Expert systems incorporating medical knowledge on cholecystitis can provide diagnostic decision support, recommending relevant lab tests and optimal imaging modalities based on patient symptoms and risk factors. This supports evidence-based care (Kunyuan et al. 2023). AI can integrate diverse patient data from notes, labs, imaging and 'omics to predict optimal individualized treatment plans. Machine learning models can continually refine predictions as new data emerges. Robotic surgical platforms like the da Vinci system may increasingly incorporate real-time AI guidance to augment the surgeon's capabilities and improve procedural accuracy. The rise of smart robotics can enhance precision in laparoscopic cholecystectomy (Filiberto et al. 2022). AI chatbots and virtual assistants can help educate patients about cholecystitis, answer questions, monitor symptoms, and facilitate follow-up care. This promotes patient engagement and recovery (Endo et al. 2023). However, significant challenges remain in implementing AI in clinical practice including regulatory hurdles, transparency, and explainability of model predictions. Physician oversight and validation is critical to ensure safety and efficacy.

Importance of biotechnology and future directions

The advent of cutting-edge biotechnology tools is revolutionizing the diagnosis and management of cholecystitis in numerous ways. Molecular techniques like PCR and DNA sequencing allow rapid, sensitive identification of bacterial, viral, fungal, and parasitic pathogens from gallbladder specimens within hours. This facilitates prompt initiation of targeted antimicrobial therapy. Next-generation sequencing methods provide in-depth characterization of the microbial communities inhabiting the gallbladder. This microbiome analysis gives insights into dysbiotic states underlying chronic inflammation and acute exacerbations. Gene sequencing also detects antimicrobial resistance threats to guide treatment (Benharash et al. 2023). Innovative new treatments on the horizon include phage therapy, which utilizes viruses that infect and destroy specific bacterial strains causing cholecystitis. Early clinical trials show promise in eliminating drug-resistant microbes. Other biotherapeutics like CRISPR gene editing and RNA

interference (RNAi) allow precise disabling of pathogen virulence factors underlying cholecystitis pathogenesis. Point-of-care diagnostics like nanopore sequencing and microfluidic PCR chips performed at the bedside will enable ultra-rapid molecular diagnosis and personalized prescription of targeted therapies within hours of presentation. Machine learning integration will further optimize predictive analytics (Irani et al. 2023).

Precision medicine and personalized care for cholecystitis.

Molecular diagnostic techniques for individual patients

Molecular diagnostic techniques for individual patients utilize next-generation sequencing to rapidly identify causative microbes from gallbladder specimens. This allows for targeted antimicrobial therapy tailored to the patient's specific infection. Metagenomic analysis of bile samples can reveal the microbiome composition, guiding probiotic interventions (He et al. 2023).

Tailored treatment plans based on genetic or biomarker profiles

Tailored treatment plans based on genetic or biomarker profiles involve analyzing patient-specific genetic variations that influence drug metabolism and efficacy. Pharmacogenomic testing identifies polymorphisms in cytochrome P450 enzymes, enabling personalized dosing of pain medications and antibiotics. Biomarker profiling, such as measuring C-reactive protein or procalcitonin levels, helps assess inflammation severity and guide treatment intensity (Georgescu et al. 2023).

Personalized drug therapies or dosing

Personalized drug therapies consider individual patient factors like age, weight, liver function, and comorbidities to optimize dosing regimens. Therapeutic drug monitoring ensures appropriate antibiotic concentrations in bile. Novel approaches include nanoparticle-mediated drug delivery systems targeted to the gallbladder (Park et al. 2023).

Patient-specific risk assessment and prevention strategies

Patient-specific risk assessment utilizes machine learning algorithms to integrate clinical, genetic, and lifestyle data, generating personalized risk scores for cholecystitis development. Prevention strategies are tailored based on these risk profiles, potentially including customized dietary interventions, targeted probiotic supplementation, or prophylactic use of ursodeoxycholic acid in high-risk individuals. Wearable devices monitoring physiological parameters can provide early warning signs of gallbladder inflammation, enabling proactive interventions (Fehring et al. 2024).

Conclusions

The integration of advanced biotechnologies holds significant promise for revolutionizing the diagnosis, treatment, and management of cholecystitis. Molecular techniques like PCR and next-generation sequencing enable rapid, precise identification of causative pathogens, facilitating targeted antimicrobial therapy. Novel therapeutic approaches such as phage therapy, CRISPR gene editing, and RNA interference show potential for overcoming antibiotic resistance and precisely targeting microbial virulence factors. Innovations in minimally invasive surgical techniques, including robotic-assisted procedures and fluorescence-guided imaging, are enhancing the safety and efficacy of cholecystectomy. The application of artificial intelligence in imaging analysis, treatment planning, and surgical guidance further augments clinical decision-making and procedural outcomes. However, despite these advancements, challenges remain in translating many of these technologies from preclinical studies to clinical practice. Further research is needed to optimize delivery methods, assess long-term efficacy and safety, and navigate regulatory hurdles for emerging biotherapeutics and AI-integrated systems.

Recommendations

To fully realize the potential of biotechnology in cholecystitis management, several key areas warrant further investigation and development. Firstly, large-scale clinical trials are needed to validate the efficacy and safety of novel biotherapeutics like phage therapy and CRISPR-based treatments in human patients. Secondly, efforts should be made to standardize and streamline the implementation of rapid molecular diagnostics in clinical settings, including the development of user-friendly, cost-effective point-of-care devices. Thirdly, interdisciplinary collaboration between clinicians, bioengineers, and data scientists should be fostered to accelerate the integration of AI and machine learning into clinical decision support systems and surgical platforms. Additionally, research into the long-term outcomes of stem cell therapies and tissue engineering approaches for gallbladder regeneration should be prioritized. Finally, ethical considerations and regulatory frameworks need to be proactively addressed to ensure the responsible development and implementation of these advanced biotechnologies in cholecystitis care.

Abbreviations

CH	Acute cholecystitis
E. coli	*Escherichia coli*
C. albicans	*Candida albicans*
US	Ultrasound
CT	Computed tomography

MRI	Magnetic resonance imaging
HIDA	Hepatobiliary iminodiacetic acid
PCR	Polymerase chain reaction
FISH	Fluorescent in situ hybridization
NGS	Next generation sequencing
IV	Intravenous
NSAIDs	Nonsteroidal anti-inflammatory drugs
MICs	Minimum inhibitory concentrations
CRISPR	Clustered regularly interspaced short palindromic repeats
siRNA	Small interfering RNA
MSCs	Mesenchymal stem cells
NOTES	Natural orifice transluminal endoscopic surgery
NIR	Near infrared
AI	Artificial intelligence
3D	Three dimensional
NETs	Neutrophil extracellular traps
IL-1	Interleukin-1
IL-6	Interleukin-6
TNF-α	Tumor necrosis factor alpha
IFN-γ	Interferon gamma
IL-10	Interleukin-10
PET	Positron emission tomography
ipaH gene	Invasion plasmid antigen H

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