Review

Newer Therapeutics to Selectively Kill *Clostridioides difficile* and Restore the Microbiome

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Review

Newer Therapeutics to Selectively Kill *Clostridioides difficile* and Restore the Microbiome

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Abstract

Background/ Objectives: The antibiotic ibezapolstat and the live biotherapeutic product live-JSLM are promising future approaches for treating *Clostridioides* difficile infection. We summarised the available data on ibezapolstat and live-JSLM to treat *Clostridioides difficile* infection. **Methods**: Data sources: PubMed and SCOPUS databases were searched from The MEDLINE and SCOPUS databases from 1 January 2012 to 15 November 2025. Original articles reporting data on ibezapolstat and live-JSLM. Both quantitative and qualitative information were summarised by means of textual descriptions. **Results**: 32 studies were included, published from 15 March 2012 to 15 September 2025. When compared to conventional anti-*Clostridioides* difficile antibiotics ibezapolstat had similar level of effectiveness and minimal impact on the gut microbiota. The available data confirm live-JSLM safety and efficacy in restoring the gut microbiota following the conclusion of the standard anti-*Clostridioides difficile* antibiotic regimen. **Discussion**: The results on ibezapolstat efficacy are promising, but require confirmation in larger patient populations through double-blind, randomized phase III trials. In the near future an integrated approach may enhance the management of *Clostridioides difficile* infection: starting with highly specific antibiotics, i.e. ibezapolstat, followed by microbiome-based therapies such as live-JSLM.

Keywords: *Clostridioides difficile* infection management; prevention of recurrence of *Clostridioides difficile* infection; ibezapolstat; clinical trials; long term patients' outcome; quality of life; live-JSLM; Rebyota; microbiome-based therapies; fecal microbiota transplantation; live biotherapeutic product; novel antimicrobials; human gut microbiota; bile acids

1. Introduction

Clostridioides difficile (*C. difficile*) is a gram-positive, anaerobic, spore-forming bacterium, responsible for 12% of all healthcare-associated infections and recurrence rates reaching approximately 25% after an initial episode, escalating to 40% following the first recurrence [1].

Currently, *Clostridioides difficile* infection (CDI) represents a substantial burden worldwide, with over 400,000 infections and 200,000 hospitalizations annually in the United States [2] and around 124,000 cases and 3700 deaths annually in Europe [3].

Despite the considerable evolution of the therapeutic landscape for CDI in recent years, the management of CDI continues to represent a persistent challenge in daily clinical practice. Whilst antibiotics such as vancomycin and fidaxomicin remain the preferred treatment options, there are limitations to their effectiveness in preventing recurrence of CDI (rCDI) [4].

The high rate of rCDI with the currently available treatments has been demonstrated to have a detrimental effect on patients' quality of life, to cause additional costs and to increase overall mortality [5]. Furthermore, the occurrence of rCDI can lead to an increased risk of further rCDI episodes, often resulting in deterioration of frailty CDI patients experiencing a spiral of disease.

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The occurrence of rCDI is driven by the disruption of the gut microbiota [6]. This disruption can be induced by broad-spectrum antibiotic treatment, leading to a loss of bacterial diversity and a decrease in the proportion of bacteria that are essential for bile acid metabolism [6]. These changes may result in elevated primary bile acid levels, facilitating *C. difficile* spore germination and onset or rCDI [6,7]. The disruption of microbiota is characterised by a weakening of antagonistic relationships between microbes and impaired metabolic functions, including a decrease in the production of secondary bile acids. Secondary acids play a crucial role in the inhibition of the germination and growth of *C. difficile* spores, and therefore their decline can promote both CDI and rCDI [6,7]. To note, in CDI patients the reconstitution of a balanced, species-rich gut microbiota has been demonstrated to reduce the likelihood of rCDI [6,7].

The fecal microbiota transplantation demonstrated to be effective in reconstitution gut microbiota and treating rCDI [8]. However, the implementation of fecal microbiota transplantation is complicated by logistical issues, including donor screening, stool processing, and patient monitoring for adverse effects. Additionally, the lack of standardisation across centres further complicates the process.

In an ideal scenario, a new innovative pharmaceutical compound should demonstrate comparable levels of activity against *C. difficile* than traditional anti-CDI antibiotics, yet not exhibit any activity against the essential gut microbiota, thereby preventing gut dysbiosis. As an alternative approach, the ideal compound should rapidly and safely restore a healthy microbiota following the conclusion of the standard anti-CDI antibiotic regimen, thereby halting the germination of *C. difficile* and preventing rCDI.

Among the most promising future approaches for treating CDI and rCDI there are highly specific anti-CD antibiotics with minimal impact on the intestinal bacterial flora, such as ibezapolstat. Furthermore, approaches designed to restore the microbiota are promising, such as live-JSLM [10].

Therefore, ibezapolstat and live-JSLM represent two novel promising approaches to treat CDI and prevent rCDI.

This review has the aim to summarise the available data on the antibiotic ibezapolstat and the live biotherapeutic product live-JSLM to treat CDI and prevent rCDI.

2. Results

2.1. Included Studies

Supplementary Figure 1 shows the selection process of the included studies. After duplicate removal 37 studies were identified, published from the 15th March 2012 to the 15th of September 2025.

Three studies were excluded because review articles, editorials and clinical trial protocols. Three studies were excluded for not reporting data on ibezapolstat or live-JSLM.

Therefore, 31 studies were included in this review [11–41]. Of the 31 included studies, 11 provided data on ibezapolstat [11–21] and 20 studies evaluated live-JSLM [22–41].

Among the included studies on ibezapolstat, 4 studies reported on phase I or phase II clinical trials [18–21]; 5 were *in vitro* studies assessing ibezapolstat activity [11–14,16] and 2 were studies in the animal model [15,17]. Among the 20 included studies on live-JSLM, 8 reported on phase II trials [22–28,40], 10 reported on phase III clinical trials [29–31,33–37,39,41], 2 were retrospective observational studies [32,38].

A summary description of the included studies is reported in Supplementary Table S2 and Table S3.

2.2. Quality of the Included Studies

The quality appraisal of the studies is reported in Supplementary Table S1 and Table S2. From a qualitative standpoint, all the included studies appeared to fulfil most of the criteria.

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2.3. Ibezapolstat

Ibezapolstat is a novel antibiotic and dichlorobenzyl purine analogue with a unique mechanism of action. Ibezapolstat binds to and inhibits the deoxyribonucleic acid (DNA) polymerase IIIC component of aerobic, gram-positive bacteria, including *C. difficile* [11].

DNA polymerase IIIC is essential for replicative DNA synthesis in gram-positive bacteria of the phylum Firmicutes, with a low guanine and cytosine content (i.e. fewer guanine and cytosine bases than adenine and thymine bases). Ibezapolstat binds to bacterial DNA polymerase III via an enzyme-specific aryl domain. Binding of the compound to this domain results in the formation of an inactive ternary complex comprising the inhibitor, DNA and DNA polymerase IIIC. DNA polymerase IIIC is absent from the genomes of gram-negative bacteria, making ibezapolstat a target with a restricted phylogeny and a narrow spectrum of activity [11].

Ibezapolstat spares common, low guanine and cytosine content gut commensal bacteria such as *Lachnospiraceae, Oscillospiraceae* and *Coprobacillaceae* [12]. An *in vitro* study suggests that these bacteria are spared due to the phylogenetic variation of their DNA polymerase IIIC binding pocket residues [12].

A microbiological study conducted on a large collection of bacterial isolates confirmed ibezapolstat activity against *C. difficile* [13] and a study collecting 313 isolates from both in-hospital and community CDI patients, reported ibezapolstat MIC^{50} and MIC^{90} of 4 mg/L [14].

Other studies confirmed that ibezapolstat exhibits high in vitro activity against *C. difficile* isolates belonging to various serotypes, with an MIC^{50} of 4 mg/L and an MIC90 ranging from 4 to 8 mg/L [11,15,16].

A study was conducted to compare the changes in the microbiome associated with ibespopolstat with those associated with other traditional anti-CDI antibiotics. In the study, germ-free mice received a faecal microbiota transplant from healthy human donors and were then exposed to ibezapolstat, vancomycin, fidaxomicin or metronidazole. Each antibiotic produced a different outcome, but the changes in alpha and beta diversities following treatment with fidaxomicin and ibezapolstat were less pronounced than those observed with vancomycin or metronidazole [17].

A phase I trial assessed the pharmacokinetic profile of ibezapolstat in humans, with low systemic absorption and high concentrations in the colon, making ibezapolostat pharmacokinetics optimal for treating CDI [18].

A subsequent study was performed in order to assess the changes in the microbiome and bile acid changes associated with the administration of ibezapolstat, in comparison to those associated with vancomycin [19]. The fecal samples were obtained from the phase I healthy volunteer study [18]. Utilising metagenomic sequencing and mass spectrometry, the study found that both ibezapolstat and vancomycin exerted an effect on the human microbiome, but in distinct manners. A rapid increase in alpha diversity in the fecal microbiome was noted after starting ibezapolstat therapy, which was maintained after completion of therapy. After starting ibezapolstat, a decrease in *Bacteroidetes* phylum was observed, with a concomitantly increased proportion of *Firmicutes* phylum. Compared with baseline, total primary bile acids decreased by a mean of 40.1 ng/mg of stool during ibezapolstat therapy ($p<0.001$) [19].

A single-arm, open-label phase II trial was conducted to assess the clinical cure rates of CDI two days after the conclusion of treatment with ibezapolstat, as well as the ibezapolstat safety [20]. The trial enrolled 10 adult CDI patients who received 450 mg of ibezapolstat orally at 12-hour intervals for a period of 10 days. The patients were then observed to a 28-day follow-up period to assess the clinical response, identify any adverse events, and ascertain the status of the fecal microbiome. The initial clinical cure rate was reported in 10 out of 10 patients (100%). The mean time to resolution of diarrhoea was 5 days (range, 3–7 days). The sustained cure rate at 28 days was also 100% [20].

Recently, a larger phase II, randomised, double-blind, active-controlled trial compared ibezapolstat to vancomycin as a treatment for CDI patients [21]. The objective of the trial was to evaluate the efficacy, safety, pharmacokinetics, and associated microbiome changes of ibezapolstat in comparison with vancomycin for the treatment of adult CDI patients [21].

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In this trial, 32 adult CDI patients were randomly assigned (1:1) to receive either ibezapolstat, 450 mg orally twice daily for 10 days, or vancomycin, 125 mg orally four times daily for 10 days. Participants were assessed for clinical response to treatment 2 days after the end of therapy and at a follow-up study visit to assess for sustained clinical cure at day 38. A subset of participants who volunteered for long-term follow-up were also reevaluated at days 56 and 84 [21]. Overall, 16 patients in the ibezapolstat arm and 14 CDI patients in the vancomycin arm were included in the efficacy evaluation. 15 (94%) of the 16 participants in the ibezapolstat group had initial clinical cure, compared with 14 (100%) of 14 participants in the vancomycin group (treatment difference: −6.3%, 95% confidence interval: −30.7 to 19.4, p: 1.0). No participants in the ibezapolstat group had a recurrence assessed on day 28 after the end of treatment compared with 14% in the vancomycin group [21]. Moreover, 15 (94%) participants in the ibezapolstat group had sustained clinical cure compared with 12 (86%) in the vancomycin group (treatment difference: 8.0%, 95% confidence interval: -19.4 to 38.0, p: 0.59). Regarding safety analysis, both treatments were well tolerated with no drug-related serious adverse events. 18% (3/17 participants) in the ibezapolstat group had mild adverse events, including gastro-oesophageal reflux disease and nausea. Bile acid analysis showed that participants in the vancomycin group had decreased primary bile acid conversion, leading to decreased ratios of secondary to primary bile acids, a shift not observed in the ibezapolstat group [21].

2.4. Live-JSLM

Fecal microbiota, live-JSLM, previously designated as RBX2660, is a single-dose, live biotherapeutic product comprising a consortium of microbes prepared from human faeces [10]. Live-JSLM is constituted of a liquid suspension, comprising a diverse set of microorganisms and is administered via rectal administration, with the objective of reducing the recurrence rate of CDI in adults CDI patients [10].

The phase II, double-blind, randomised, placebo-controlled PUNCH CD2 trial enrolled and randomised 127 patients with recurrent CDI, demonstrating efficacy in restoring the composition of the gut microbiome and reducing the risk of recurrent CDI [22,23].

A substudy of this trial was conducted to characterise the fecal bacterial microbiome before and after the live-JSLM administration [24,25]. Live-JSLM was found to be more effective than the placebo at restoring participant microbiomes [24,25].

Subsequent to this trial, an open-label phase II clinical trials were performed [26]. The open-label trials comprised a total of 149 participants who were treated with live-JSLM, in comparison to a historical control group of patients. The trial comprised patients who had experienced two or more rCDI. The participants were administered two doses of live-JSLM rectally, with the doses administered seven days apart. The trial demonstrated that 78.9% (112/142) of patients administered with live-JSLM experienced no further instances of recurrent CDI for a period of 8 weeks following the conclusion of the study treatment, in contrast to the 30.7% (23/75) observed in the historical control group ($p<0.0001$) [26].

Theoretically, exposure to broad-spectrum antibiotics after live-JSLM administration may diminish its beneficial effects. A post-hoc analysis was performed on a subgroup of patients from the PUNCH open label study who subsequently received non-CDI antibiotics after live-JSLM administration [27]. This subgroup comprised 43 patients who primarily received fluoroquinolones, cephalosporins or penicillins to treat urinary or respiratory tract infections. The results were reassuring, with treatment response rates at eight weeks and one year higher than 90% [27]. A different post hoc analysis on a subgroup of 29 patients from the PUNCH CD2 trial confirmed the safety and efficacy of live-JSLM in rCDI and observed that live-JSLM reduces the abundance of antibiotic-resistant *Enterobacteriaceae* at 2 months after administration [28].

Subsequently, the PUNCH CD3 trial was conducted to demonstrate the effectiveness and safety of live-JSLM in treating patients with recurrent CDI. It was a large, prospective, randomised, double-blind, placebo-controlled, phase III study [29]. The participants were adults with rCDI who had completed one or more rounds of standard antibiotic therapy or had experienced two or more severe

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episodes of CDI resulting in hospitalisation within the past year. 267 patients were randomised 2:1 to receive live-JSLM or a placebo rectally, after completing a full course of antibiotic treatment for rCDI. Treatment success was defined as an absence of diarrhoea within 8 weeks from the treatment. Live-JSLM had a higher treatment success rate than placebo (70.6% versus 57.5%), and was well tolerated [29].

Post hoc analysis of the PUNCH CD3 trial demonstrated significant improvements in the quality of life of patients with a first recurrence of CDI who received live-JSLM [30,31].

Following the results of the PUNCH CD3 trial, the US Food and Drug Administration approved live-JSLM for the prevention of recurrent CDI in adults following standard anti-CDI antibiotic treatment.

Afterwards, a retrospective study was conducted, with the main aim to confirm the safety of live-JSLM in CDI patients [32]. Of 94 patients who were treated with live-JSLM, 82.8% responded at 8 weeks, and 88.7% of these patients had a sustained response at 6 months [32].

The PUNCH CD3-OLS was an open-label trial designed to assess the safety and efficacy of live-JSLM for prevention of rCDI in a large real-world, clinical practice population [33].

A total of 793 participants were enrolled in the study, 697 of whom received live-JSLM [33]. At 8 weeks from the live-JSLM administration, 47.3% of the study participants had experienced mild or moderate gastrointestinal adverse events. Serious adverse events were reported by 3.9% of participants. In the PUNCH CD3-OLS study, live-JSLM achieved a sustained clinical response in 91% of participants at 6 months [33].

A post-hoc subgroup analysis of the PUNCH CD3-OLS trial indicated that live-JSLM is also safe and effective as adjunctive treatment to prevent rCDI in patients with inflammatory bowel disease [34]. A post-hoc analysis confirmed the safety and efficacy of live-JSLM in moderately immunocompromised CDI patients, including those with neoplasms, renal failure, HIV infection, and those assuming concomitant immunosuppressants, either glucocorticoids or selective immunosuppressants [35].

In the five clinical trials conducted to evaluate live-JSLM, it was found to be safe in rCDI patients [36,37].

Moreover, 2 studies were conducted to further assess the safety and clinical effectiveness of live-JSLM when administered via colonoscopy [38,39]. The CDI-SCOPE trial (NCT05831189) was a single-arm study that evaluated the administration of live-JSLM via colonoscopy [39]. The trial comprised 41 rCDI adult patients. The analysis revealed that 39 participants (95.1%) experienced treatment success. A total of 5 treatment-emergent adverse events were reported in 4/41 participants (9.8%), all of which were of a gastrointestinal nature and mild in severity [39].

The results of the studies which evaluated the microbiome changes in patients enrolled in the PUNCH trials demonstrated that the alterations in the microbiome of patients receiving live-JSLM were associated with a clinical response [40,41]. The patients who exhibited a positive response to the administration of live-JSLM had higher proportion of *Bacteroidia* and *Clostridia* bacteria, accompanied by an escalation in alpha diversity [40,41]. Concurrently, a transition from primary to secondary bile acid dominance was reported [40,41].

3. Discussion

We conducted this review with the objective of synthesising the available data on the antibiotic ibezapolstat and the live biotherapeutic product live-JSLM for the treatment of *Clostridioides difficile* infection and the prevention of *Clostridioides difficile* recurrence. Overall, the current evidence regarding the novel antibiotic ibezapolstat appears encouraging.

Ibezapolstat was reported to have similar level of effectiveness against *Clostridioides difficile* when compared to conventional anti-CDI antibiotics [11,15–17]. However, it does not impact the beneficial gut microbiota, thereby helping to avoid gut dysbiosis [20].

Notably, the recent phase II clinical trial comparing ibezapolstat with vancomycin for CDI treatment showed positive effects on the gut microbiome [20]. These included the preservation of

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bacterial taxa involved in converting primary bile acids into secondary bile acids through the 7-α dehydroxylation pathway [20]. The observed reduction in primary bile acids, along with an improved secondary-to-primary bile acid ratio, suggests that ibezapolstat administration may lower the risk of CDI recurrence [6].

Although the results on ibezapolstat efficacy are promising, they require confirmation in larger patient populations through double-blind, randomized phase III trials.

As for live-JSLM, the available data confirm its efficacy in restore a healthy microbiota following the conclusion of the standard anti-CDI antibiotic regimen, thereby halting the germination of C. difficile and preventing rCDI [33–35].

Importantly, clinical trials assessing live-JSLM's safety have consistently confirmed its favorable safety profile [36]. The administration of live-JSLM rectally or via colonoscopy is practical, safe, and effective [33,39]. These findings suggest that live-JSLM could streamline the process of fecal microbiota transplantation, addressing current barriers that limit its broader use [42].

One limitation of our review is that the included studies were highly diverse and had different objectives. Furthermore, some included studies had a small patient's population. Acknowledging these pitfalls, the current evidence concerning the novel antibiotic ibezapolstat and the live biotherapeutic agent live-JSLM is promising.

In our opinion, in the near future an integrated approach will enhance the management of CDI and better address the underlying pathophysiology of microbiome dysbiosis, preventing CDI recurrence [9]. A future approach could involve a two-step treatment: starting with anti-C. difficile antibiotics, i.e. ibezapolstat, followed by microbiome-based therapies such as live-JSLM [9].

In our view, a combined therapeutic strategy may soon become the standard for managing CDI more effectively, targeting both the infection and the underlying microbiome imbalance to prevent the CDI recurrence.

4. Materials and Methods

The MEDLINE and SCOPUS databases were used to search for published articles on ibezapolstat and live-JSLM from the 1st of January 2012 to the 15th of November 2025.

The search terms [(ibezapolstat)] and [(Rebyota) AND (live-jslm) AND (RBX2660)] were used in the MEDLINE database in separate searches.

The SCOPUS database was searched using the following terms: ibezapolstat and Rebyota and live-jslm and RBX2660 in multiple searches.

No attempt was made to obtain information from unpublished studies. Review articles, correction articles, editorials, commentaries, case reports and clinical trial protocols were excluded from further assessment.

4.1. Eligibility Criteria

Original articles reporting data on ibezapolstat and live-JSLM were eligible for inclusion.

4.2. Quality Assessment

To evaluate the study quality of the selected papers, the Cochrane risk of bias tool for clinical trials in its updated version (RoB2) was used for randomized trials [43]. Observational studies were appraised through an adapted version of the Newcastle-Ottawa Scale (NOS) [44]. In this scale, observational studies were scored across three domains: selection (four questions), comparability (two questions), and ascertainment of the outcome of interest (three questions). Studies with fewer than 5 stars were considered of low quality (or a high risk of bias), 5 to 7 stars of moderate quality (and the same applies to the risk of bias), and more than 7 stars of high quality (low risk of bias). In addition to the score, a downgrading was performed if the study was not comparative.

2.3. Data Synthesis

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Quantitative and qualitative information were summarized by means of textual descriptions.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org. Figure S1: Search strategy through electronic databases. Table S2. Description of the included studies on ibezapolstat. Table S3. Description of the included studies on live-JSLM.

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