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Review

Clostridioides difficile: Modern Approaches in Pathogenesis, Diagnosis, Treatment, Prevention, Emerging Perspectives and Health Economics

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Abstract

Introduction: *Clostridioides difficile* (*C. difficile*) is a major cause of antibiotic-associated diarrhea and healthcare-associated infections, with rising global incidence and severity due to the emergence of hypervirulent strains. **Methods**: This review synthesizes recent literature on the epidemiology, pathogenesis, diagnostic approaches, and therapeutic strategies related to *C. difficile* infection (CDI). Sources were selected from peer-reviewed journals, clinical guidelines, and emerging research between 2020 and 2025. **Results**: Advances in molecular diagnostics have improved the accuracy and speed of CDI detection. New therapeutic options such as fidaxomicin offer narrower-spectrum antibiotic activity with reduced recurrence rates. Fecal microbiota transplantation (FMT) has emerged as a highly effective option for recurrent CDI. Preventive efforts, including antibiotic stewardship programs and early-phase vaccine trials, show potential in reducing infection rates. **Discussion**: The management of CDI is evolving rapidly with the integration of precision diagnostics, targeted therapies, and microbiome-based interventions. Preventive strategies are critical, particularly in healthcare settings where *C. difficile* persists in the environment. Continued research and coordinated public health efforts are essential to reduce disease burden, improve outcomes, and limit transmission. **Conclusions**: *Clostridioides difficile* infections remain a major healthcare challenge with rising incidence and recurrent cases. Fidaxomicin has become the preferred first-line therapy. Microbiota-based therapies (like FMT, Rebyota, and Vowst) and Lipopolysaccharide Binding Protein (LBP) are highly effective for recurrent CDI prevention. Diagnostic strategies have improved with multi-step testing, enhancing accuracy and reducing overtreatment. Future focus lies in vaccines, targeted antimicrobials, and stricter prevention through antibiotic stewardship and hygiene.

Keywords: *Clostridioides difficile*; fidaxomicin; fecal microbiota transplantation; CDI diagnosis; antibiotic stewardship; vaccine development

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1. Introduction

Clostridioides difficile (formerly *Clostridium difficile*) is a Gram-positive, anaerobic, spore-forming bacterium responsible for a wide spectrum of gastrointestinal diseases, ranging from mild antibiotic-associated diarrhea to life-threatening pseudomembranous colitis. Although once considered primarily a hospital-acquired infection, CDI is increasingly reported in community settings, affecting not only older adults but also younger and otherwise healthy individuals [1].

The virulence of *C. difficile* is mainly attributed to two exotoxins, TcdA and TcdB, which disrupt intestinal epithelial cells and provoke severe inflammation. Its ability to form durable spores allows the bacterium to persist in hospital environments and on surfaces, making immunocompromised patients and those receiving broad-spectrum antibiotics particularly vulnerable [2].

Hypervirulent lineages such as ribotype 027/NAP1/BI, characterized by elevated toxin production and fluoroquinolone resistance, have been associated with higher morbidity and mortality [3]. Recent studies have also revealed additional modes of transmission, including zoonotic and foodborne routes [4,5].

Genomic analyses show overlap between strains from humans and companion animals, while novel toxigenic ribotypes have been identified in swine production environments, highlighting CDI as a potential One Health challenge [5,6].

In the United States, CDI causes over 450,000 cases and approximately 20,000 deaths annually, with average hospital costs surpassing $15,000 per patient. Contemporary data confirm that CDI continues to impose a considerable burden on working-age adults, leading to both direct healthcare costs and indirect socioeconomic impacts [7]. Community-acquired CDI is strongly associated with prior exposure to certain antibiotics, underlining the importance of stewardship not only in hospitals but also in outpatient settings [8].

On a global scale, the emergence of novel ribotypes and regional variations in strain distribution demonstrate the dynamic epidemiology of CDI and emphasize the need for international surveillance efforts [9,10].

Recurrent disease represents one of the most pressing clinical challenges, with up to 30% of patients experiencing relapse after standard treatment. This is largely due to spore persistence and disruption of the gut microbiota [11]. FMT has proven to be highly effective for recurrence, outperforming conventional therapies [12].

Both capsule-based and colonoscopic FMT approaches have been shown to be safe and effective [13,14].

Meanwhile, microbiota-based risk models offer promising predictive tools for identifying patients at greatest risk of relapse [3].

Risk factors for CDI extend beyond antibiotic use and include advanced age, prolonged hospitalization, immunosuppressive therapy, and gastrointestinal surgery. Furthermore, asymptomatic carriers (LBPs) serve as reservoirs for transmission in both healthcare and community environments [2]. The COVID-19 pandemic further complicated CDI surveillance and diagnosis, as overlapping gastrointestinal symptoms and strained hospital resources led to under-detection in many cases [15].

Therapeutic options continue to evolve. Although vancomycin and fidaxomicin remain standard treatments, new strategies aim to improve outcomes and reduce recurrence. Oral microbiota-based therapeutics such as Vowst (SER-109) (approved in 2023) have demonstrated efficacy in lowering recurrence rates following antibiotic treatment (enema administration) [16].

While the FDA's (Food and Drug Administration) approval of Rebyota in 2022 introduced the first standardized FMT-derived product into clinical practice [17].

Alongside these, advances in microbiome-based diagnostics, vaccine development, and alternative microbiota restoration therapies are under active investigation [3,18].

Vaccination strategies against *C. difficile* have focused on neutralizing toxins A and B. Conventional toxoid vaccines, including Sanofi Pasteur's candidate and Pfizer's PF-06425090 (CLOVER trial), failed to achieve primary endpoints in phase 3 studies, although earlier-phase trials demonstrated immunogenicity [19]. Studies in older adults showed strong antibody responses to both toxins, highlighting the potential for vaccine-mediated immunity [20]. More recently, mRNA vaccines delivered via lipid nanoparticles have elicited robust systemic and mucosal immunity in preclinical models, demonstrating protection against toxin-induced injury [21]. While no vaccine has yet received approval and human trials for mRNA candidates are ongoing, these approaches represent a promising avenue for preventive immunization in high-risk populations.

From a public health perspective, CDI represents a multifaceted challenge at the intersection of pathogen biology, healthcare practice, and host vulnerability. The increase in community-acquired cases, coupled with evidence of zoonotic and foodborne spread, underscores the importance of a One Health framework that integrates human, animal, and environmental surveillance [4–6,22].

Beyond its role in infectious disease, emerging evidence suggests that toxigenic strains may contribute to colorectal carcinogenesis, further broadening CDI's clinical significance [23].

Addressing CDI effectively will therefore require coordinated strategies that combine antimicrobial stewardship, novel therapeutics, robust infection control, and interdisciplinary collaboration across the One Health spectrum [9,18,22].

2. Epidemiology

The epidemiology of CDI varies widely across geographic regions and demographic groups. While traditionally regarded as a hospital-associated infection, recent evidence highlights increasing community-acquired cases and the international spread of hypervirulent strains [24].

Beyond traditional hospital-associated infections, surveillance programs and genomic sequencing have revealed diverse sources of CDI. These now include contaminated food, animal reservoirs, and asymptomatic human carriers, underscoring its complex epidemiology and the challenges of effective control [25].

Reported incidence and mortality rates differ across regions, as shown in Table 1.

Table 1. CDI incidence and mortality across regions.

Reference	Predominant Strain	Mortality Rate (%)	Incidence (per 100,000)	Region	Rank
[12]	RT027	4.5	143	USA	1
[6]	RT027	3.8	123	Canada	2
[9]	RT001, RT027	3.5	91–117	Europe	3
[11]	RT014	1.5	42	Australia	4
[10]	RT017	1.2	33–75	Asia	5

2.1. Healthcare-Associated Clostridioides difficile Infection (HA-CDI)

Healthcare-associated *Clostridioides difficile* infection (HA-CDI) continues to represent the dominant form of CDI worldwide and accounts for the majority of severe cases, hospital admissions, and infection-related mortality. Epidemiological surveillance studies indicate that approximately 70–75% of CDI cases occur in healthcare environments, highlighting the

major influence of hospitalization, antimicrobial exposure, and underlying comorbidities on disease development and transmission [26].

Molecular epidemiological investigations have identified several ribotypes that are frequently linked with HA-CDI, including RT027, RT106, RT014, and RT020 [4]. Among these, the hypervirulent RT027 lineage has historically been associated with increased disease severity, enhanced toxin production, and elevated mortality rates. Nevertheless, its prevalence has declined in certain regions in recent years, likely reflecting improvements in infection prevention strategies and antimicrobial stewardship programs [27].

Regional surveillance across Europe and North America has demonstrated that RT027 and RT001 have been dominant strains in healthcare settings, while other ribotypes such as RT014 remain widely distributed across multiple countries [28]. These strains are frequently implicated in hospital outbreaks and increased healthcare utilization, emphasizing the importance of effective infection control measures, early diagnostic testing, and prudent antimicrobial use.

2.2. Community-Associated Clostridioides difficile Infection (CA-CDI)

In contrast to HA-CDI, community-associated CDI (CA-CDI) has increasingly been recognized over the past decade and now represents a growing proportion of total CDI cases. Epidemiological data suggest that approximately 20–30% of CDI cases arise in community settings, affecting individuals without recent hospitalization or traditional healthcare exposures [29].

CA-CDI is often observed in younger populations and may involve a broader range of circulating strains compared with healthcare-associated infections. Molecular surveillance studies have identified ribotypes such as RT106, RT020, RT014, and RT027 among the most frequently detected strains in community-associated cases [30].

Furthermore, several epidemiological analyses indicate that the proportion of CA-CDI cases has increased over time, even in regions where healthcare-associated incidence has declined. This trend suggests evolving transmission patterns and highlights the potential role of environmental, foodborne, or zoonotic reservoirs in the spread of the pathogen [31].

Overall, these findings demonstrate that the epidemiology of CDI continues to evolve globally, with both healthcare-associated and community-associated transmission contributing substantially to the total disease burden. Consequently, differentiating between HA-CDI and CA-CDI is essential for accurate epidemiological interpretation and for the implementation of targeted prevention strategies.

These variations highlight differences in healthcare infrastructure, antimicrobial prescribing practices, diagnostic capacity, and infection-control measures across regions [32].

Furthermore, the detection of CDI outside healthcare facilities emphasizes the growing role of community transmission and zoonotic potential [33].

Ongoing genomic surveillance and international collaboration remain essential for monitoring strain evolution, guiding antimicrobial stewardship, and informing vaccine and therapeutic development strategies [24,25,32,33].

CDI exhibits notable variation across demographic and clinical populations. Advanced age represents the most significant risk factor, with individuals aged $\geq$65 years experiencing higher incidence, greater disease severity, and increased mortality, likely due to immunosenescence and more frequent healthcare exposure [29]. Differences related to sex have also been observed, with slightly higher incidence reported among females, possibly reflecting variations in antibiotic use and patterns of healthcare utilization [24].

Moreover, CDI is more prevalent among individuals with increased healthcare exposure, including hospitalized patients and residents of long-term care facilities, as well as among immunocompromised groups such as transplant recipients, oncology patients,

and those receiving immunosuppressive therapies [29]. At a broader level, disparities in CDI burden and outcomes have also been linked to social vulnerability and inequalities in healthcare access [24,25].

To complement Table 1, world map visualization is included, using color intensity ("heat zones") to represent CDI incidence per 100,000 population by region. Overlaying this with markers indicating predominant ribotypes (e.g., RT027 in North America, RT017 in Asia, RT014 in Australia) would provide an at-a-glance understanding of global distribution patterns. Such a figure would visually emphasize the regional contrasts in both disease burden and strain diversity, making the epidemiological trends more accessible [34].

Here is the world map visualization (Figure 1) with a color gradient (heat scale) to represent CDI incidence per 100,000 population.

- Dark red: ≥40 per 100,000;
- Medium red: 20–39 per 100,000;
- Light pink: <20 per 100,000.

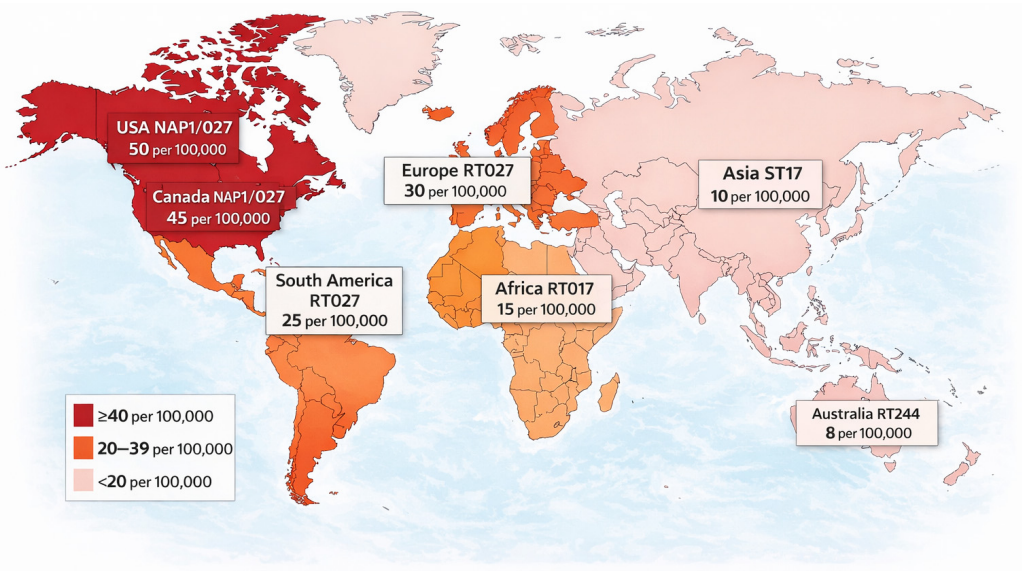

Figure 1. Global Distribution of *Clostridioides difficile* Infection (CDI) and Predominant Strains.

2.3. Emergence, Diffusion, and Characteristics of Recent Hypervirulent C. difficile Types

The emergence and global dissemination of hypervirulent *Clostridioides difficile* strains constitute a major epidemiological and clinical concern that merits dedicated discussion. Epidemic lineages such as PCR ribotype 027 (RT027) have played a central role in large healthcare-associated outbreaks, largely due to enhanced toxin production and the presence of binary toxin, which contribute to increased pathogenicity and disease severity. Genomic investigations suggest that these hypervirulent strains have arisen through multiple evolutionary events and have subsequently spread across diverse geographic regions. Recent surveillance data indicate continued expansion of hypervirulent lineages beyond historically affected areas, with reports documenting their detection in new settings and populations. In addition, clinical studies demonstrate that infections caused by hypervirulent strains are associated with higher complication rates and increased short-term mortality compared with non-hypervirulent strains, underscoring their substantial clinical impact. Advances in molecular diagnostics, including enhanced ribotyping methods and mass spectrometry-based approaches, are improving the identification and epidemiological tracking of high-risk strains such as RT027, RT078, RT176, and related lineages. Collectively, these findings highlight the importance of continuous surveillance, molecular characteri-

zation, and targeted infection control strategies to mitigate the ongoing threat posed by hypervirulent *C. difficile* strains [27,35].

3. Pathogenesis

CDI begins when spores survive gastric acidity and reach the colon. Primary bile acids, such as taurocholate, stimulate spore germination, while secondary bile acids produced by commensal bacteria inhibit this process [36]. Antibiotic-induced disruption of the gut microbiota reduces secondary bile acid levels, creating an environment conducive to spore germination and bacterial proliferation [37,38].

The glucosyltransferase activity of TcdA and TcdB can suppress toxin-specific adaptive immune responses, further promoting disease progression [39].

Once germinated, vegetative *C. difficile* secretes TcdA and TcdB, which inactivate Rho GTPases. This causes cytoskeletal collapse, epithelial barrier damage, and an inflammatory cascade involving IL-1β, TNF-α, and IL-8 [34,39].

Host immunity is critical in disease severity. Neutrophils and macrophages infiltrate the gut rapidly after toxin-mediated damage; while initially protective, excessive neutrophilic inflammation can worsen tissue injury and contribute to pseudomembrane formation. Adaptive immunity is also important: high serum antibody levels against TcdA and TcdB correlate with reduced recurrence, whereas insufficient immune responses increase susceptibility [39,40].

IL-33 has been shown to protect against recurrent CDI by promoting mucosal repair and immune regulation [41].

A healthy gut microbiota confers colonization resistance through nutrient competition, production of inhibitory metabolites such as short-chain fatty acids, and restoration of secondary bile acids, often mediated by bacteria like *Clostridium scindens*. Butyrate, for example, suppresses inflammation and directly impacts *C. difficile* physiology [34,37,42–46]. *C. difficile* demonstrates metabolic adaptability, utilizing simple carbohydrates and amino acids via Stickland fermentation, which supports colonization and allows it to compete against commensal microbes [38].

These insights have informed therapeutic strategies, including toxin-neutralizing monoclonal antibodies, microbiome restoration via fecal microbiota transplantation or live biotherapeutics, modulation of bile acid metabolism, and targeting metabolic and immunometabolic pathways to prevent germination and recurrence [33,34,43–46].

Spores and Toxins as Key Virulence Factors of Clostridioides difficile

A hallmark of *Clostridioides difficile* is its ability to form highly resilient spores, which are central to environmental persistence and transmission. These spores survive harsh conditions, including oxygen exposure, desiccation, disinfectants, and antibiotics, allowing prolonged survival on hospital surfaces and facilitating fecal–oral transmission [31,47]. The spore's multilayered structure, comprising the exosporium, coat, cortex, and core, protects against environmental stresses and supports persistence in healthcare settings [47]. Certain epidemic lineages, particularly RT027, demonstrate enhanced sporulation, contributing to higher transmissibility and global spread [27].

The primary virulence determinants of *C. difficile* are its exotoxins, TcdA and TcdB, encoded within the pathogenicity locus (PaLoc). These toxins glucosylate host Rho GTPases, disrupting the cytoskeleton, damaging epithelial barriers, and inducing inflammatory responses, which drive diarrhea and colitis [48].

There is considerable heterogeneity in toxin production. Over 34 toxinotypes exist, reflecting structural and regulatory differences in TcdA and TcdB, as well as variations

in toxin expression. Distinct phenotypes—A+B+, A−B+, and A−B−—influence both epidemiology and disease severity [31,48].

Some strains also produce binary toxin (CDT), encoded by cdtA and cdtB, which modifies host actin and enhances bacterial adherence and virulence [48]. The combination of high sporulation, toxin production, and CDT characterizes hypervirulent ribotypes, particularly RT027 and RT078, associated with outbreaks and severe disease [27].

4. Diagnosis

4.1. Clinical Assessment

The accurate diagnosis of CDI relies on both clinical evaluation and laboratory testing, as asymptomatic colonization is common and laboratory results alone cannot distinguish infection from carriage [49–51].

Clinical evaluation should focus on the patient's symptoms and risk factors:

- Symptoms: ≥3 unformed stools in 24 h, abdominal pain or cramping, fever, leukocytosis, and elevated creatinine.
- Severe or complicated CDI: hypotension, ileus, toxic megacolon, perforation, or sepsis.
- Risk factors: recent antibiotic use, hospitalization, older age, immunosuppression, and proton pump inhibitor therapy [51].

Testing patients without symptoms or with formed stool may result in false-positive results and unnecessary treatment [50,52].

4.2. Laboratory Testing

Laboratory tests complement clinical assessment:

- Screening Tests: Glutamate dehydrogenase (GDH) EIA (Enzyme immunoassay) detects a highly conserved enzyme present in all *C. difficile* strains. It is highly sensitive but not specific, making it suitable as an initial screening tool [49,53].
- Toxin Detection: EIAs for toxins A and B are highly specific and rapid but have moderate sensitivity; a positive result confirms active toxin production [49,53].
- Molecular Methods: NAAT/PCR (Nucleic Acid Amplification Tests/Polymerase chain amplification) detect toxin genes such as tcdB with high sensitivity and specificity. However, NAATs may identify asymptomatic carriers, potentially leading to overdiagnosis if used in isolation [54,55].

4.3. Multistep Testing Algorithms

Most laboratories improve diagnostic accuracy using two-step or three-step algorithms [49,50,54]:

a. GDH + Toxin EIA:

- Both positive → CDI confirmed;
- Both negative → CDI excluded;
- Discordant (GDH+/Toxin−) → proceed to NAAT.

b. NAAT/PCR for toxin genes:

- Positive → toxigenic strain detected; interpret in clinical context;
- Negative → CDI excluded.

This approach balances sensitivity and specificity, minimizing missed diagnoses and unnecessary treatment [49,54], see Figure 2.

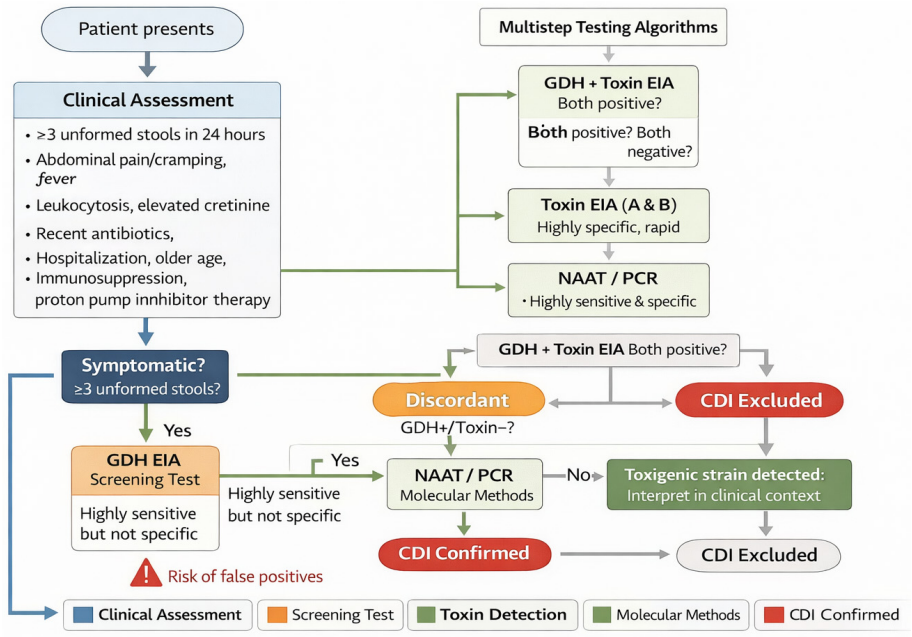

Figure 2. Diagnostic Flowchart for *Clostridioides difficile* Infection (CDI).

4.4. Emerging and Innovative Diagnostics

Recent technological advancements have enhanced the speed and accuracy of CDI diagnosis:

- LAMP (loop-mediated isothermal amplification): rapid, sensitive, suitable for point-of-care testing [55].
- Next-generation sequencing (NGS): enables detailed strain typing and antimicrobial resistance profiling [55].
- MALDI-TOF MS (Matrix-Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) Mass Spectrometry): fast bacterial identification, potential for rapid CDI detection [6].
- Novel immunoassays (SIMOA-Single Molecule Array, Singulex Clarity): automated toxin detection with near-perfect sensitivity and specificity (see Table 2) [55].

Table 2. Characteristics of Diagnostic Tests and Algorithms for CDI.

Test/Strategy	Sensitivity	Specificity	Turnaround	Advantages	Limitations	References
GDH EIA	~83–93%	Moderate	Hours	Good screening tool; rapid	Does not confirm toxin; moderate specificity	[49,53]
Toxin A/B EIA	~75%	~95%	Hours	Highly specific; rapid	Moderate sensitivity	[49,53]
NAAT/PCR	~90%	~95%	Hours	High sensitivity; detects toxigenic strains	Risk of overdiagnosis in asymptomatic carriers	[54]
GDH + Toxin + NAAT algorithm	—	Very high	Hours–1 day	Widely recommended; balances sensitivity and specificity	Slightly longer workflow; requires multiple steps	[49,50,54]
LAMP	90–100%	94–99%	Minutes–Hours	Rapid; point-of-care potential; high sensitivity	May require specialized equipment; cost considerations	[55]
MALDI-TOF	90–100%	90–100%	Minutes	Fast strain identification	Limited to laboratory settings; does not detect toxins	[55]
SIMOA/Singulex Clarity	~100%	~100%	<40 min	Automated toxin detection; near-perfect sensitivity and specificity	High cost; limited availability	[55]

Accurate differentiation of *Clostridioides difficile* strains is essential for outbreak investigation and understanding strain-specific virulence and resistance. PCR ribotyping remains widely used for rapid epidemiologic discrimination [56]. For higher-resolution analyses, multilocus sequence typing (MLST) and whole-genome sequencing (WGS) enable precise distinction between hypervirulent strains such as R20291 and reference strains like 630, providing insights into evolutionary relationships, toxin variants, and antimicrobial resistance genes [57]. Emerging rapid sequencing technologies, including nanopore sequencing, facilitate near real-time strain typing directly from stool samples, significantly reducing turnaround times for surveillance [58]. Integrated bioinformatics pipelines now combine ribotyping, resistome profiling, and virulence factor analysis to provide a comprehensive framework for high-resolution characterization in clinical and epidemiologic settings.

4.5. Imaging and Endoscopy

Computed Tomography (CT) scans: indicated for complicated cases such as toxic megacolon, perforation, or severe colitis [51].

- Endoscopy: reserved for inconclusive stool tests or urgent diagnostic needs; visualization of pseudomembranes is diagnostic but carries procedural risk in unstable patients [51].

4.6. Diagnostic Stewardship and Guidelines

Guidelines from IDSA (Infectious Diseases Society of America) and ESCMID (European Society for Clinical Microbiology and Infectious Diseases) emphasize judicious testing to reduce overdiagnosis and unnecessary therapy [50,52]:

- Only test patients with clinically significant symptoms (Bristol Stool Scale).
- Avoid testing formed stool or asymptomatic individuals.
- Repeat testing within 7 days should be avoided unless symptoms persist or worsen.
- Proper diagnostic stewardship prevents unnecessary antibiotic use and limits antimicrobial resistance [50–52].

This structured approach ensures that CDI is diagnosed accurately based on both clinical context and laboratory evidence, reduces false positives, and supports rational use of antibiotics, minimizing harm to patients and the gut microbiome.

5. Treatment

Effective management of CDI aims to relieve symptoms, prevent recurrence, preserve gut microbiota, and support host immune defenses.

a. First-Line Antibiotic Therapy

For mild to moderate CDI, first-line therapy includes oral vancomycin or fidaxomicin, which specifically target *C. difficile* [59].

- Fidaxomicin, a narrow-spectrum macrocyclic antibiotic, reduces recurrence more effectively than vancomycin, making it preferred for initial episodes, particularly in high-risk patients [59,60].
- Severe or fulminant CDI often requires high-dose oral vancomycin, sometimes combined with IV metronidazole. Tapered fidaxomicin therapy is mainly reserved for recurrent CDI.

Surgical intervention may be needed for complications such as toxic megacolon or intestinal perforation (see Table 3) [61].

Table 3. Summary of First-Line Antibiotic Therapy.

Severity	Antibiotic	Dosage/Duration	Notes
Mild–Moderate	Fidaxomicin	200 mg orally twice daily ×10 days	Preferred; reduces recurrence
Mild–Moderate	Vancomycin	125 mg orally 4× daily ×10 days	Alternative if fidaxomicin unavailable
Severe	Vancomycin	125–500 mg orally 4× daily ×10–14 days	High-dose for severe cases
Fulminant	Vancomycin + IV Metronidazole	Vancomycin 500 mg orally/NG tube 4× daily + Metronidazole 500 mg IV 8–12 h	Consider surgery if complicated

b. Management of Recurrent CDI

Patients with multiple recurrences benefit from FMT, which restores microbial diversity and reduces relapse rates to <10% [17,62,63].

- Standardized FMT-derived products such as SER-109 and CP101 provide safer, controlled alternatives [17].
- Recurrent CDI management combines microbiota restoration, targeted antimicrobial therapy, and immune support to break the cycle of relapse and improve outcomes.

c. Adjunctive and Emerging Therapies

Additional strategies aim to reduce recurrence while preserving gut microbiota:

- Bezlotoxumab: monoclonal antibody against toxin B; lowers recurrence when combined with antibiotics [61].
- Ridinilazole: narrow-spectrum antimicrobial selectively targeting *C. difficile*, sparing beneficial flora [59].
- Probiotics: may offer supportive benefits, though evidence is limited; not routine standard therapy [61].
- mRNA vaccines: under early investigation, aim to provide immune protection in high-risk populations [64].

d. Treatment Goals

Effective CDI management focuses on four main objectives:

1. Relieve symptoms: reduce diarrhea, abdominal pain, fever, and systemic manifestations; prevent dehydration or electrolyte imbalance [65,66].
2. Minimize recurrence: lower relapse rates using appropriate antimicrobials, microbiota restoration (FMT or standardized products), and adjunctive therapies like bezlotoxumab [65,67,68].
3. Preserve gut microbiota: maintain a healthy microbial community using narrow-spectrum antibiotics (fidaxomicin), microbiota-sparing agents (ridinilazole), and live biotherapeutics [66,67].
4. Support host immune defenses: strengthen immune response against *C. difficile* toxins via monoclonal antibodies, immunomodulation, or investigational vaccines [68,69].

By addressing both the acute infection and underlying microbial and immune vulnerabilities, these strategies aim for durable resolution and a reduction in the cycle of recurrent CDI [65–69].

6. Prevention and Control

Effective prevention of CDI requires a comprehensive, multifaceted strategy that disrupts transmission, preserves gut microbiota, and facilitates early recognition of cases [70,71].

Antibiotic stewardship remains the foundation of prevention, as broad-spectrum antimicrobials—particularly fluoroquinolones, cephalosporins, and clindamycin—are strongly linked to CDI risk. Stewardship initiatives should emphasize targeted antimicrobial selection, strict initiation criteria, and limiting treatment duration. Incorporating prescriber feedback, routine audits, and electronic medical record (EMR) alerts has been shown to reduce inappropriate antibiotic exposure and subsequently lower CDI incidence [70].

Equally critical are infection prevention and control (IPC) measures, given the organism's ability to form hardy spores. Alcohol-based hand rubs are ineffective against spores, making thorough handwashing with soap and water essential after patient contact or exposure to contaminated environments [71,72]. Environmental cleaning protocols should use sporicidal agents, such as sodium hypochlorite at concentrations of 5000 ppm, for both daily and terminal room disinfection [71,72].

Standardized cleaning procedures, combined with auditing and monitoring, enhance compliance and effectiveness. Healthcare workers should wear gloves and disposable gowns when entering the rooms of symptomatic patients and remove them prior to exit to prevent cross-transmission [72].

Early identification and isolation of symptomatic patients is vital to containing nosocomial spread. Suspected cases should be isolated in private rooms with dedicated bathrooms immediately, without waiting for laboratory confirmation. During outbreaks, cohorting may be employed if private rooms are limited. Active surveillance programs—tracking incidence, severity, and recurrence rates—are essential for identifying clusters and guiding infection control interventions. Data should be routinely reviewed by infection prevention teams and reported to institutional and public health authorities as appropriate [72].

Several emerging preventive strategies are under investigation. Vaccine candidates targeting toxins A and B have advanced to Phase III clinical trials, although one large trial was terminated due to lack of efficacy [73]. Adjunctive approaches such as probiotics and bile acid modulators show variable benefit, while the monoclonal antibody bezlotoxumab has demonstrated significant reductions in CDI recurrence, particularly among high-risk groups including older adults and immunocompromised patients [74,75]. A systematic review confirmed modest recurrence reduction even in primary CDI [76], and a Mayo Clinic multicenter real-world cohort and meta-analysis found bezlotoxumab to significantly lower recurrence compared to standard therapy [77]. Despite these developments, the most effective prevention continues to rely on robust antibiotic stewardship, strict IPC adherence, and rapid case management to protect vulnerable patient populations.

7. Emerging and Experimental Perspectives

7.1. Microbiome-Based Therapies (Prevention of Recurrence)

Restoring intestinal microbial diversity is a key strategy for preventing recurrence in Clostridioides difficile infection (CDI). FMT has demonstrated high efficacy but faces safety, regulatory, and logistical constraints [78–80].

Standardized live biotherapeutic products (LBPs):

- REBYOTA® (RBX2660)—enema-based microbiota suspension; reduced recurrence in phase 3 PUNCH-CD3 trial; long-term safety confirmed [79,80].
- VOWST™ (SER-109)—oral Firmicutes spore consortium; durable recurrence prevention in ECOSPOR III/IV trials; FDA-approved 2023 [81].
- VE303—defined 8-strain consortium; phase 2 > 80% recurrence reduction; phase 3 RESTORA-TiVE303 ongoing [82,83].
- Probiotics: Saccharomyces boulardii and Lactobacillus species have been explored for primary prevention, but recent evidence shows limited efficacy, and routine use is not recommended [78].

7.2. Vaccines (Primary Prevention)

Preventive vaccination strategies have mainly targeted toxins A and B.

- Toxoid vaccines:
  - Sanofi Pasteur candidate—terminated after phase 3 failure [84].
  - Pfizer PF-06425090 (CLOVER)—did not meet primary endpoint, secondary analyses suggested reduced illness duration [85].
- Early-phase toxoid studies: Showed strong antibody responses in older adults and healthy adults [86,87].
- mRNA vaccines: Lipid nanoparticle-encapsulated mRNA for toxins A & B induced robust systemic and mucosal immunity in preclinical models [88,89]. Human trials are not yet underway.

7.3. Narrow-Spectrum Antimicrobials (Therapy for Acute Infection/Recurrence Prevention)

These agents target *C. difficile* while sparing the gut microbiota.

- Ridinilazole: phase 3 trials—lower recurrence vs. vancomycin but no superiority in sustained clinical response [90].
- CRS3123: methionyl-tRNA synthetase inhibitor; phase 2—high initial cure, markedly reduced recurrence [91,92].
- Ibezapolstat: Gram-positive selective; phase 2b—narrow-spectrum microbiome disruption, effective against resistant strains; preclinical mouse models show distinct microbiome/metabolic effects [93].

7.4. Immunotherapies (Adjunctive/Prevention of Recurrence)

- Bezlotoxumab: monoclonal antibody targeting toxin B; reduces recurrence in high-risk patients; real-world and post-marketing data support benefit [94] (no longer being manufactured by Merck, Darmstadt, Germany).

7.5. Host-Directed Interventions (Prevention of Recurrence)

Bile acid modulation: ursodeoxycholic acid inhibits spore germination; early clinical trials show potential to prevent colonization and recurrence (see Table 4) [74,78].

Table 4. Emerging *C. difficile* therapies.

Therapeutic Class	Candidate	Mechanism of Action	Trial Phase	Key Outcomes	Current Status	Primary Goal
Microbiome-Based Therapy	REBYOTA (RBX2660)	Restores gut microbial diversity	Phase 3	Significantly reduced recurrence; durable safety	FDA-approved	Prevention of recurrence
Microbiome-Based Therapy	VOWST (SER-109)	Oral Firmicutes spore consortium	Phase 3	Reduced recurrence in recurrent CDI	FDA-approved	Prevention of recurrence
Microbiome-Based Therapy	VE303	8-strain bacterial consortium	Phase 3	>80% reduction in phase 2	Phase 3 ongoing	Prevention of recurrence
Vaccine—Toxoid	Sanofi Pasteur bivalent	Neutralizes toxins A & B	Phase 3	Failed primary end-point; immunogenicity in earlier trials	Development discontinued	Primary prevention
Vaccine—Toxoid	Pfizer PF-06425090 (CLOVER)	Neutralizes toxins A & B	Phase 3	Did not meet primary endpoint	Development discontinued	Primary prevention

Table 4. *Cont.*

Therapeutic Class	Candidate	Mechanism of Action	Trial Phase	Key Outcomes	Current Status	Primary Goal
Vaccine—mRNA	Experimental mRNA vaccines	Lipid nanoparticle mRNA encoding toxins A & B	Preclinical	Strong systemic & mucosal immunity	Preclinical	Primary prevention
Narrow-Spectrum Antimicrobial	Ridinilazole	Selective inhibition of C. difficile	Phase 3	Lower recurrence vs. vancomycin	Completed; not approved	Therapy/recurrence prevention
Narrow-Spectrum Antimicrobial	CRS3123	Methionyl-tRNA synthetase inhibitor	Phase 2	High initial cure; reduced recurrence	Phase 2 completed; further trials ongoing	Therapy/recurrence prevention
Immunotherapy	Bezlotoxumab	Monoclonal antibody against toxin B	Post-marketing	Reduces recurrence in high-risk patients	FDA-approved	Adjunctive prevention of recurrence
Host-Directed	Ursodeoxycholic acid	Modulates bile acids, inhibits spore germination	Early clinical trials	Preliminarily prevents colonization and recurrence	Experimental/ongoing	Prevention of recurrence

The therapeutic landscape for CDI is rapidly advancing. FDA approval of microbiome-based therapies such as REBYOTA and VOWST represents a major milestone, VE303 may provide a defined-consortium alternative if phase 3 trials are successful. Narrow-spectrum antimicrobials such as CRS3123 show potential to reduce recurrence by sparing the commensal microbiota. Despite the failure of toxoid vaccines in late-stage studies, mRNA-based vaccine platforms offer a novel preventive strategy. Immunotherapies such as bezlotoxumab remain integral in high-risk patients, and host-targeted interventions, particularly those modulating bile acid pathways, may represent the next frontier in recurrence prevention.

Collectively, these approaches signify a shift from broad-spectrum antimicrobial strategies toward precision modulation of the microbiota–host–pathogen axis in CDI management.

7.6. Omics Approaches and Emerging Microbiota-Restoration Strategies

Recent advances in omics technologies have greatly enhanced our understanding of the gut microbiota in CDI. Shotgun metagenomic sequencing has revealed distinct microbial community structures and functional capacities in CDI patients compared with asymptomatic carriers or individuals with non-CDI diarrhea, highlighting specific taxa and metabolic pathways that could guide both diagnosis and therapy Integration of metagenomics with other omics layers allows for detailed mapping of microbial interactions and resistance mechanisms disrupted in CDI, providing targets for innovative interventions [95].

Emerging microbiota-restoration strategies are also being explored. Bacteriophage therapy offers a means to selectively reduce pathogenic bacteria, although limitations include the scarcity of strictly lytic phages and narrow host specificity [96,97]. Meanwhile, bacterial consortium transplantation (BCT)—using defined mixtures of commensal bacteria—represents a controlled evolution of FMT, supporting gut microbial restoration and functional recovery as demonstrated by sequencing-based tracking of engraftment [98].

Overall, these integrative *omics* approaches provide critical insights into CDI pathogenesis, recurrence risk, and response to microbiome-directed therapies. They also establish a foundation for innovative interventions, including BCT and phage therapy, as precision strategies to restore microbiota balance and prevent recurrence.

7.7. Emerging Technologies for Rapid Point-of-Care Testing (POCT) in Clostridioides difficile Diagnosis

Recent developments in POCT for *Clostridioides difficile* aim to provide rapid, sensitive, and field-deployable diagnostics. CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)-Cas-based assays integrated with isothermal amplification techniques such as recombinase polymerase amplification (RPA) or LAMP can detect toxin genes (tcdA, tcdB) in under an hour with visual readouts, minimizing reliance on laboratory infrastructure while maintaining high sensitivity and specificity [99,100].

Single-cell Raman spectroscopy, coupled with machine learning, has demonstrated the ability to distinguish toxigenic *C. difficile* strains directly from stool samples, offering rapid, label-free detection that could complement molecular approaches [101]. Additional platforms, including microfluidic CRISPR-based systems and AI-assisted readouts, show promise for scalable, automated POCT solutions, although many remain in early developmental stages and require validation in clinical cohorts [102].

While these technologies offer faster turnaround times and decentralization potential, further studies are needed to confirm diagnostic accuracy, usability, and cost-effectiveness compared with conventional laboratory methods. Overall, emerging POCT platforms, particularly CRISPR-Cas-based assays and Raman spectroscopy, represent a paradigm shift in rapid CDI diagnostics, with potential to improve early detection, surveillance, and infection control in both hospital and community settings [101,102].

8. Health Economics and Outcomes

CDI imposes substantial clinical, economic, and humanistic burdens. Hospital-attributable costs often exceed USD 20,000 per case, primarily driven by recurrent episodes, which affect approximately 20–30% of patients following initial infection. Fidaxomicin has demonstrated superior sustained cure rates and significantly reduced recurrence compared with vancomycin, as shown in randomized trials and meta-analyses [103]. Economic evaluations conducted in diverse settings—such as Japan and Spain—illustrate that despite higher acquisition costs, fidaxomicin's improved outcomes offset expenses, leading to favorable cost-effectiveness. For example, a Japanese semi-Markov model estimated an ICER of JPY 5,715,183 per QALY gained, approaching national willingness-to-pay thresholds [104]. In Spain, an extended-pulsed fidaxomicin regimen showed improved sustained cure and reduced recurrences compared with vancomycin in patients aged $\geq$ 60 years, providing economic justification for its usage [105].

Further, a lifetime-horizon Markov model assessed cost-effectiveness of standard and extended-pulsed fidaxomicin, and bezlotoxumab plus vancomycin versus standard vancomycin in the US societal context. Extended-pulsed fidaxomicin dominated vancomycin (i.e., lower cost, higher QALYs). Standard fidaxomicin achieved an Incremental Cost-Effectiveness Ratio (ICER) of only USD 495 per QALY gained, while bezlotoxumab added to vancomycin yielded an ICER of USD 17,746 per QALY—below the US willingness-to-pay threshold of USD 150,000 [106].

Equally important are recurrence-prevention strategies beyond pharmacologic agents. A decision-analytic model evaluating FMT (via colonoscopy or oral capsules) versus fidaxomicin, vancomycin, and bezlotoxumab in first recurrent CDI found FMT strategies to be cost-effective—colonoscopic FMT incurring the lowest cost (~USD 5250) and oral-capsule FMT yielding an ICER of USD 31,205 per QALY. At current pricing, bezlotoxumab was not cost-effective in this model [107].

The humanistic impact of CDI is profound. A French prospective study using EQ-5D-3L reported utility scores declining sharply from 0.542 at baseline to 0.050 during acute infection, resulting in a mean decrement of 0.492 and an estimated loss of approximately

0.028 QALYs per episode [108]. This underscores the significant quality-of-life burden associated with CDI, reinforcing the value of recurrence prevention.

Collectively, recent evidence affirms that preventing recurrence is the principal driver of both economic efficiency and quality-of-life gains in CDI management. Fidaxomicin—particularly in extended-pulsed regimens—offers superior clinical outcomes and cost-effectiveness under many scenarios. Adjunctive therapies such as bezlotoxumab provide added preventive value at acceptable cost in high-risk groups, while FMT (especially via capsules or colonoscopy) presents as a cost-effective option for recurrence [106,107]. Incorporating quantified utility losses into economic models remains essential to fully capture the humanistic burden of CDI [108].

These analyses introduce an innovative framework for integrating multi-omics data with categorical health-related quality of life measures, offering fresh perspectives to inform future clinical research.

The microbiota–gut–brain axis is thought to influence health-related quality of life, as well as shifts in the microbiome and metabolome, through interactions involving the immune system, gastrointestinal tract, and central nervous system in patients with recurrent *C. difficile* infection after RBL treatment (as reflected in *Cdiff* 32 mental domain HRQOL scores) [109,110].

C. difficile infection imposes a substantial burden on patients' quality of life, both during the acute phase and in the period that follows. These findings underscore the urgent need to develop improved strategies for prevention, treatment, communication, and patient education-addressing care not only during active CDI but also in long-term management [111].

Health policy strategies that use prevention-focused, high-value treatments targeted at vulnerable populations are likely to optimize both clinical outcomes and economic resource utilization [103–107].

9. One Health

Recent studies published in 2025 and beyond have advanced understanding of *Clostridioides difficile* epidemiology, transmission pathways, and preventive strategies, with growing attention to a One Health framework. Emerging evidence highlights the zoonotic potential of *C. difficile*, identifying livestock, companion animals, and environmental reservoirs as possible contributors to human infection and underscoring the need for coordinated surveillance across human and veterinary sectors. Contemporary genomic investigations further demonstrate interconnections among healthcare, community, animal, food, and environmental sources, reinforcing the importance of integrated infection control measures. In parallel, research into host–microbiome interactions continues to clarify how disruptions in gut microbial communities promote colonization and toxin production, supporting the development of targeted microbiota-based therapies to reduce recurrence. Additionally, novel preventive approaches, including mucosal vaccination strategies explored in recent experimental models, suggest promising future directions for limiting colonization and preventing relapse. Collectively, these findings reflect the multifactorial nature of *C. difficile* infection and support multidisciplinary, cross-sector strategies in both research and clinical practice [112,113].

10. Discussion

CDI continues to pose a substantial clinical and economic burden worldwide. Despite advances in antimicrobial therapy and infection control, recurrence rates remain high, with 20–30% of patients experiencing relapse after an initial episode [114]. This underscores the necessity for strategies that go beyond symptom management to include prevention of

recurrence, preservation of the gut microbiota, and host-directed interventions. Antibiotics remain the mainstay of CDI treatment, with fidaxomicin and vancomycin recommended as first-line agents. Fidaxomicin offers advantages over vancomycin due to its narrower spectrum and lower recurrence rates, particularly in patients at high risk of relapse, despite higher acquisition costs [114]. Cost-effectiveness analyses indicate that the long-term benefits of sustained cure often outweigh the initial expense [114]. Extended-pulsed fidaxomicin regimens have been shown to be more effective than standard vancomycin therapy, and adjunctive treatments such as bezlotoxumab can further reduce the likelihood of recurrence in vulnerable populations [115].

Restoring the gut microbiota has emerged as a transformative approach in CDI management. FMT demonstrates consistently high efficacy in preventing recurrent CDI, with cure rates exceeding 90% in refractory cases [107]. However, donor variability and regulatory challenges have limited its widespread adoption. Standardized live biotherapeutic products (LBPs), such as REBYOTA and VOWST, offer reproducible and controlled alternatives. Emerging next-generation therapies, including VE303, aim to refine this approach by providing precision-engineered microbial consortia [116].

Prevention is a central goal at both individual and population levels. While traditional toxoid-based vaccines have faced setbacks, newer platforms such as mRNA vaccines show promise in eliciting durable immune responses in high-risk populations [86]. Host-directed therapies, including bile acid modulators and monoclonal antibodies like bezlotoxumab, complement antibiotic therapy by enhancing colonization resistance and neutralizing toxins [115]. These strategies reflect a paradigm shift from solely targeting the pathogen toward modulating the microbiota–host–pathogen axis.

System-level strategies are critical to CDI control. Antibiotic stewardship, rigorous hand hygiene, sporicidal cleaning, and rapid case identification are essential to limit transmission and preserve the effectiveness of novel therapies [65]. Reducing inappropriate antibiotic exposure not only prevents CDI but also mitigates selective pressures that could undermine future interventions.

CDI imposes significant economic and humanistic burdens. Sustained cure achieved through fidaxomicin, FMT, or LBPs has been identified as the primary driver of cost-effectiveness and improved patient outcomes [113,114,116,117]. Incorporating patient-reported outcomes into economic analyses highlights the profound functional and societal impact of recurrent CDI, emphasizing the value of preventive strategies.

The optimal management of CDI will likely involve an integrated, precision-based framework combining targeted antibiotics, microbiome restoration, immune modulation, and comprehensive public health measures [115–117]. Advances in rapid diagnostics, microbiome and genomic profiling, global surveillance, and vaccine development will be critical in achieving sustainable reductions in CDI incidence and recurrence [118].

Approximately one-quarter of patients experience recurrence of *C. difficile* infection following successful treatment of their initial episode, and the likelihood of additional recurrences rises sharply after the first relapse, with secondary recurrence rates reported to reach 40–60% in later episodes [119].

CDI remains a complex challenge at the intersection of antimicrobial efficacy, microbial ecology, and healthcare economics. The growing focus on microbiota-sparing therapies, preventive immunization, and host-directed interventions marks a new era in CDI management, offering the potential to significantly improve patient outcomes and reduce the burden on healthcare systems [65,113–117].

11. Future Directions

As the clinical and public health burden of CDI continues to grow, innovative strategies are needed to improve prevention, diagnosis, and treatment. Several promising avenues warrant focused research and investment:

- Personalized medicine: The heterogeneity of patient responses to CDI therapies highlights the need for individualized approaches. Profiling a patient's gut microbiome, immune status, and genetic susceptibility could guide targeted interventions—ranging from tailored antibiotic regimens to microbiome-modulating therapies—potentially reducing recurrence rates and improving outcomes.
- Rapid point-of-care diagnostics: Early detection is critical to preventing the spread of CDI and initiating timely treatment. Advances in portable, rapid diagnostic tools—such as molecular assays or biosensors—could enable near-instant identification of infection in community and outpatient settings, reducing delays associated with laboratory-based testing and limiting nosocomial transmission.
- Standardized FMT products: FMT has emerged as a highly effective therapy for recurrent CDI, but variability in donor material and protocols limits its widespread adoption. Developing standardized, well-characterized microbial consortia or "off-the-shelf" microbiota products could improve safety, reproducibility, and regulatory compliance, enabling broader and more consistent clinical application.
- Global surveillance networks: Coordinated international monitoring of CDI strains, antimicrobial resistance patterns, and outbreak clusters is essential for timely public health responses. Leveraging genomic sequencing and real-time data sharing can facilitate early identification of hypervirulent or resistant strains, guiding infection control strategies and informing global treatment guidelines.
- Prophylactic strategies: Prevention remains the ultimate goal in CDI management. Promising avenues include vaccine development targeting key bacterial toxins, bile acid-based therapies that restore colonization resistance, and precision probiotics designed to reinforce the gut microbiome. Rigorous clinical validation of these approaches could significantly reduce both primary infections and recurrences, particularly in high-risk populations.

Investment in these areas will be critical not only for improving patient outcomes but also for achieving long-term control of CDI. By integrating precision medicine, advanced diagnostics, microbiome-based therapies, and global surveillance, the field moves closer to mitigating the impact of this persistent healthcare challenge and ultimately reducing CDI to a manageable, low-burden infection (see Table 5).

Table 5. Future Directions for *Clostridioides difficile*.

Research Priorities	Potential Benefits	Focus	Future Direction
Microbiome profiling, host-genetics studies	Reduced recurrence, optimized treatment efficacy	Tailoring therapy using microbiome and host genetics	Personalized Medicine
Development of portable molecular/biosensor assays	Timely treatment, limited transmission	Faster, accessible testing for early detection	Rapid Diagnostics
Defining microbial consortia, regulatory frameworks	Greater safety, consistency, and clinical adoption	Safe, reproducible microbiota-based therapies	Standardized FMT Products
Genomic sequencing and real-time reporting	Early outbreak detection, global preparedness	Cross-border monitoring of strains and resistance	Global Surveillance
Large-scale clinical trials, mechanistic studies	Prevention of primary and recurrent CDI	Vaccines, bile acid therapies, targeted probiotics	Prophylactic Strategies

12. Conclusions

CDI remains a formidable challenge at the intersection of antimicrobial stewardship, microbial ecology, and healthcare resource management. Despite substantial advances in antibiotic therapy, recurrence rates remain unacceptably high, underscoring the limitations of pathogen-targeted approaches alone. Emerging strategies that prioritize microbiome preservation, host-directed interventions, and precision therapeutics—including live biotherapeutic products, fecal microbiota transplantation, and monoclonal antibodies—offer the potential to transform outcomes for high-risk patients. Concurrently, system-level interventions such as rigorous infection prevention protocols and antibiotic stewardship are critical to sustaining the efficacy of these novel therapies.

The integration of antimicrobial therapy, microbiome restoration, immunoprophylaxis, and rapid diagnostics represents a paradigm shift toward a holistic, patient-centered model of CDI management. Economic and quality-of-life analyses further reinforce that strategies aimed at a sustained cure—not merely symptomatic relief—provide the greatest value to patients and healthcare systems alike. Looking forward, precision medicine approaches leveraging microbiome and genomic profiling, combined with ongoing vaccine development and global surveillance, are likely to define the next era of CDI management. Collectively, these advances signal a transition from reactive treatment to proactive, recurrence-preventive care, with the potential to substantially reduce the clinical and economic burden of CDI worldwide.

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Abbreviations

The following abbreviations are used in this manuscript:

FDA	Food and Drug Administration
CDI	Clostridioides difficile infection
GDH	Glutamate dehydrogenase
LAMP	Loop-mediated isothermal amplification
NGS	Next-generation sequencing
FMT	Fecal microbiota transplantation

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