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MTT Clinical Protocol Guide

MTT Clinical treatment principles and protocol

For healthcare professionals

Microbiota transfer therapy (MTT) programme

MicroBiome Bank

v7.1 — 2026-08-09

This edition is bound to the normative core of the SIS v7.1 — Severity Scale and Normative Dose Axis (approved by Dr. Gábor Patay on 2026-08-09 at 08:08). The severity bands and the doses come from that document; the present guide attempts to describe the full clinical context of the MTT therapy and contains no band or dose definition of its own.

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Document details

Version	Status	Approved by	Approval timestamp	Normative source
7.1	Approved	Dr. Patay Gábor	2026-08-09 08:08	SIS v7.1 Severity Scale

What is this document about? This is the complete clinical handbook of microbiota transfer therapy: the hyperbolic treatment principle, the dysbiosis–CDI continuum, the preparation portfolio (HospBiome, DiffBiome, FindBiome, TransferBiome), the DEMI and DynaMAP frameworks, the three patient pathways, the 32-day diary, donor quality and the contraindications. The SIS system is one chapter among many here. The normative source of the severity bands and the doses is the SIS v7.1 Severity Scale; the scoring methodology of the questionnaire is described by the SIS v7.1 Clinical Guide.

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Contents

Introduction — audience and scope

This Guide is addressed to clinicians who already have some practical exposure to faecal microbiota transplantation (FMT) – or who are actively seeking to acquire it. It is not an introduction to the field for general audiences, nor is it a regulatory document. It is the operational reference of the MicroBiome Bank clinical programme: the framework we use day-to-day to triage patients, select preparations, and guide therapy through to resolution.

The content distilled in these pages is the product of twelve years of accumulated clinical experience – patient cases ranging from first-presentation CDI to refractory recurrence, from straightforward dysbiosis to chronic-disease pathways extending over years. Threaded into this experiential foundation are the materials that were presented at international conferences over the past decade, the personal consultations we have held with clinicians and researchers in our field, and our own internal observations from continuous donor-screening, capsule-manufacturing, and patient-monitoring practice.

Where the published peer-reviewed literature offers relevant evidence, we have integrated it with appropriate caution – taking into account both the level of the underlying study and the operational context in which the evidence was generated. We do not treat published findings as automatically applicable; we treat them as inputs that must be reconciled with what we observe directly in our clinical work. Where the literature supports a position, we cite it (Sections X–XI). Where the literature is silent or inconsistent, we state our operational position openly.

This document is not a final or definitive text, and never can be. The clinical understanding of microbiota-based therapy is evolving rapidly, and our own protocols are refined continuously as new patient data, donor cohorts, and external research accumulate. We rewrite sections of this Guide as our understanding deepens; the version number and date in the cover reflect the state at the time of issue, not a frozen consensus.

We are open to feedback, dialogue, and the integration of further clinical experience – particularly where that experience is progressive, well-documented, and grounded in direct patient observation. Clinicians who wish to contribute observations, propose refinements, or raise concerns are invited to contact the MicroBiome Bank clinical team directly. The Guide improves through the same mechanism that built it: structured, evidence-aware exchange between practitioners.

I. Foundational Concepts – the Hyperbolic Treatment Principle

I.1 The Dysbiosis – *C. difficile* Continuum

The clinical framework adopted by MicroBiome Bank rests on a single foundational reframing: *Clostridioides difficile* infection is not an isolated, externally acquired disease, but the acute exacerbation of an underlying dysbiotic state [001], [002]. Dysbiosis – the loss of microbial diversity and the disruption of competitive ecological balance within the colonic microbiota – is the soil. *C. difficile* overgrowth, toxin production, and the resulting clinical picture are the inevitable consequences of that soil reaching a critical degradation threshold [044], [003].

This reframing has direct therapeutic implications. If CDI is the acute manifestation of a chronic ecological disruption, then any intervention that targets only the acute episode – without addressing the underlying dysbiosis – is, by definition, incomplete. Recurrence is not a treatment failure; it is the natural behaviour of an unresolved system returning to its previous state. The aim of MTT (Microbiota Transfer Therapy – restoration of microbiota including the use of FMT), in this framework, is not to suppress an organism but to restore an ecology [051], [057], [048].

I.2 The Hyperbolic Treatment Curve

Dose intensity in MTT does not scale linearly with disease severity. Clinical observation across a decade of practice has shown that effective treatment follows a hyperbolic relationship between symptom severity and required microbiota density: as severity increases, the dose required for stabilisation increases sharply, and as symptoms recede, the maintenance dose declines disproportionately fast. The seminal evidence that microbiota restoration – not pathogen suppression – produces durable resolution comes from the van Nood randomised trial [029], from its ten-year follow-up [030], and from real-world capsule-based FMT cohorts [052] together with their median 182-week follow-up [049]. The curve is presented here as a clinical observation drawn from operational practice rather than as a formal dose-finding study. The published lyophilised-capsule FMT cohorts use a fixed dose and report no dose-reduction data [049], [052]; the operational thresholds applied below therefore derive from our own clinical practice, and no published dose-response data currently supports them.

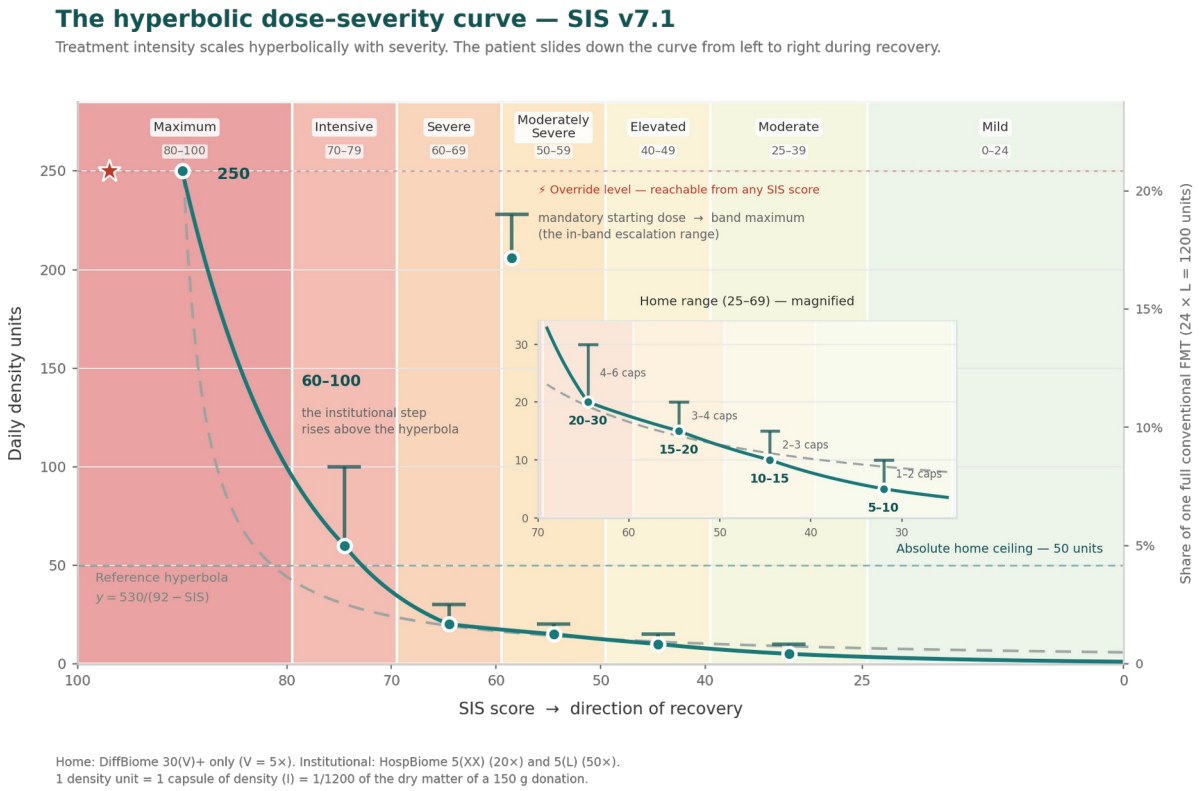

Figure 1.1 – The hyperbolic treatment curve. Dose intensity scales non-linearly with symptom severity. Patients "descend" the curve as the underlying ecology stabilises.

The treatment metaphor we use clinically is that of "sledding" down the curve: the patient enters at the steep, high-dose end during the acute phase and gradually slides toward the asymptotic, low-dose tail as the microbiota recovers its functional diversity. The endpoint is not zero capsule consumption per se, but a self-sustaining ecology that no longer requires exogenous support to maintain its competitive structure against opportunistic colonisation.

Key Principle

Treatment intensity must match the position of the patient on the hyperbolic curve. An under-dosed acute phase fails to stabilise; an over-dosed maintenance phase displaces functional recovery and inflates cost without therapeutic benefit. The SIS scoring system (Section III) exists precisely to localise the patient on this curve and to ensure the efficacy of treatment while keeping the financial burden to a minimum.

I.3 Why Eradication Is Not the Goal

A common misconception – both in clinical practice and in patient expectation – is that the success of MTT is measurable by the disappearance of *C. difficile* from stool testing. This metric is misleading for two reasons.

First, *C. difficile* is part of the commensal microbiota of a non-trivial fraction of the healthy adult population. Its mere presence is not pathognomonic; its overgrowth and toxin expression in the context of dysbiosis is what produces disease. A negative test in a still-dysbiotic patient is to be regarded as a fragile result.

Second, the high-dose, narrow-window suppression of *C. difficile* – whether by antibiotics or by aggressive single-shot MTT – can produce short-term test negativity while leaving the underlying ecological vacuum unaddressed. The patient appears cured, but the dysbiotic terrain is intact [033], and recurrence is statistically highly likely [031], [028]. In the United States the overall burden of CDI declined between 2011 and 2017 — chiefly through the retreat of ribotype 027 — but the estimated number of recurrent cases did not [032].

The clinical aim, therefore, is not eradication but resolution: returning the patient to a state in which (a) symptoms are absent, (b) functional digestive and immunological parameters are restored, and (c) the residual microbiological risk profile is comparable to that of the general population. The patient is sledded down the curve until they are symptom-free, complaint-free, and risk-free. Defined bacterial consortia follow the same logic: in a phase 2 dose-ranging trial the eight-strain VE303 reduced recurrence in high-risk patients — the phase 3 trial is still under way [056].

II. The Disease Continuum – Stations and Trajectories

II.1 From Dysbiosis to Pseudomembranous Colitis

The clinical picture associated with *C. difficile* is not a binary – either present or absent – but a progression. We treat it as a series of stations along a single trajectory, with movement between stations driven by the depth of dysbiosis, the persistence of triggering factors (most commonly antibiotic exposure), and the underlying immunological reserve of the host.

The acute trajectory, where it goes unchecked, leads through clinically distinct stations:

- Mild dysbiosis: subclinical loss of diversity, often asymptomatic or presenting only as transient bowel irregularity.
- Symptomatic dysbiosis: persistent loose stools, bloating, post-prandial discomfort; functional but increasingly fragile microbiota.
- Toxin-positive: *C. difficile* overgrowth – the diagnostic threshold of CDI, with watery diarrhoea, abdominal cramping, and elevated inflammatory markers.
- Severe CDI: high stool frequency, dehydration risk, marked leucocytosis, often febrile.
- Pseudomembranous colitis: endoscopically observable mucosal pseudomembranes, transmural inflammation.
- Toxic megacolon, perforation, and death: the terminal acute station. Fulminant CDI accounts for 3–5% of all CDI cases and carries a mortality of 30–40% [058]; among patients requiring colectomy, in-hospital mortality rose from 21.0% to 30.7% between 2018 and 2022 [059] — current antibiotic-led management has not brought this rate down.

II.2 The Chronic Trajectory: Toward Colorectal Carcinoma

The chronic trajectory is less acutely visible but no less important. Persistent low-grade dysbiosis sustains a state of chronic mucosal inflammation, oxidative stress, and altered metabolite production — notably reduced short-chain fatty acid synthesis [044], [010]. The direction of the bile acid shift here is the opposite of the one seen in the dysbiosis that leads to CDI: there it is the loss of secondary bile acids

that permits *C. difficile* germination [003], whereas in the pattern associated with a high-fat diet deoxycholic and lithocholic acid accumulate and, being more hydrophobic, are more carcinogenic than primary bile acids — releasing reactive oxygen species that damage DNA [060]. Over years to decades, this terrain is associated with:

- Inflammatory bowel disease (IBD): CDI is more frequent in IBD [040] and follows a more severe course [039];
- Functional disorders such as IBS with refractory symptoms;
- Metabolic dysregulation contributing to obesity and insulin resistance [045], [046];
- Neurological disturbance via the gut-brain axis (Parkinson's disease prodromal signs, ASD-related symptoms, age-related cognitive decline);
- **Colorectal carcinoma – the most consequential chronic endpoint, with growing evidence linking microbial composition and metabolic output to mucosal carcinogenesis, so far at the level of association [042], [043], [050], [054].**

Both trajectories – acute decompensation toward fatal colitis and chronic progression toward malignancy – share a common origin in the dysbiotic state. This is why we view CDI not as a disease in isolation, but as one possible indicator of a much larger condition, and why our treatment principle prioritises the resolution of the underlying ecology rather than the suppression of any single endpoint.

II.3 Stations on the Curve

A patient may enter our clinical pathway at any station. Equally, under treatment they move between stations – sometimes in the desirable direction (from the more severe stations toward the milder ones), but occasionally in the reverse, when the underlying triggers (antibiotic re-exposure, dietary disruption, immunological insult) reactivate the trajectory. The reclassification rules in Section III.5 address precisely this bidirectional movement.

Clinical implication: A patient currently classified as "Dysbiosis" or "IBS/IBD" in our system is not in a different disease from a CDI patient – they are at a different station on the same continuum. Our protocols allow movement between treatment groups as the underlying state changes (Section VI.4).

III. The Symptom Importance Scale (SIS)

III.1 Purpose and Structure

The Symptom Importance Scale (SIS) is a structured clinical scoring instrument developed and maintained by MicroBiome Bank to localise a patient on the hyperbolic treatment curve described in Section I.2. It integrates 23 weighted variables drawn from four domains:

- Patient-reported symptoms (stool frequency, abdominal pain, fatigue, etc.);
- Laboratory findings (toxin assay status, inflammatory markers, electrolyte disturbance);
- Prior treatment data (number of antibiotic courses, prior MTT episodes, recurrence count);
- Known risk factors (age, comorbidity profile, immunological state, hospitalisation history).

The output is two independent sub-scores (A-SIS and P-SIS, see III.2 and III.3), with a combined maximum of 100 points. The questionnaire is anonymised at the patient end and reviewed by a physician before any treatment decision is finalised.

III.2 A-SIS – Acute Severity Index

Maximum: 44 points. The A-SIS measures current clinical severity – in plain terms, how unwell the patient is right now. It determines whether immediate intervention is required and, if so, what dose intensity is needed in the initial phase (a minimum of 10 days at the starting dose).

Clinical question: How sick is the patient right now?

Variables contributing to A-SIS include stool frequency, consistency and formedness (Bristol scale), abdominal pain severity, oral intake tolerance, dehydration risk, leucocytosis, and presence of systemic inflammatory signs. A-SIS is the variable most responsive to short-term treatment effect; it should fall measurably within the first treatment cycle if the protocol is correctly matched.

III.3 P-SIS – Prognostic Risk Index

Maximum: 56 points. The P-SIS measures long-term risk and likelihood of therapy resistance – how easily, or with what difficulty, the patient is expected to respond to treatment. It is calculated from prior antibiotic exposure, comorbidity burden, recurrence history, age, immunological state, and lifestyle factors.

Clinical question: How dangerous is the situation long-term?

P-SIS is comparatively stable in the short term and changes principally with sustained MTT, comorbidity management, and lifestyle modification. A patient with a low A-SIS but high P-SIS is in remission of acute symptoms but remains at elevated risk of recurrence and warrants extended maintenance or compatibility-driven TransferBiome therapy (Section IV.5).

Two patients with identical total SIS scores may receive markedly different protocols if the acute and prognostic components differ in dominance. The SIS is best understood not as a single number but as a two-dimensional clinical characterisation.

III.4 Severity Categories and Recommended Actions

The combined SIS total (0–100, complemented by the option of a clinical override) defines seven treatment levels. **The table below is adopted from Chapter III of the SIS v7.1 Severity Scale; the normative source of the band boundaries and the doses is that document, and the present Guide defines none of its own.** The scale has been aligned with current IDSA/SHEA severity stratification [023], [028]; physicians may deviate from it where warranted on the basis of overall clinical judgment.

#	SIS	Name	Care setting	Preparation	Starting dose	Band max.	Density units
1	0–24	Mild	Home observation	—	—	—	—
2	25–39	Moderate	Home treatment	DiffBiome 30(V)+	**1**/day	2/day	**5 → 10**
3	40–49	Elevated	Home treatment with medical consultation	DiffBiome 30(V)+	**2**/day	3/day	**10 → 15**
4	50–59	Moderately Severe	Home treatment under close supervision	DiffBiome 30(V)+	**3**/day	4/day	**15 → 20**
5	60–69	Severe	Hospital assessment; may be continued at home	DiffBiome 30(V)+	**4**/day	6/day	**20 → 30**
6	70–79	Intensive	Hospital treatment	HospBiome 5(XX)	**3**/day	5/day	**60 → 100**
7	80–100	Maximum	Immediate hospital admission	HospBiome 5(L)	**5**/day, min. 3 days	—	**250**
⚡	override	HospBiome override	Immediate hospital admission	HospBiome 5(L)	**5**/day	—	**250**

The **starting dose is a mandatory initial value** – the course begins with it. The **band maximum is strictly an escalation value** in the event of non-response within the band, not a freely selectable range. If daily stool frequency does not decrease beyond 48 hours, the dose may be raised to the band maximum; if there is no response there either, the starting dose of the next level or a LOT switch comes into consideration, and the SIS must be re-administered. In the home bands, non-response persisting at 10 capsules/day (50 units) warrants institutional referral.

1 density unit = 1 capsule of density (I) = 1/1200 of the dry-matter content of the 150 g donation. V = 5×, XX = 20×, L = 50×. Absolute home ceiling: 10 capsules/day (50 units) — exclusively in an exceptional situation, under medical supervision.

In the 0–24 (mild) band no capsule is required: the emphasis is on fluid replacement, observation, and lifestyle change. The levels are identical to the triage used in the patient guide (DiffBiome Handbook, I.2) and to the seven bands of the figure in Section I.2. The clinical override (suspected toxic megacolon, fulminant colitis, severe dehydration, sepsis criteria) can require immediate hospital admission independently of the calculated score. The SIS is a decision-support, not a decision-making tool.

III.5 Reclassification Rules

Re-administration of the SIS is recommended in the event of non-response; the normative source deliberately prescribes no fixed interval — the timing is a clinical decision. The purpose of re-scoring is to track movement on the disease continuum. The mutually aggravating relationship between CDI and IBD — CDI is more frequent in IBD [040] and follows a more severe course [039] — makes this dynamic reclassification clinically essential; **three triggers** govern movement between treatment groups. **This table is the single normative source of pathway reclassification within the present Guide; Section VI.4 adopts the same triggers and adds only the operational action.**

Trigger	From → To	Action
SIS falls below 40 in a patient on the CDI protocol	Acute CDI → General dysbiosis	Switch to the dysbiosis protocol; FindBiome compatibility test for personalised TransferBiome therapy.
SIS reaches or exceeds the 50–60 band in a patient on a dysbiosis or IBS/IBD protocol	Dysbiosis / IBS / IBD → Acute CDI	Switch to the CDI protocol. Prescribe DiffBiome or HospBiome according to the score. Specialist supervision required.
SIS remains persistently below 40 after a CDI episode	Acute CDI → return to the original protocol	Continue the FindBiome / TransferBiome process where applicable. Continue SIS monitoring.

The "50–60" band is an indeterminate value, and is cited as such in the normative source as well. It is to be resolved to a single, specific score at the time the SIS is administered.

III.6 Physician Override and Validation

The completed SIS is, in every case, reviewed by a physician before treatment is finalised. The physician may adjust the assigned scores based on additional clinical data not captured by the questionnaire, and may activate the "HospBiome override" classification – which necessitates institutional care – regardless of computed score where critical signs are present (toxic megacolon suspicion, fulminant colitis, severe dehydration, sepsis criteria).

In addition to the hard override, the normative source also prescribes a **soft override**. The soft override serves to protect the fulminant first episode: the patient who is acutely severe but, in the absence of recurrence, produces a low P-SIS, was previously positioned in the middle range.

Condition	Minimum classification
A-SIS ≥ 30	Level 5 (60–69, Severe)
A-SIS ≥ 38	Level 6 (70–79, Intensive)

Hierarchy: hard override > soft override > score.

The thresholds (30 and 38) are not ROC-optimised. The mechanism takes the seven-band system as its basis: the thresholds are tied to the 60 and 70 band boundaries, and are not interpretable under the earlier five bands.

Internal prospective validation of the SIS – including refinement of weighting coefficients and category thresholds against treatment outcomes – is ongoing at MicroBiome Bank, with periodic re-evaluation of operational thresholds based on accumulated clinical practice. The instrument is therefore best understood as a working clinical tool refined through continuous use, not a frozen scoring system.

Important: The SIS is a decision-support instrument, not a decision-making instrument. The automated category assignment must never be acted upon without physician review. Treatment decisions are made under medical supervision; the score provides guidance.

IV. Treatment Protocol & Capsule Portfolio

IV.1 The Sliding Scale Concept

The capsule portfolio is structured to follow the hyperbolic curve from acute hospital management through home treatment to long-term maintenance. Each preparation is differentiated principally by microbiota density (dry-matter concentration in the lyophilised material), expressed by Roman-numeral suffixes attached to the product name, and by packaging unit count (Arabic numerals).

The treating physician selects a starting product based on SIS classification, then steps the patient down through preparations of decreasing density as A-SIS falls. This sliding scale is the operational expression of the hyperbolic principle described in Section I.2.

The protocol fixes the dose in daily **density units**, from which the capsule count is derived: daily density units = daily capsule count × density factor (V = 5×, XX = 20×, L = 50×). When changing preparation — in escalation and in dose reduction alike — the clinician calculates the density equivalent (density × capsule count) and raises or lowers within that. Viewed in capsule counts, the same step is sometimes an eightfold increase and sometimes a reduction, which is why the capsule count in itself cannot be used as a basis for decision.

The administration regimen is a property of the portfolio, not part of the description of any one preparation, which is why it is given here and applies equally to every preparation in the portfolio: first thing in the morning, on an empty stomach, swallowed whole with plenty of water; further fluid intake approximately 30 minutes later. Simultaneous consumption with alcoholic or hot liquids is to be avoided. The unopened bottle is to be kept in the refrigerator, in a dark place, standing upright; the freezer is appropriate for long-term storage. The capsule is not damaged by brief periods at room temperature, but storage must, at minimum, be in a refrigerator.

IV.2 HospBiome – Hospital Treatment

HospBiome 5(L) – 5 capsules per packaging unit, 50× lyophilised density, therapy initiation. Indicated for hospitalised CDI patients unable to consume liquids orally or at risk of dehydration. Administered for a minimum of 3 days until reduction in stool frequency is achieved. May be opened and emulsified in a neutral liquid (water, cold tea, plain yogurt) for nasogastric tube administration if oral administration is compromised.

HospBiome 5(XX) – 5 capsules per packaging unit, 20× density. **The entry point at level 6 (SIS 70–79)**: starting dose 3 capsules/day, band maximum 5 capsules/day. It is at the same time the first dose-reduction step of a therapy initiated at level 7: indicated for continuation of treatment initiated with HospBiome 5(L), once stool frequency has demonstrably decreased. Administered to patients capable of oral fluid intake and not at risk of dehydration. Continue until clinically significant symptoms are fully resolved.

HospBiome 5(V) – 5 capsules per packaging unit, 5× density, late hospital phase. Indicated for the late phase of hospital treatment, and for continuation of HospBiome 5(XX) once stool frequency has further decreased; it is at the same time the transition to the home preparation.

HospBiome 15(V) – 15 capsules per packaging unit, 5× density. Clinically identical to 5(V): **the same capsule, differing solely in pack size** (an economical pack designed for Hungary). Pack size is a matter of procurement quantity and cost, and therefore does not appear in the treatment matrix.

Discharge: DiffBiome 30(V)+ at 4 capsules per day — the starting dose of level 5 — then dose reduction according to Section IV.6.

IV.3 DiffBiome – Home Treatment

Only DiffBiome 30(V)+ may be given at home; there is no other home preparation. The former DiffBiome 30(V) and DiffBiome 15(V) packs have been withdrawn and can no longer be ordered.

DiffBiome 30(V)+ – 30 capsules per packaging unit, fivefold (V) density, pooled graft. The "+" indicates a homogenised mixture of raw materials collected over several days from the same donor. This is the home preparation at levels 2–5 (SIS 25–69); the daily capsule count is given by the matrix in Section III.4 (starting dose, and the band maximum in the event of non-response).

The administration regimen is given in Section IV.1; it applies equally to every preparation in the portfolio.

IV.4 FindBiome – Donor Compatibility Test

FindBiome is a 4×15-capsule diagnostic preparation designed to identify the donor LOT most compatible with the recipient prior to MTT in chronic-disease patients. Each of the four boxes contains capsules from a different donor LOT. The patient takes one box at a time, separated by a 3-day washout, while completing a structured 32-day diary recording stool, symptoms, hydration, mood, sleep, and dietary intake. The principle that mucosal compatibility is highly person-specific – and not predicted by stool sampling alone – is supported by direct mucosal sampling studies [034].

The compatibility analysis is not a measure of efficacy – it serves to minimise side effects. The LOT that produces the fewest adverse effects in the patient becomes the substrate for subsequent TransferBiome therapy. This step is essential before initiating long-term MTT for chronic conditions, where the duration of treatment makes side-effect tolerance the dominant clinical concern.

IV.5 TransferBiome – Long-Term MTT

TransferBiome is the long-term therapeutic preparation, prepared from the donor LOT identified through FindBiome compatibility testing. The standard course is the administration of a defined number and density of capsules over a minimum of 60 days. For chronic conditions and for the dysbiosis pathway following CDI resolution, this is the principal therapy. The combination of compatibility-matched donor material and long duration is what enables the underlying ecology to regain its functional structure rather than merely being recolonised; longitudinal microbiota data show that after MTT the recipient microbiota returns to the healthy range within days, but then fluctuates dynamically over both the short and the long term and diverges from the original donor implant — dynamic behaviour being an intrinsic property of healthy faecal microbiota [048]. Successful MTT therefore establishes a self-sustaining recipient ecosystem rather than a permanent donor-shaped imprint.

IV.6 Tapering and Dose Reduction

Tapering follows the descending arm of the hyperbolic curve. Its pace is set by the patient's condition, not by the calendar. **There is no fixed day-ladder.** The minimum course length in every band is 10 days at the starting (or escalated) dose; dose reduction below this is not recommended, however quickly the patient improves.

Reduction may only be initiated once **every** condition of the symptomatic gate is met:

Criterion	Requirement
Elapsed time	≥ 10 days at the starting (or escalated) dose
Bristol trend	Sustained shift from the baseline 6–7 range toward 5–6, and from the 5–6 range toward 4–5
Stool volume / frequency	The extreme daily stool count has decreased substantially relative to baseline
Blood in the stool	None

The Bristol trend and the stool count are to be read together: an improvement in the Bristol value on its own, without a decreasing stool count, is not sufficient.

The procedure:

- −1 capsule every 3–4 days, for as long as the gate holds;
- If stool consistency returns to types 5–6 or 6–7 or the stool count rises: back to the band maximum, hold, consider a LOT switch; if recurrence is repeated, a different LOT must be used;
- Follow-up within 1–2 weeks after the final dose, to confirm the therapeutic outcome.

Caution: Tapering must not be prematurely terminated. Premature cessation in a patient with elevated P-SIS (high prognostic risk) is the most common cause of recurrence. P-SIS reduction lags A-SIS reduction by weeks to months and should govern the maintenance phase duration.

V. The DEMI Framework & DynaMAP Analysis

V.1 The DEMI Questionnaire

DEMI – Dysbiosis: Etiology, Manifestation and Intervention – is the structured clinical questionnaire developed by MicroBiome Bank to characterise the dysbiotic state in patients pursuing the chronic disease pathway. It is administered as part of the "exposome consultation", a 70-minute clinical consultation during which etiological factors (prior antibiotic exposure, dietary history, environmental exposures, etc.), manifest symptoms (digestive, neurological, metabolic, immunological, etc.), and prior interventional history (probiotics, dietary changes, prior MTT, surgical history) are documented.

The output of the DEMI questionnaire feeds into the patient's clinical record and informs the interpretation of the DynaMAP profile (Section V.2). It does not produce a single severity score; it is a multidimensional characterisation document.

V.2 The DynaMAP Profile

DynaMAP – Dynamic Microbiota Allocation Profile – is a microbiota composition analysis based on a patient stool sample, presenting results across functional taxonomic groups rather than as a static species inventory. The profile emphasises the dynamic aspect: which bacterial families are functionally dominant, which are depleted, and which compensatory shifts have occurred. Results are typically available within 16–20 days.

V.3 Clinical Application

DEMI and DynaMAP together produce a clinical characterisation that allows three things which the SIS alone cannot provide:

- Identification of specific functional deficits that the donor microbiota should address – informing LOT selection beyond the compatibility test result alone;
- Recognition of etiological factors that must be removed from the patient's environment for treatment to hold (typically dietary or pharmacological);
- Establishment of a baseline against which post-MTT recovery can be measured objectively.

For acute CDI patients, DEMI/DynaMAP is generally deferred until the acute phase has resolved, that is, until the **A-SIS** has fallen substantially. This is a matter of timing and is not the same as the pathway reclassification threshold of Section III.5, which applies to the **total SIS score**. For chronic-disease patients on the compatibility-based pathway (Section VI.3), DEMI/DynaMAP is performed as a parallel diagnostic alongside FindBiome compatibility testing.

VI. Patient Pathways

VI.1 Acute CDI Pathway

Entry: laboratory-confirmed CDI, or clinically dominant CDI presentation. The pathway consists of five steps:

1. Patient registration and intake assessment;
2. SIS questionnaire (acute focus, A-SIS dominant);
3. Capsule order: HospBiome (hospital) or DiffBiome (home) – selected by SIS category;
4. Initial therapy at the starting dose for the assigned category, for a minimum of 10 days; tapering begins only once the symptomatic gate of Section IV.6 is met;
5. Re-assessment by SIS; on stable SIS < 40, transition to the dysbiosis pathway (Section VI.4).

VI.2 SIS-Screened Pathway

Entry: IBS, IBD, or general dysbiosis presentation, with possible underlying CDI risk. The pathway consists of seven steps:

1. Patient registration and intake assessment;
2. Goals & Expectations questionnaire (clinical context, realistic targets, measurability);
3. SIS questionnaire (full, both A-SIS and P-SIS);
4. SIS-driven decision: if SIS < 40, proceed to the FindBiome compatibility test; if the SIS reaches or exceeds the 50–60 band, divert to the acute CDI pathway temporarily;
5. FindBiome compatibility test (4 LOTs over 32 days with diary);
6. TransferBiome MTT with the matched LOT (over a minimum of 60 days);
7. Tapering, antibiotic-free nutrition, and long-term maintenance.

VI.3 Compatibility-Based Pathway

Entry: chronic conditions with established dysbiotic terrain – ASD/ADHD, obesity / type II diabetes, Parkinson's disease, multiple sclerosis, malignant tumour. In these indications MTT is an experimental intervention: controlled clinical evidence is limited or absent, and where randomised trials have been conducted — in obesity, type 2 diabetes and autism spectrum disorder — the primary endpoints did not improve. In Parkinson's disease three randomised trials have been published, with contradictory results. In malignant tumour the strongest available data concern not standalone MTT but FMT added to immune checkpoint inhibitor therapy: in a phase 2 trial without a control arm, objective response rates of 80 percent in non-small cell lung cancer and 75 percent in metastatic melanoma were measured, alongside a grade 3 or higher adverse event rate of 65 percent in the melanoma cohort [053]. The pathway is therefore to be initiated only on individual assessment and with informed consent. Six steps, no SIS gating:

1. Patient registration and intake assessment;
2. Goals & Expectations questionnaire;
3. FindBiome compatibility test (32 days, 4 LOTs, full diary);
4. DynaMAP analysis and DEMI consultation (in parallel);
5. TransferBiome MTT with the matched LOT (minimum 60 days, though usually considerably longer);

6. Tapering, antibiotic-free nutrition, and long-term maintenance.

VI.4 Reclassification Between Pathways

The continuum framing (Section II) allows formalised reclassification when a patient's state changes. **The source of the triggers is Section III.5; the table below adopts the same ones and adds only the operational action.** The greater frequency of CDI in IBD [040] and its more severe course [039] make formalised reclassification a clinical safety mechanism rather than an administrative convenience:

Trigger	From	To	Operational action
SIS falls below 40 in a patient on the CDI protocol	Acute CDI pathway	Dysbiosis (compatibility-based)	"Switch to Dysbiosis" notification, followed by a FindBiome ordering link.
SIS reaches or exceeds the 50–60 band in a patient on a dysbiosis or IBS/IBD protocol	SIS-screened or compatibility-based	Acute CDI (temporary)	"Switch to CDI" notification; specialist supervision; DiffBiome/HospBiome prescribed.
SIS remains persistently below 40 after a CDI episode	Acute CDI pathway	Original pathway (resumed)	Continue the FindBiome / TransferBiome process where applicable.

VII. Patient Monitoring & Follow-Up

VII.1 The 32-Day Diary Protocol

During the FindBiome compatibility test and the early phase of TransferBiome therapy, the patient maintains a structured 32-day diary recording, daily, the following variables:

- Capsule intake: capsule count, LOT identifier, time of dosing;
- Concurrent medications (any change must be flagged);
- Hydration: fluid intake total in litres, distribution across the day (on request);
- Stool: frequency, consistency on the Bristol scale, urgency, any unusual features;
- Symptom intensity: bloating, abdominal pain, fatigue, mood, sleep quality;
- Diet: meal composition, antibiotic-free protein source confirmation, any deviations;
- General health: blood pressure, temperature, body weight (weekly), exercise.

Diary entries are logged through a structured questionnaire linked to the patient identifier (userID). The diary is not optional – it is the substrate for clinical interpretation. Rest days are recorded with the same rigor as treatment days.

VII.2 Bristol Stool Scale

Stool consistency is recorded against the standard Bristol scale (types 1–7), the validated stool-form instrument originally described by Lewis and Heaton [020]. For the purposes of MTT monitoring:

- Types 1–2: constipation; uncommon during MTT, may indicate delayed transit;
- Types 3–4: normal, the target consistency;
- Type 5: mild loose stool, usually transient;
- Types 6–7: loose to watery; expected during the acute phase, persistent presence after day 10 indicates inadequate response.

VII.3 LOT Tracking and Compatibility

Every capsule consumed during FindBiome and TransferBiome therapy is logged using its LOT identifier. This is a requirement that cannot be bypassed, for two reasons. First, the analysis of compatibility relies on attributing observed adverse effects to the correct donor LOT. Second, in the

event of an adverse outcome, the LOT identifier permits traceability to the donor and the manufacturing batch.

If a relapse occurs during tapering or after treatment, repeat treatment may be initiated – but on a different LOT. Repeated relapse on the same LOT is an indication that the matched donor material is no longer adequate for the patient's evolved state, and re-testing through a fresh FindBiome cycle is warranted.

VIII. Donor Quality & the Food–Microbiota Link

VIII.1 The Donor Screening Framework

Donor quality at MicroBiome Bank is governed by a formalised screening instrument – the Donor Screening Questionnaire (DSQ) – currently in version 4.0. The DSQ produces a Total Score for each donor candidate composed of three additive components: a Base Score (145–165 points, age-adjusted), Weighted Points (–127.2 to +61.4, derived from 99 clinical questions across 7 weighted categories), and an SD Bonus (0–95 points) recognising elite microbiota-relevant health markers. The total scale spans 18 to 321 points, with eligible donors typically scoring between 150 and 280 points in practice.

The seven scoring categories reflect the relative clinical importance of different determinants of donor microbiota quality: Digestion carries the highest weight (×1.5) as gastrointestinal function is the primary determinant of preparation quality; Diet is weighted ×1.3, reflecting the dominant role of dietary diversity in shaping microbial composition; LifeStyle is weighted ×1.2 in recognition of the role of physical activity, substance avoidance, and stress regulation in microbiota resilience. The remaining categories – EarlyLife, BodyMass, Health, and Sleep – carry equal ×1.0 weight as background determinants.

Beyond the additive scoring, the DSQ contains 20 binary exclusion gates covering:

- congenital abnormalities (Q5),
- recent gastrointestinal infection (Q36),
- active GERD (Q54),
- antibiotic exposure within 3 months (Q62),
- diagnosed IBD or coeliac or structural GI disease (Q64),
- specific organ-system pathologies (Q67–Q72),
- GI surgery (Q73),
- cancer (Q76),
- chronic disease requiring medication (Q77),
- psychiatric treatment (Q78),
- autoimmune disease (Q81),
- blood-borne infection (Q82),
- blood transfusion (Q83),
- active smoking (Q84),
- recreational drug use (Q88),
- tropical travel within 12 months (Q90),
- steroid use (Q93),
- high-risk sexual behaviour (Q95),
- STI diagnosis (Q96),
- and recent tattoo or piercing within 6 months (Q97).

Triggering any single exclusion gate disqualifies the candidate regardless of total score.

Donors who clear the exclusion gates are stratified by SD Bonus into three tiers:

- Standard Donor (SD 0–39),

- Super-Donor (SD 40–59),
- and Elite Super-Donor (SD 60–95).

Monte Carlo validation across 5,000 simulated donor profiles confirms the calibration: 69.1% pass all gates, mean Total Score 186.3 (SD 18.6), with the SD-tier thresholds producing the intended selectivity (~92% Standard, ~8% Super, <0.2% Elite). The full DSQ scoring system is documented in a dedicated companion guide.

The clinical implications discussed in the remainder of this section – donor diet, antibiotic-free nutrition, and the food–dysbiosis loop – apply to donors who have already cleared the DSQ screening framework. The DSQ defines who is eligible to donate at all; this section addresses how the eligible donor's microbiota quality is then maintained through the operational period.

VIII.2 Why Donor Diet Defines Therapy Quality

An MTT preparation is, mechanically, the lyophilised microbiota of a screened human donor. The quality of that preparation is therefore an exact function of the donor's microbiota at the time of donation, which is in turn an exact function of the donor's recent dietary intake, lifestyle, and environmental exposure. The therapy cannot be of higher quality than the state of the donor's gut at the moment of donation. This is an operating principle of the donor screening programme; no direct clinical study linking donor quality to outcome is available. The recipient microbiota is closest to the donor material one day after the intervention and diverges from it thereafter [048].

This produces a clinical principle that is often under-appreciated: the upstream determinants of donor microbiota quality – what the donor eats, the integrity of the food supply they rely on – are equally clinical determinants of therapy outcome. The DSQ Diet category (×1.3 weight) and the operational dietary requirements detailed below are two sides of the same clinical commitment: ensuring that the donor's microbiota at the moment of donation is in the best achievable state.

VIII.3 Antibiotic-Free and Antihelmintic-Free Nutrition

Conventional commercially-produced animal protein contains residual antibiotic and antihelmintic compounds, even within regulatory limits. Within the context of donor preparation suitable for microbiota transfer, even sub-clinical residues are clinically significant: they perturb the donor's gut ecology in ways that are both detectable in the donatum and consequential for the recipient. Empirical work has shown that residue concentrations within current regulatory MRLs are sufficient to produce measurable microbiota disturbance and metabolic dysfunction in animal models [046], while livestock antibiotic use is linked to gut dysbiosis so far by ecological-level correlates and mechanistic animal work [045], [047].

It should be noted that the population-level human causal evidence linking dietary antibiotic-residue exposure to recipient-relevant dysbiosis is at present indirect: the literature rests predominantly on animal models and on epidemiological correlations rather than on prospective interventional trials in humans. The pattern is, however, structurally analogous to the *C. difficile* / colorectal carcinoma case discussed in Section II.2 – where mechanistic and animal-model evidence accumulated before the population-level human data, and that human data so far supports the link at the level of association: the risk of colorectal carcinoma was elevated in patients with repeatedly positive *C. difficile* tests but not in those with a single positive test [054]; direct causal evidence in humans is not yet available [050]. The accumulating mechanistic, animal-model, and epidemiological evidence over the past decade points consistently in the same direction, even if a definitive human interventional confirmation is still pending.

Given this evidence pattern, the operational position adopted here is conservative in the precautionary sense: where an intervention serves the patient's and donor's microbiota integrity and carries no offsetting clinical risk, the asymmetry of evidence supports adoption rather than deferral. For this reason, MicroBiome Bank donors follow a prescribed diet excluding antibiotic- and antihelmintic-treated animal protein. The antibiotic-free animal protein source for the donor population is procured through

Cservölgy Major as a scientific collaboration partner. The same source is recommended to MTT recipients during the maintenance phase, on the principle that a recovering microbiota cannot be sustained on the same dietary input that contributed to its disruption.

This operational dietary requirement aligns with the DSQ's exclusion gate at Q62 (any antibiotic use within 3 months disqualifies the candidate) and the SD Bonus criterion that awards +20 points to donors who report never having taken antibiotics. The dietary policy described here applies to the period after a donor has been screened in: it ensures that the gut state which qualified them for donation is preserved through the operational donation cycle.

VIII.4 The Food–Dysbiosis Causal Loop

The clinical argument generalises beyond antibiotics. Industrial food production causes a range of microbiota-relevant perturbations – emulsifiers [036], [037], [038], preservatives, residual pesticide and herbicide compounds, and not-yet-characterised degradation products of the compounds that provide long shelf life. Each is a low-grade, chronic input into the dysbiotic state described in Section II. Each contributes to the soil out of which the disease continuum grows. Of particular note, common dietary emulsifiers carboxymethylcellulose (E466) and polysorbate-80 (E433) even at relatively low concentrations produce low-grade intestinal inflammation, shift microbiota composition toward pro-inflammatory profiles, and trigger metabolic syndrome and colitis in susceptible animal models [036], [037].

Key Principle: If the food supply does not improve, neither donors nor recipients can sustain a healthy microbiota. Dietary reform is not adjunct to MTT – it is part of the therapy.

This is also the reason that recurrence after an apparently successful MTT course is most often traceable, on careful history, to dietary normalisation: the patient returns to their previous dietary input, and the dysbiotic substrate begins to re-establish itself. The maintenance phase is, in clinical practice, principally a dietary phase.

IX. Warnings, Contraindications, and Special Populations

IX.1 Concurrent Antibiotics

Warning: MTT must not be administered concurrently with antibiotic therapy. Antibiotics damage the live microorganisms in the capsule, both economically wasteful and clinically counterproductive. Antibiotic-induced microbiota disruption is profound (within 3–4 days of initiation), often only partially recoverable; repeated exposure leads to a persistent ecological regime shift [033].

If antibiotic therapy is unavoidable (e.g. organ-specific infection requiring targeted antimicrobial coverage), MTT is to be suspended as the default. Antibiotic treatment started during the course warrants suspension of the course; continuation at an increased dose comes into consideration only in a life-threatening situation, following prior consultation, and even then must be regarded as partly compromised. Antibiotic courses should be completed at least 48 hours before MTT initiation where possible.

IX.2 Probiotics

Warning: The use of probiotic supplements must be suspended immediately upon initiation of MTT. Continuation during the course comes into consideration only where the treating physician expressly so orders — this is not recommended. In the context of established dysbiosis or CDI, probiotics may aggravate the underlying dysbiotic state [018], [034], and following antibiotic treatment they may contribute to expansion of the antibiotic resistance gene reservoir [055]. The vast majority of the trials included in meta-analyses showed no significant effect on the incidence of *C. difficile*-associated diarrhoea [035]. Probiotics therefore have no place in the MicroBiome Bank protocol — an operational

clinical position warranted by the absence of evidence set out above, not a recommendation drawn from any of the cited publications.

The scope of this position requires clarification. The clinical position of MicroBiome Bank applies specifically to the context of established dysbiosis or CDI – the patient population to which this Guide is addressed – and is not a general statement against all probiotic use across all clinical contexts. Within this specific population, the current evidence base supports the position that probiotics – as currently formulated and marketed – are clinically not recommended. Direct mucosal sampling has shown that probiotic colonisation is highly person-specific [034], that probiotics delay rather than support post-antibiotic microbiome reconstitution [018], and that the broader evidence base for probiotic clinical efficacy in dysbiotic and CDI contexts is substantially weaker than commonly assumed [035]. The preparation of MTT capsules is not a probiotic, and the two should not be conflated.

IX.3 Immunocompromised, Pregnant, and Pediatric Patients

Of the recommendations below, the paediatric and severity-related considerations follow current IDSA/SHEA guidance [023], [028], adapted to the MTT context; the position on immunocompromised, pregnant and breastfeeding patients reflects our own operational caution, as the guidelines do not cover them.

Immunocompromised patients: Severe immunodeficiency or active immunosuppressive therapy require close monitoring due to elevated infection risk. MTT may still be indicated, but only under specialist supervision and with intensified surveillance.

Pregnancy and lactation: Limited data are available. Treatment should only be initiated after careful individual assessment by the treating physician. The default position is deferral unless clinical urgency dictates otherwise.

Pediatric use: No known conditions related to the use of DiffBiome or HospBiome capsules currently contraindicate the application of MTT in patients under 18 years of age. Standard physician supervision applies.

Elderly patients: Frequently present with multiple comorbidities and concurrent medication. MTT should be used with caution, accompanied by closer monitoring for adverse effects or interactions. Dosage adjustment may be warranted.

Severe gastrointestinal conditions: Toxic megacolon, fulminant colitis, or suspected bloodstream infection require thorough evaluation before MTT is considered. In these contexts, hospital-based protocols and adjunct interventions take precedence over standard MTT initiation.

X. References

The numbering of the entries below follows the shared MicroBiome Bank bibliography; the same number identifies the source in every other MBB document.

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XI. Reference summaries

[001] Seekatz A. et al. (2022) — The role of the gut microbiome in colonization resistance and…

Colonization resistance of the gut microbiota and FMT therapy in rCDI — A healthy microbiota inhibits *C. difficile* colonization (nutrient competition, bile acids, SCFAs, bacteriocins). Antibiotics → dysbiosis → CDI. FMT restores diversity. Monoclonal antibodies do not treat dysbiosis.

https://doi.org/10.1177/17562848221134396

[002] Britton R. & Young V. (2014) — Role of the intestinal microbiota in resistance to…

Intestinal microbiota and *C. difficile* colonization resistance — fundamental mechanisms — The native microbiota inhibits germination and growth of *C. difficile* spores. Antibiotics impair this defense. Key mechanisms: bile acid metabolism, nutrient competition. FMT restores colonization resistance.

https://doi.org/10.1053/j.gastro.2014.01.059

[003] Reed A.D. & Theriot C.M. (2021) — Contribution of Inhibitory Metabolites and Competition…

This review examines how commensal *Clostridium* species mediate colonization resistance against *C. difficile*. Commensal *Clostridia* modify primary bile acids into secondary bile acids that suppress *C. difficile* spore germination and vegetative outgrowth. They additionally produce antimicrobial peptides and short-chain fatty acids that directly inhibit *C. difficile* and compete for limiting nutrients such as proline, important for *C. difficile* growth via Stickland fermentation. Loss of commensal *Clostridia* after broad-spectrum antibiotics is a key mechanistic step toward CDI susceptibility. The authors conclude from this that new therapies against CDI are urgently needed; the clinical validation of defined *Clostridium* consortia comes not from this review but from the VE303 phase 2 trial [056].

https://doi.org/10.3390/microorganisms9020371

[010] Gregory A.L. et al. (2021) — A short chain fatty acid-centric view of…

This review synthesizes the role of short-chain fatty acids (SCFAs) in *C. difficile* colonization resistance. The gut microbiome produces acetate, propionate and butyrate via fiber fermentation; these SCFAs maintain epithelial barrier function, modulate immunity and signal directly to *C. difficile*. The authors propose a conceptual model in which *C. difficile* senses the SCFAs themselves as a marker of a healthy, competitive gut environment and, in response, increases toxin production in order to sustain the dysbiotic state that favours it. SCFAs influence toxin production and competition with commensals. The review argues that targeting SCFA pathways — through dietary intervention, next-generation probiotics or other targeted approaches — offers a precision, non-antibiotic strategy against CDI distinct from both antibiotics and faecal transplant.

https://doi.org/10.1371/journal.ppat.1009959

[018] Suez J. et al. (2018) — Post-Antibiotic Gut Mucosal Microbiome Reconstitution Is Impaired…

Human and mouse study of what happens to the gut mucosal microbiome after a course of antibiotics. Three routes were compared: spontaneous recovery, an 11-strain probiotic preparation, and autologous faecal transplantation, that is, return of the person's own flora frozen before the antibiotic. The probiotic colonised the mucosa but delayed the return of the indigenous microbiome and of host mucosal gene expression, and the difference persisted for months. Autologous transplantation, by contrast, produced near-complete recovery within days. Conclusion: after antibiotics a probiotic is not a neutral supplement but may slow the return of the native flora; it is a full microbiota graft that replaces the missing community.

https://doi.org/10.1016/j.cell.2018.08.047

[020] Lewis S.J. & Heaton K.W. (1997) — Stool form scale as a useful guide to intestinal transit…

The original validation of the Bristol Stool Form Scale. Whole-gut transit time was measured with radio-opaque marker pellets in 66 volunteers, who kept a diary of stool form on a 7-point scale and of

defecation frequency. Transit time correlated most closely with stool form (r = -0.54), more strongly than with stool frequency or stool output. When transit time was altered with senna and with loperamide, stool form tracked the change (r = -0.65). Conclusion: recording stool form is a simple and responsive way to monitor change in bowel function — which is why it is suitable for the patient's own diary.

https://doi.org/10.3109/00365529709011203

[023] McDonald L.C. et al. (2018) — Clinical Practice Guidelines for *Clostridium* difficile…

Comprehensive IDSA/SHEA clinical practice guideline on the diagnosis, treatment and prevention of *C. difficile* infection in adults and children. It defines severity categories (non-severe, severe, fulminant) and characterises fulminant disease by hypotension or shock, ileus or toxic megacolon — findings that require inpatient care, intravenous therapy and surgical consultation. For multiply recurrent infection in which antibiotic therapy has repeatedly failed, faecal microbiota transplantation is recommended. This document provides the international frame to which the book's red flags and hospital-referral signs are aligned.

https://doi.org/10.1093/cid/cix1085

[028] Johnson S. et al. (2021) — Clinical Practice Guideline by the Infectious Diseases Society…

A focused update of the 2017 IDSA/SHEA guideline, limited to treatment recommendations. The principal change is that fidaxomicin is now preferred over vancomycin for an initial episode — a conditional recommendation with moderate certainty — because although initial cure is similar, sustained cure is better. For recurrent episodes fidaxomicin is likewise preferred, as is a tapered and pulsed vancomycin regimen. Bezlotoxumab is offered as an adjunct for patients at high risk of recurrence. The guideline states explicitly that vancomycin remains an acceptable choice where fidaxomicin is unavailable, and metronidazole has receded even for mild disease. For the SIS the guideline matters because it confirms that the treatment decision depends not only on current severity but on the risk of recurrence — the duality that underlies the acute and prognostic subscores of the SIS.

https://doi.org/10.1093/cid/ciab549

[029] van Nood E. et al. (2013) — Duodenal infusion of donor feces for recurrent *Clostridium*…

The first randomised controlled trial comparing faecal microbiota transplantation with standard antibiotic therapy in recurrent *Clostridioides difficile* infection. Patients were assigned to three arms: donor faeces given through a duodenal tube after a short course of vancomycin, vancomycin alone, or vancomycin with bowel lavage. The trial was stopped early because the difference was so large: in the transplantation arm 81 percent of patients were cured after the first infusion and 94 percent including repeat infusions, against 31 and 23 percent in the two vancomycin arms. After treatment the diversity of the patients' faecal flora came to resemble that of the donors. This paper turned microbiota transplantation into an evidence-based treatment and is the starting point for the dosing regimen of the present protocol.

https://doi.org/10.1056/NEJMoa1205037

[030] Ooijevaar R.E. et al. (2021) — Ten-Year Follow-Up of Patients Treated with Fecal…

This long-term follow-up study tracked the 34 patients who received FMT among the 43 randomised in the original van Nood et al. (2013) trial; the median follow-up was 3.5 years, and seven of the patients reached the ten-year mark. The authors document sustained clinical efficacy with no late safety signals – no transmissible infections attributable to donor stool and no new immune-mediated disorders were identified; in the case of one death from urosepsis the authors note that, in the absence of microbiological data, a relationship to FMT cannot be ruled out with complete certainty. No stool samples were available to examine the long-term microbiota effect; the authors themselves note this as a limitation of the study. The paper also synthesises a literature review of accumulated rCDI–FMT outcomes globally. It supports the position that microbiota transfer therapy is durable when correctly

applied; the ecological nature of the resolution, however, cannot be inferred from this study, because no microbiota sampling was performed.

https://doi.org/10.3390/microorganisms9030548

[031] Lessa F.C. et al. (2015) — Burden of *Clostridium* difficile infection in the United States

Population-based surveillance from ten US geographic areas in 2011 estimated the national CDI burden at 453,000 infections and 29,300 deaths annually. The study established that approximately two-thirds of cases were healthcare-associated, with the hypervirulent NAP1 strain disproportionately represented. Recurrence affected 83,000 patients in the same year. These figures underline why CDI remains a high-priority target for ecologically restorative therapies rather than antibiotic-only management.

https://doi.org/10.1056/NEJMoa1408913

[032] Guh A.Y. et al. (2020) — Trends in U.S. Burden of *Clostridioides difficile* Infection and…

Updated US epidemiological surveillance demonstrating that the burden of healthcare-associated CDI declined substantially between 2011 and 2017 – by 36 percent according to the adjusted estimate (95% CI 24 to 54), according to the authors primarily as a result of the decline of ribotype 027 and, alongside it, improved infection control – while community-associated CDI remained stable. The estimated burden of first recurrences and of in-hospital mortality, however, did not change meaningfully. The authors conclude that better identification of patients at high risk of recurrence is needed, together with further strengthening of infection control and antibiotic stewardship; the paper does not discuss microbiota-restorative therapy. The unchanged burden of recurrences is the clinical gap that compatibility-based MTT addresses.

https://doi.org/10.1056/NEJMoa1910215

[033] Dethlefsen L. & Relman D.A. (2011) — Incomplete recovery and individualized responses of…

Stanford-based longitudinal study tracking the gut microbiota of three individuals over 10 months across two consecutive courses of ciprofloxacin, with deep 16S rRNA sequencing. Loss of bacterial diversity was profound and rapid, occurring within 3-4 days of antibiotic initiation, and recovery toward the pre-treatment state was often incomplete months after cessation. Repeated antibiotic exposure produced incremental, non-recoverable shifts in community composition. With over 2,000 citations, this paper is the canonical reference establishing that antibiotic-induced dysbiosis is not a self-correcting disturbance but can leave a lasting ecological imprint – central to the case for MTT in patients with cumulative antibiotic exposure history.

https://doi.org/10.1073/pnas.1000087107

[034] Zmora N. et al. (2018) — Personalized Gut Mucosal Colonization Resistance to Empiric…

Companion paper to Suez et al. (2018) demonstrating that probiotic colonisation of the gut mucosa is highly person-specific: some individuals are 'permissive' colonisers, while others are 'resistant', and oral probiotic intake exerted a transient and individually variable effect on mucosal community structure and the gut transcriptome. Stool sampling alone fails to detect this heterogeneity – direct mucosal sampling was required. The result undermines the case for empiric probiotic use as a generic intervention. It also provides the conceptual basis for personalised, compatibility-tested microbiota therapy of the kind operationalised by FindBiome.

https://doi.org/10.1016/j.cell.2018.08.041

[035] Suez J. et al. (2019) — The pros, cons, and many unknowns of probiotics

Comprehensive Nature Medicine review evaluating the clinical evidence base for probiotic supplementation across multiple indications. The authors document the heterogeneity of probiotic preparations, and the fact that across most of the indications examined the results are conflicting and that no probiotic preparation has been approved as a medical intervention. They highlight specific risks, above all delayed microbiome recovery after antibiotics. Its most important finding for the CDI context is

that the overwhelming majority of the studies included in the meta-analyses showed no significant effect on the incidence of *C. difficile*-associated diarrhoea. The authors do not, however, reject probiotics: in their view, with rigorous research and regulation they may become an effective intervention in selected indications.

https://doi.org/10.1038/s41591-019-0439-x

[036] Chassaing B. et al. (2015) — Dietary emulsifiers impact the mouse gut microbiota promoting…

Landmark Nature paper showing that two ubiquitous dietary emulsifiers – carboxymethylcellulose (CMC, E466) and polysorbate-80 (P80, E433) – induced low-grade intestinal inflammation even at relatively low concentrations, altered microbiota composition, and produced obesity/metabolic syndrome in wild-type mice. The authors themselves note that the extent of human emulsifier consumption is not tracked, but that, given how widespread emulsifiers are in food production, actual human exposure may exceed the 1.0 percent level used in the experiment. In mice predisposed to colitis, the same compounds triggered overt colonic inflammation. The authors propose emulsifier exposure as a contributor to the post-1950 rise in IBD and metabolic disease. A corrigendum has been issued for the paper (Corrigendum: *Nature* 2016;536(7615):238). This is the central evidence cited in Section 8.3 of this Guide regarding industrial food production as a chronic input into dysbiosis.

https://doi.org/10.1038/nature14232

[037] Chassaing B. et al. (2017) — Dietary emulsifiers directly alter human microbiota…

Follow-up to Chassaing 2015 extending the findings from mice to a human-microbiota model (the M-SHIME ex vivo system). Both emulsifiers increased the pro-inflammatory potential of the microbiota, demonstrably so through elevated flagellin levels; the composition of the community, however, was altered only by P80, while the effect of CMC operated through microbiota gene expression. The rise in lipopolysaccharide levels occurred only with P80, and at higher doses. When a suspension of the emulsifier-treated microbiota was administered to immunodeficient (RAG−/−) mice, serum IL-6 levels rose significantly. The work confirms that the murine findings translate to human gut ecology and reinforces the argument that dietary reform is a clinical, not an aesthetic, component of the maintenance phase.

https://doi.org/10.1136/gutjnl-2016-313099

[038] Naimi S. et al. (2021) — Direct impact of commonly used dietary emulsifiers on human gut…

Systematic ex vivo screening of 20 commonly used dietary emulsifiers in the MiniBioReactor Array model of human gut microbiota. Both CMC and P80 again reproduced their adverse effects, while a subset of additional emulsifiers also produced inflammation-relevant shifts in microbial composition and gene expression. The study demonstrates that the toxicological problem extends beyond the two best-studied compounds and supports a precautionary stance toward broad classes of food additives. Provides the empirical breadth behind the claim in Section 8.3 that industrial food production introduces a range of microbiota-relevant perturbations.

https://doi.org/10.1186/s40168-020-00996-6

[039] Ananthakrishnan A.N. et al. (2009) — *Clostridium* difficile and inflammatory bowel disease

Reference review establishing the bidirectional clinical relationship between CDI and inflammatory bowel disease. IBD patients are at substantially elevated risk of CDI, and incident CDI in IBD precipitates flares, prolongs hospitalisation, increases colectomy rates, and worsens mortality. The authors highlight that the clinical picture in IBD-CDI overlap is often atypical – pseudomembranes are uncommon, and standard endoscopic markers are unreliable. This is the basis for the reclassification rules in Sections 3.5 and 6.4 governing movement between the IBS/IBD and CDI treatment groups.

https://doi.org/10.1016/j.gtc.2009.07.003

[040] Amakye D. et al. (2025) — Global Patterns of *Clostridioides difficile* Infection in…

Recent systematic review and meta-analysis of 28 studies from 11 countries comprising over 796,000 IBD patients, quantifying the global pooled CDI rate in IBD at 8.84%. The authors also present regional variation in prevalence (Asia 11%, North America 7.85%, Europe 7.92%). This is the most current epidemiological reference supporting the IBD-to-CDI reclassification logic operationalised in this Guide.

https://doi.org/10.1093/crocol/otaf024

[042] Yin H. et al. (2022) — *Fusobacterium nucleatum* promotes liver metastasis in colorectal…

Mechanistic study demonstrating that oral administration of *Fusobacterium nucleatum* in mice exacerbates colorectal cancer (CRC) liver metastasis through alterations in the hepatic immune niche and gut microbiota composition. The paper documents systemic elevation of pro-inflammatory cytokines and recruitment of myeloid-derived suppressor cells with reduced NK and Th17 infiltration. The work supports a causal – not merely correlative – role for specific dysbiotic species in CRC progression. It is the kind of evidence underlying the chronic-trajectory narrative in Section 2.2 of this Guide.

https://doi.org/10.18632/aging.203914

[043] Ranjbar M. et al. (2021) — The dysbiosis signature of *Fusobacterium nucleatum* in…

Systematic review consolidating the evidence on *Fusobacterium nucleatum* as both a marker and a driver of colorectal cancer. The authors discuss the bacterium's virulence factors (FadA, Fap2, MORN2), its established overrepresentation in CRC tissue, and its prognostic value as a biomarker of CRC risk. They examine the directionality question – cause versus consequence – and find evidence supporting both roles, with emerging consensus on a contributory pathogenic role in mucosal carcinogenesis. A correction has been issued for the paper (Correction: *Cancer Cell Int* 2022;22(1):134). Provides the scoping reference for the dysbiosis-to-CRC trajectory described in Section 2.2.

https://doi.org/10.1186/s12935-021-01886-z

[044] Wang S. et al. (2023) — Butyrate Protects against *Clostridium* difficile Infection by…

Mechanistic CDI study showing that butyrate, a short-chain fatty acid produced by commensal Firmicutes, exerts a protective effect against CDI through multiple synergistic pathways: enhanced gut barrier integrity, anti-inflammatory action, and modulation of bile acid metabolism via bile salt hydrolase regulation and FXR activation. CDI patients have markedly reduced fecal butyrate compared to controls. The findings establish a metabolic mechanism by which a healthy, diverse microbiota resists CDI colonisation – and by which a dysbiotic microbiota becomes permissive. This underpins the SCFA-related reasoning in Section 2.2 about depleted SCFA synthesis in chronic dysbiosis.

https://doi.org/10.1128/spectrum.04479-22

[045] Riley L.W. et al. (2013) — Obesity in the United States – Dysbiosis from Exposure to…

Hypothesis paper from the UC Berkeley School of Public Health linking the temporal trajectory of US obesity to the parallel intensification of subtherapeutic antibiotic use in concentrated animal feeding operations (CAFOs) over the same period. The authors synthesise epidemiological data on residue exposure with experimental microbiome literature documenting how low-dose antibiotic exposure perturbs gut ecology. They argue chronic dietary exposure to antibiotic residues is a plausible, under-investigated driver of population-level metabolic disease. This is one of the foundational references for Section 8.2 of this Guide regarding antibiotic-free animal protein.

https://doi.org/10.3389/fpubh.2013.00069

[046] Chen R.A. et al. (2022) — Dietary Exposure to Antibiotic Residues Facilitates Metabolic…

Empirical study quantifying how chronic dietary exposure to subtherapeutic antibiotic residues (specifically tylosin at theoretical maximum daily intake, or TMDI, doses) alters gut microbiota composition and bile acid metabolism, producing measurable metabolic dysfunction in animal models.

The obesity-related phenotype was transferable to germ-free recipient mice, indicating the effect is mediated by the altered microbiota itself. Early-life exposure produced lasting metabolic consequences via FGF15 signalling. Provides the most current empirical evidence cited in Section 8.2 supporting the categorical exclusion of conventional antibiotic-treated animal protein from donor and post-MTT recipient diets.

https://doi.org/10.1128/msystems.00172-22

[047] Silvestro S. et al. (2025) — The Role of Livestock Antibiotic Use in Microbiota Dysbiosis…

Recent comprehensive review examining the One Health implications of livestock antibiotic use – connecting animal husbandry practices to human gut microbiota disturbance, antimicrobial resistance gene proliferation, and downstream neuroinflammatory effects through the gut-brain axis. The authors argue that food-chain antibiotic exposure is a clinically relevant, chronic, low-grade input into population microbiome health. The review extends the metabolic-disease argument of earlier work into the domain of neurological and neurodegenerative disease aetiology. Supports the broader chronic-trajectory framing in Section 2.2 of this Guide.

https://doi.org/10.3390/antibiotics14060608

[048] Weingarden A. et al. (2015) — Dynamic changes in short- and long-term bacterial…

Longitudinal microbiome characterisation following FMT in four patients with recurrent CDI, sampling daily for 28 days and weekly to 84 days post-treatment, over a total of 151 days. The recipient microbiota rapidly normalised from a markedly dysbiotic state to a healthy-range composition within days. Composition continued to change thereafter, diverging from the original donor implant material and fluctuating dynamically over both the short and the long term – while remaining throughout within the cloud of healthy microbiota. The paper supports the framing in this Guide that successful MTT produces a self-sustaining recipient ecology, not a permanent donor-tracked imprint.

https://doi.org/10.1186/s40168-015-0070-0

[049] Hecker M.T. et al. (2026) — Long-Term Follow-Up After Fecal Microbiota Transplantation via…

This 2026 retrospective cohort study from two US academic hospitals followed 129 patients with recurrent CDI treated with freeze-dried oral FMT capsules over a median follow-up of 182 weeks – the longest published follow-up of capsule-based FMT to date and a near-perfect match for the lyophilised capsule format used in the MicroBiome Bank protocol. Of the cohort, 89% had experienced three or more prior CDI episodes; at three months, 80% of patients had no recurrence, and the 20% who failed initial therapy were largely successfully managed with repeat FMT. Across the long follow-up, only 17% of patients experienced any additional CDI episode, and most of those were again successfully managed with repeat FMT. The study confirms that lyophilised oral capsule FMT is a durable, repeatable, real-world therapy for recurrent CDI in heavily pre-treated patients.

https://doi.org/10.20411/pai.v11i1.868

[050] Zou J. et al. (2026) — *Clostridioides difficile*

This 2026 Chinese Medical Journal review synthesises emerging mouse-model and clinical evidence that toxigenic *C. difficile* is not merely an acute pathogen but a plausible chronic pro-carcinogenic driver in colorectal cancer. The authors integrate findings on TcdA/TcdB-induced epithelial damage, cellular senescence, oxidative stress, biofilm formation, and metabolic reprogramming, arguing that chronic asymptomatic *C. difficile* carriage may participate in the dysbiotic terrain that promotes CRC initiation. Notably, the review reports that *C. difficile* prevalence in cancerous colorectal tissue reaches 60% versus 20% in adjacent healthy tissue, and that anti-TcdB IgG titres above threshold are present in 66.7% of CRC patients versus 30.8% of healthy controls. The paper formalises the conceptual link between the acute and chronic trajectories described in Section 2 of this Guide.

https://doi.org/10.1097/CM9.0000000000003834

[051] Bø S. et al. (2025) — Faecal microbiota transplantation for primary…

This Norwegian case report describes a patient in her sixties who developed her first episode of CDI and elected to receive FMT rather than the standard antibiotic course. The patient achieved complete resolution within two days after a single rectal FMT, with no recurrence during the two months of follow-up. The authors – themselves investigators in the Norwegian phase III trial – place the case in the context of their own, then still unpublished trial, which demonstrated non-inferiority of FMT to standard antibiotic treatment (67% vs. 61%); its full report has since been published [057]. Together, these data extend the clinical role of FMT beyond the recurrent-CDI niche into primary infection management – a position increasingly relevant for ecological-restoration treatment frameworks such as the one used in this Guide.

https://doi.org/10.4045/tidsskr.25.0276

[052] Daneri L. et al. (2025) — Lyophilised fecal microbiota transfer in capsules for recurrent…

This 2025 Spanish retrospective cohort study covered 36 patients with multiply-recurrent CDI and 38 procedures, with a median age of 78.5 years and a median of four prior episodes; the great majority of the authors work at a single Madrid centre. The lyophilised, encapsulated formulation achieved a 75 percent cure rate at three months in this elderly, refractory population, with no serious adverse events attributable to the procedure. The authors note that FMT failures could be successfully resolved by additional therapy that need not necessarily be a further FMT, supporting flexible escalation pathways. The paper validates capsule-based, off-the-shelf FMT as a logistically simpler alternative to colonoscopic delivery – directly aligned with the format used in the MicroBiome Bank protocol described in Section IV of this Guide.

https://doi.org/10.1016/j.ijantimicag.2025.107561

[053] Duttagupta S. et al. (2026) — Fecal microbiota transplantation plus immunotherapy in…

This 2026 Nature Medicine phase 2 trial assessed healthy-donor FMT delivered as a single oral capsule dose prior to first-line immune checkpoint inhibitor therapy in 20 patients with non-small cell lung cancer (anti-PD-1) and 20 with metastatic melanoma (anti-PD-1 plus anti-CTLA-4). The primary endpoint was the objective response rate in the lung cancer cohort: 80 percent (95% CI 58.4 to 91.9); in the melanoma cohort it was a key secondary endpoint, at 75 percent (53.1 to 88.8). Responders developed a distinct gut microbiota composition after FMT, but in the authors' interpretation the clinical benefit does not depend on engraftment of donor strains: strain-level engraftment did not differ between responders and non-responders. In the melanoma cohort, 65 percent of patients experienced grade 3 or higher adverse events, with myocarditis in 15 percent of them; in the lung cancer cohort no such event occurred, and the independent safety monitoring committee deemed both cohorts safe. The melanoma toxicity profile matches the known profile of the ipilimumab–nivolumab combination. The trial extends FMT from CDI into oncology and supports the broader paradigm that microbiota composition is a clinically meaningful determinant of immune-mediated disease outcomes – providing a forward-looking validation for the chronic-disease pathway (Section 6.3) and the long-term implications of resolving dysbiosis.

https://doi.org/10.1038/s41591-025-04186-5

[054] Rifkin S. et al. (2026) — Association between *Clostridioides difficile* Test Positivity and…

This 2026 retrospective cohort study, spanning two US academic centres, examined the association between *C. difficile* test positivity and subsequent incident colorectal cancer in 100,181 individuals with over one million person-years of follow-up. In a Cox model adjusted for age, sex, diabetes, inflammatory bowel disease, family history of colorectal cancer and race, a repeat positive test at least thirty days apart was associated with increased CRC risk (aHR 2.05; 95% CI 1.27 to 3.29), whereas a single positive test was not (aHR 0.70; 95% CI 0.45 to 1.10). The study bridges the gap between mechanism – toxin-mediated tumorigenesis described in mouse models – and population-level human risk, and reinforces

the clinical case for resolving, not merely treating, recurrent CDI. This item is the peer-reviewed full paper of the previously cited IDWeek 2025 conference abstract.

https://doi.org/10.1158/2767-9764.CRC-25-0606

[055] Montassier E. et al. (2021) — Probiotics impact the antibiotic resistance gene reservoir…

This study used direct endoscopic mucosal sampling to track how probiotic intake affects the antibiotic resistance gene (ARG) reservoir along the full length of the gastrointestinal tract. In healthy individuals not receiving antibiotics, an eleven-strain probiotic reduced ARG counts – but only in those in whom the strains actually colonised. After a course of antibiotics the picture reversed: the course expanded the resistome of the lower gut, and while autologous microbiota transplantation or spontaneous recovery attenuated this, the probiotic aggravated it further – through the expansion of strains carrying vancomycin resistance genes, not through genes of the probiotic strains themselves. Neither effect was detectable in stool samples; both required direct mucosal sampling. This paper is the source of the antibiotic-resistance claim in Section IX.2.

https://doi.org/10.1038/s41564-021-00920-0

[056] Louie T. et al. (2023) — VE303, a Defined Bacterial Consortium, for Prevention of…

This phase 2, dose-finding randomised trial evaluated VE303 – a defined bacterial consortium of eight commensal *Clostridia* strains – in 79 adults at high risk of recurrent CDI, following standard antibiotic treatment. In the high-dose arm, recurrence at eight weeks was 13.8 percent (4/29), against 45.5 percent (10/22) with placebo. The trial provides clinical validation that rationally composed, defined *Clostridium* consortia offer a mechanism-driven alternative to stool-based transplantation for preventing recurrent CDI – a claim that the mechanistic review cited under [003] does not itself make. A phase 3 trial of the preparation is ongoing, so the result still awaits confirmation.

https://doi.org/10.1001/jama.2023.4314

[057] Juul F.E. et al. (2025) — Fecal Microbiota Transplantation Versus Vancomycin for Primary…

COLONIZE (NCT03796650) is a Norwegian, 18-centre, open-label, non-inferiority phase III trial comparing a single rectal FMT without pre-treatment with ten days of oral vancomycin (125 mg four times daily) in primary CDI. According to the trial registry, the study was halted at the pre-specified interim analysis because the non-inferiority criterion had been met; 104 patients were randomised, of whom 100 were analysable. The primary endpoint – clinical cure at fourteen days and freedom from recurrence within sixty days without further treatment – was reached by 34/51 (66.7%) of patients in the FMT arm and 30/49 (61.2%) in the vancomycin arm (difference 5.4 percentage points; 95.2% CI -13.5 to 24.4; $p < 0.001$ for non-inferiority). The non-inferiority margin was 25 percentage points and the confidence interval was wide, so the result must be interpreted with caution. The authors conclude that FMT may also be considered as a first-line treatment in primary CDI.

https://doi.org/10.7326/ANNALS-24-03285

[058] Carlson T.J. et al. (2022) — Fulminant *Clostridioides difficile* Infection

A review of the treatment options for fulminant *Clostridioides difficile* infection. The authors put the fulminant form at 3–5% of all CDI cases and its associated mortality at 30–40% — an order of magnitude above CDI mortality at population level. The review works through the pharmacological and surgical options for managing the fulminant case. In this document the item supplies the mortality figure for the terminal station of Section II. 1.; its denominator is all fulminant cases, not only the patients who proceed to surgery.

https://doi.org/10.1055/s-0041-1740973

[059] Chen Z. et al. (2026) — Trends, predictors, and association of surgical timing with…

A cohort study based on the national inpatient database (National Inpatient Sample) covering 2018 to 2022: of 240,564 CDI hospitalisations, colectomy was performed in 717 cases. Among the patients

operated on within eight days of admission, in-hospital mortality was 26.5% overall and rose from 21.0% to 30.7% over the period. The study also analyses the association between the timing of surgery and mortality. In this document the item supports the statement that current antibiotic-led management has not brought down the mortality of fulminant CDI; the denominator here is the group of patients who proceed to colectomy.

https://doi.org/10.1017/ice.2026.10411

[060] Collins S.L. et al. (2023) — Bile acids and the gut microbiota

A review of the metabolic interactions between bile acids and the gut microbiota. It is of key importance for this document because it states both directions of the bile acid shift within a single publication. The authors link recurrent CDI to antibiotic-induced elevated primary and reduced secondary bile acid levels — this is the basis of the mechanistic description in section II. 1 and chapter IV. In people consuming a high-fat diet, by contrast, it is precisely the secondary and unconjugated bile acids that accumulate, owing to the expansion of 7α-dehydroxylase- and BSH-producing bacteria; the two major secondary bile acids, deoxycholic acid and lithocholic acid, have long been associated with gastrointestinal cancers, above all colorectal carcinoma, and being hydrophobic they are more carcinogenic than primary bile acids. The mechanism is the release of reactive oxygen species that damage DNA and cause mutations. The source of the bile acid statement in section II. 2.

https://doi.org/10.1038/s41579-022-00805-x