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Narrative review

How to develop a controlled human infection model for *Clostridioides difficile*

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ABSTRACT

Background: *Clostridioides difficile (C. difficile)* remains the leading cause of healthcare-associated diarrhoea, posing treatment challenges because of antibiotic resistance and high relapse rates. Faecal microbiota transplantation is a novel treatment strategy to prevent relapses of *C. difficile* infection (CDI), however, the exact components conferring colonization resistance are unknown, hampering its translation to a medicinal product. The development of novel products independent of antibiotics, which increase colonization resistance or induce protective immune mechanisms is urgently needed.
Objectives: To establish a framework for a Controlled Human Infection Model (CHIM) of *C. difficile*, in which healthy volunteers are exposed to toxigenic *C. difficile* spores, offering the possibility to test novel approaches and identify microbiota and immunological targets. Whereas experimental exposure to non-toxigenic *C. difficile* has been done before, a toxigenic *C. difficile* CHIM faces ethical, scientific, logistical, and biosafety challenges.
Sources: Specific challenges in developing a *C. difficile* CHIM were discussed by a group of international experts during a workshop organized by Inno4Vac, an Innovative Health Initiative-funded consortium.
Content: The experts agreed that the main challenges are: developing a clinically relevant CHIM that induces mild to moderate CDI symptoms but not severe CDI, determining the optimal *C. difficile* inoculum dose, and understanding the timing and duration of antibiotic pretreatment in inducing susceptibility to CDI in healthy volunteers.
Implications: Should these challenges be tackled, a *C. difficile* CHIM will not only provide a way forward for the testing of novel products but also offer a framework for a better understanding of the

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pathophysiology, pathogenesis, and immunology of *C. difficile* colonization and infection. **Annefleur D.O. Hensen, Clin Microbiol Infect 2024;▪:1**

Introduction

Clostridioides difficile (*C. difficile*) is the leading cause of healthcare-associated diarrhoea with a total annual number of *C. difficile* infection (CDI) cases of almost 190 000 in the European Union and up to 500 000 in the United States, which causes substantial clinical, social, and economic burdens [1–3]. Because of high relapse rates of CDI after antibiotic treatment (ranging from 20% after treatment of a first episode, up to 60% after multiple recurrences) [4,5], limited approved treatment modalities [6,7] and the rise of antibiotic resistance [8], the need for novel treatment strategies has become pressing. The most important advancement in this area has been the prevention of recurrence by microbial restoration therapies such as faecal microbiota transplantation (FMT) and new commercially developed human microbiota-derived medicinal products [9–12]. However, the essential components of FMT as well as the specific markers that define microbiota susceptibility for CDI are still largely unknown. The development of a Controlled Human Infection Model (CHIM) for *C. difficile*, in which healthy adult volunteers are experimentally exposed to spores of toxigenic *C. difficile* with the purpose of inducing bacterial colonization, infection, and potential symptoms in trial participants, offers the possibility to investigate colonization resistance and susceptibility conferred by the microbiota by relating baseline microbiota to trial endpoints. As such, it allows for the identification of microbiota, metabolites, and immunological susceptibility markers, which can accelerate the identification of new targets.

Moreover, the efficacy of novel preventive products can be evaluated with the use of a CHIM. Although CHIMs have not yet been used for toxigenic *C. difficile* before, they have proven to be useful in many other disease areas [13,14] and have even led to the registration of a novel vaccine for cholera [15].

Inno4Vac, an Innovative Health Initiative-funded project, hosted a workshop on 9–10 February 2023 to discuss together with international experts and key players in the field of *C. difficile* and CHIMS, the design, set-up, and challenges of a *C. difficile* CHIM. The major recommendations and outcomes of this workshop are summarized in this article with the aim to inform and guide the development of a new CHIM for *C. difficile*.

Ethical considerations

The ethical admissibility of a *C. difficile* CHIM depends on the balance between the scientific and social benefits and the potential risks and burdens of the study. The WHO guidance on the ethical conduct of controlled human infection studies [16] states that human challenge studies, just like other health-related research with human participants, can only be conducted when they satisfy research ethics standards, including those requiring that the potential risks and burdens of the research are systematically identified, evaluated, minimized, and considered reasonable and justified in terms of the social and scientific value of the research. Fig. 1 shows an overview of the potential benefits and risks of a *C. difficile* CHIM. The expert group considers the social and scientific value of the research high, since (a) there are no animal models that fully capture the complex interaction between *C. difficile* and its human host and (b) the disease burden underpins the need to rapidly evaluate novel preventive products. As with any CHIM, the *C. difficile* CHIM will be established in young healthy adults to minimize risk. Clearly, this population does not represent a high-risk group for CDI, which means that findings from CHIM studies may not fully replicate in patient populations. The expert group nevertheless considers data from an experimental human population of significant interest, as the heterogeneity between volunteers is expected to provide a framework for a better understanding of

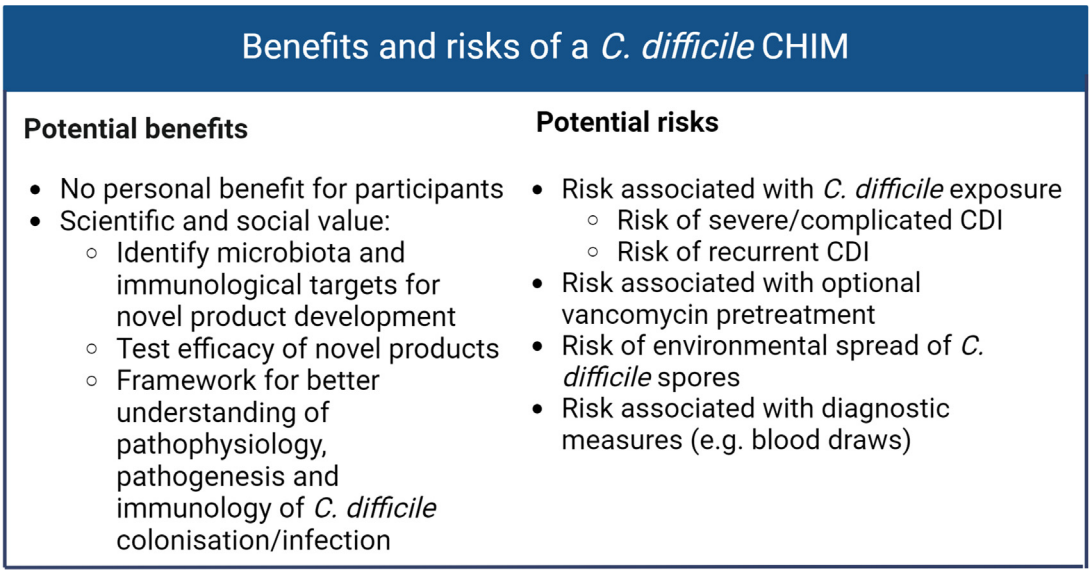

Fig. 1. The potential benefits and risks of a *C. difficile* CHIM. *Figure created with* BioRender.com.

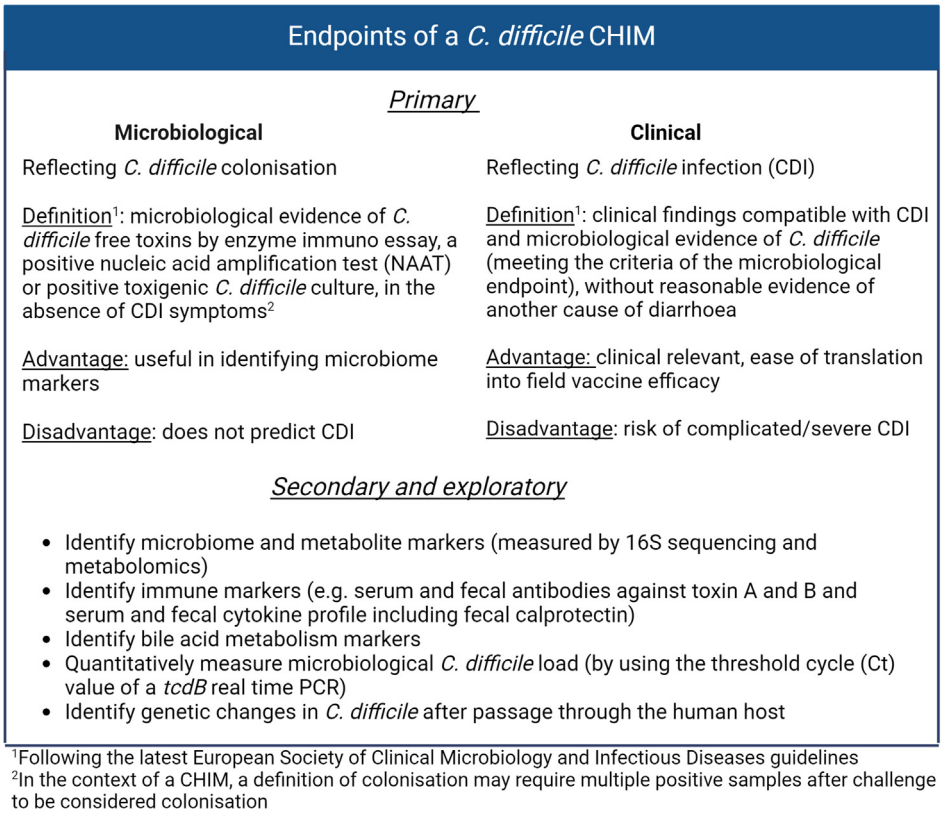

Fig. 2. The proposed endpoints of a *C. difficile* CHIM. *Figure created with* BioRender.com.

the pathophysiology, pathogenesis, and immunology of *C. difficile* colonization and infection.

With regards to risks, the expert group evaluated three risks of *C. difficile* CHIM. First, the risk of severe CDI in a *C. difficile* CHIM is estimated to be extremely low, as (a) at-risk individuals can be excluded from participation (see section '*Study population*'), (b) the *C. difficile* strain can be selected based on its safety profile (see section '*Challenge product*'), and (c) participants can be closely monitored to facilitate rapid rescue treatment. Second, the risk of subsequent CDI recurrence can be mitigated by appropriate highly effective rescue treatment with FMT (see section '*Rescue treatment*'). Lastly, the risk of environmental spread of *C. difficile* can be reduced by strict hygienic measures for participants and by the fast treatment of symptomatic participants. Moreover, toxigenic *C. difficile* spores are already highly common in the natural environment [17–19] (see section '*Study design – follow up*'), therefore the added third-party risk of a *C. difficile* CHIM to the community is estimated to be relatively small.

In conclusion, experts agree that the anticipated scientific and social value of a *C. difficile* CHIM is considerable and justifies conducting a *C. difficile* CHIM where risks and burdens are minimized.

Objectives and endpoints

Primary endpoint; microbiological vs. clinical endpoint

The scientific usefulness of a *C. difficile* CHIM for the testing of preventive interventions is critically dependent on the endpoint of the CHIM. The experts' definitions of a microbiological and clinical endpoint plus their advantages and disadvantages are listed in Fig. 2. An ideal *C. difficile* CHIM would result in mild to moderate symptoms in the majority of participants, but utmost care should be taken to avoid any severe or serious complications. Therefore, the expert group agreed to aim for a clinical endpoint (infection) if safety of participants will not be compromised and otherwise aim for a microbiological endpoint (colonization). As a target for the primary endpoint, an attack rate of 70% or higher should be aimed for as this infectivity rate is often used as a cut-off in CHIMs, to have a high number of volunteers reaching the endpoint and a limited overall number of exposed (and potentially at-risk) individuals.

Secondary and exploratory endpoints

The expert group emphasized that a *C. difficile* CHIM offers unprecedented opportunities to gain more knowledge about microbiota, metabolites, and immunological susceptibility markers and recommended including numerous secondary and exploratory endpoints (listed in Fig. 2) that increase the overall social value of the CHIM study.

Study design

During the workshop, key aspects relating to CHIM study design were discussed: (1) dosing of *C. difficile* spores, (2) antibiotic pretreatment, and (3) trial design. First, with regards to spore dosing, the results of non-toxigenic *C. difficile* (NTCD) colonization trials were reviewed [20,21]. In these trials, (single or repeated) varying doses of NTCD spores (10^4 to 10^8 CFU) were given to healthy adults [21] and CDI patients [20]. The doses were well tolerated but showed no dose-response relationship in NTCD colonization. Notably, colonization in healthy volunteers only occurred after oral vancomycin pretreatment [21]. The expert group concluded that host colonization resistance is probably more crucial in determining *C. difficile* susceptibility than the inoculum dose, and therefore altering colonization resistance through

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antibiotic pretreatment should be prioritized over *C. difficile* dose escalation in a *C. difficile* CHIM.

The expert group recommended the use of oral vancomycin as antibiotic pretreatment based on its safety profile and previous successful experience in the NTCD model. Because the *C. difficile* strain will be selected on the basis of vancomycin susceptibility (see section '*Rescue treatment*' and '*Challenge product*'), vancomycin pretreatment and administration of *C. difficile* spores need to be spaced sufficiently. However, the optimal duration of a wash-out for oral vancomycin is unclear, as this varies between studies [22–24] and individuals (e.g. depending on their stool frequency [24]). The expert group suggests that there should be at least 48 hours between vancomycin ingestion and *C. difficile* spore administration, and possibly even a longer period, depending on the duration and dosage of the treatment. In the NTCD trials, vancomycin 125 mg was given four times a day for 5 days, which caused mild gastrointestinal side effects, to avoid these, a shorter pretreatment can be considered [21].

Taken together, the expert group recommends an adaptive dose study design with a stepwise approach of escalating interventions, ensuring safety first, while gearing towards an optimal balance between endpoints and burden. For escalation, antibiotic pretreatment should be prioritized over increasing dosing of *C. difficile* spores, for which the expert group prefers a (repeated) low dose (10^4) over a high dose of *C. difficile* spores. To ensure safety, the expert group advises to start with a small pilot group which will be exposed to a low dose of *C. difficile* spores without antibiotic pretreatment. If this low dosing of *C. difficile* spores is safe and the target of the primary endpoint ($\geq$70%) is not reached, escalation to the next step (with antibiotic pretreatment) is advised. The expert group concurred that there is no need for the inclusion of placebo volunteers in this first dose-escalating trial.

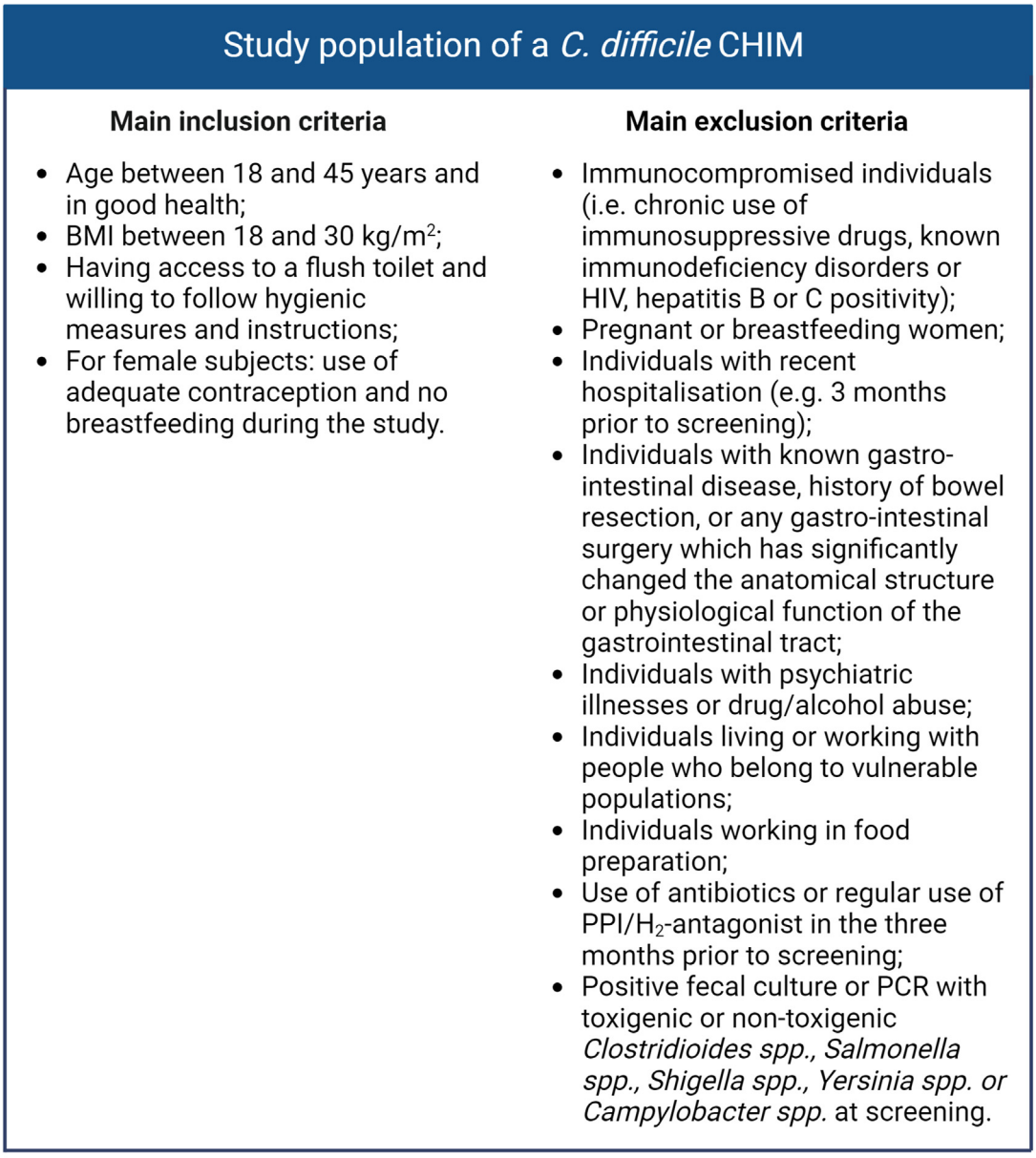

Fig. 3. The main in- and exclusion criteria for the study population of a *C. difficile* CHIM. *Figure created with* BioRender.com.

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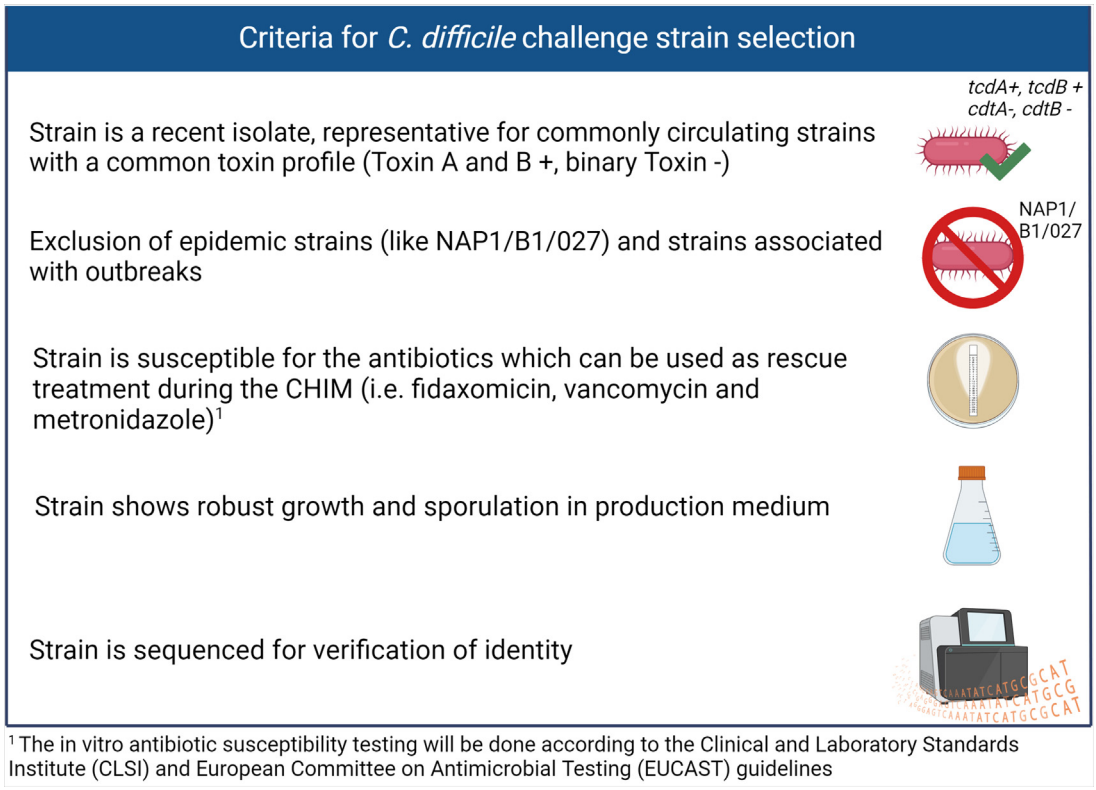

Fig. 4. The selection criteria for a *C. difficile* challenge strain. *Figure created with* BioRender.com.

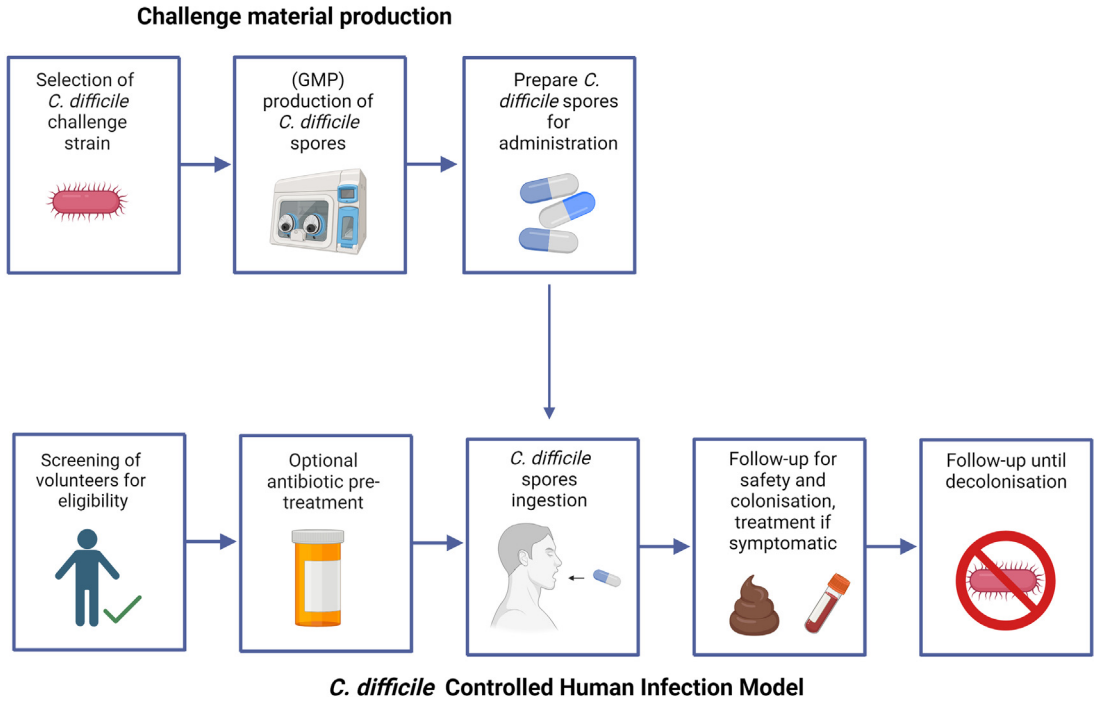

Fig. 5. Overview of the several subsequent steps of challenge material production and a *C. difficile* CHIM. *Figure created with* BioRender.com.

Follow up

To minimize the risk of severe/complicated CDI, participants should be strictly monitored for adverse events and vital signs. Moreover, during the follow-up period, stool collection for *C. difficile* testing and microbiota analysis should be performed as often as possible because of the phenomenon of intermittent shedding. If participants are still *C. difficile* positive at the last follow-up visit, these participants should be followed until decolonization (up to a certain maximum, e.g. 1 year after the start of the

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trial). According to the experts' opinion, decolonization should be defined as having a negative molecular *C. difficile* test on at least two different time points.

There was not yet consensus reached on whether participant monitoring should be in an in- or outpatient setting. The choice for one of these settings is governed by balancing multiple factors (e.g. participant safety, pathogen transmissibility, environmental contamination, infection control, available facilities, and participant engagement). Participants' safety can be secured in both settings; as this may seem more convenient in an inpatient facility with direct observation and frequent monitoring of participants, it can also be guaranteed in an outpatient setting with frequent ambulant visits, strict participant instructions, and a 24-hour available contact number, whereas this offers substantially more flexibility to the participants. However, an inpatient setting offers more control over sample collection/investigation, dietary intake, and minimizing exposure of participants to wild-type *C. difficile* strains. From a biosafety perspective, outpatient settings are justified, as recent data show that *C. difficile* strains are globally present in the natural environment, where its prevalence averages ~30%, with the most common strains being associated with human CDI [17]. Therefore, the added risk of environmental spread of *C. difficile* through a CHIM is estimated to be relatively small. This risk can be further reduced by strict hygienic measures and treatment of symptomatic participants. However, as there is no previous experience with toxigenic *C. difficile* CHIMs, the expert group could not reach a consensus on whether an inpatient setting would be needed, given the burden on participants.

Study population

To minimize the risk of a *C. difficile* CHIM, careful selection of participants is crucial. The recommended main inclusion and exclusion criteria for a *C. difficile* CHIM are listed in Fig. 3. Preferably the same ratio of male/female participants are included. In addition, the expert group agreed that a paucity of knowledge precludes selection of individuals based on microbiota composition. However, as more insight into *C. difficile* microbiota markers may be obtained with the development of a *C. difficile* CHIM, this may be a possibility for future trials.

Rescue treatment

Rescue treatment is a critical component in a *C. difficile* CHIM, as there needs to be an accurate treatment for those developing overt CDI. As rescue treatment standard of care is recommended; treating a first episode with the microbiota-sparing antibiotic fidaxomicin and as a second option with vancomycin [6]. The *C. difficile* challenge strain should test susceptible to these antibiotics before release (see section '*Challenge product*'). As FMT is highly effective for the prevention of recurrent episodes [9], any recurrence of CDI after the first episode will be treated with allogenous FMT. The expert group considered the use of autologous FMT, but as this has not been tested previously for the purpose of preventing recurrent CDI, there is a lack of clinical evidence, protocols, standardization, and experience with regards to safety.

The export group agreed to not treat participants with asymptomatic colonization because asymptomatic colonization is quite common in the general population [25], decolonization happens naturally in two-thirds of the population in 1 month [26], and the evidence of transfer of toxigenic *C. difficile* from asymptomatic colonized individuals to vulnerable populations is low [27]. This recommendation aligns with the clinical treatment guidelines [6].

Challenge product

Challenge strain selection

The selection of a *C. difficile* challenge strain is one of the first steps in developing a challenge model and plays a critical role in minimizing the risk to trial participants. For an overview of the *C. difficile* challenge strain criteria please refer to Fig. 4. The expert group underlines the importance of selecting a strain that is representative of commonly circulating strains; in Europe, the non-epidemic clade strain 1 is responsible for a substantial fraction of CDI cases [28,29]. As epidemic strains (e.g. clade 2, including NAP1/B1/027) are associated with lower curing rates, higher mortality, and increased recurrence rates [30–32], non-epidemic commonly circulating strains are preferable.

Ideally, a panel of different challenge strains should be created to increase the antigenic variance of the CHIM portfolio, increase understanding of the clinical effects of different strains, and accelerate vaccine development. However, given resource constraints and the novelty of the *C. difficile* CHIM, a stepwise approach to this is preferable.

Production of the challenge agent (C. difficile spores)

Following both the European Medicines Agency and the United States Food and Drug Administration requirements, the *C. difficile* challenge agent should be produced adhering as much as possible to the principles of Good Manufacturing Practices [33]. To minimize the risk of adventitious agents, animal-derived products should be avoided. Moreover, multiple quality controls and in-process controls should be performed, to ensure identity, purity, quantity, and safety of the challenge product and its intermediate products.

The experts advised administering *C. difficile* spores orally in direct-release capsules to mimic natural transmission and control spore dosing in the gastrointestinal tract. Because *C. difficile* spore germination requires exposure to primary bile acids in the proximal small intestine, enteric capsules were not recommended. For an overview of the several subsequent steps of challenge material production and the *C. difficile* CHIM see Fig. 5.

The expert group agreed that the challenge strain should be made available for research purposes to public and private research entities on a non-profit base, to make the CHIM as a research tool widely available and promote equitable research practices. To do so, the consortium intends to establish an access group that will monitor that sharing takes place in a fair, safe, and transparent manner, complying with frameworks for safe and secure transfer, storage and use, to minimize risks of misuse or advertent infection.

Conclusion

The development of a *C. difficile* CHIM will be an important tool to identify microbiota and immunological susceptibility markers, which can accelerate the identification of novel targets to battle *C. difficile*, which is still the leading cause of healthcare-associated diarrhoea. However, a toxigenic *C. difficile* CHIM has not been performed before and faces scientific, ethical, logistical, and biosafety challenges. Discussing these challenges raised the following main questions for a *C. difficile* CHIM; how and to what extent colonization and/or CDI can be achieved in healthy volunteers, what is the optimal dose of the *C. difficile* inoculum should be used to induce mild to moderate symptoms of CDI, what is the role of antibiotic pretreatment is in inducing susceptibility to CDI, and which biomarkers are associated with CDI. The development of a *C. difficile* CHIM offers the unique opportunity to answer these questions.

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Thus, a *C. difficile* CHIM is not only a model for the development and testing of (preventive) products, but also offers a framework for a better understanding of the pathophysiology, pathogenesis, and immunology of *C. difficile* colonization and infection.

Author contributions

A.D.O.H.: responsible for conceptualization and writing the original draft (lead). M.R., W.K.S.: responsible for conceptualization, writing-original draft (supporting), and supervision. N.C.: responsible for project administration. M.J.G.T.V., D.N.G., W.C., V.B.Y., S.T., P.S., M.M.G., C.C., S.C.J.d.K., D.D., S.M., J.-M.D., and E.J.K.: responsible for writing review and editing.

Transparency declaration

D.D, J.-M.D., and S.M. are employees of GSK and own business equities. W.K.S. performs research that is funded in part by a public–private partnership with AcurX Pharmaceuticals unrelated to the work presented here. D.N.G. has licensed NTCD technology and is a consultant for Destiny Pharma and Sebela Pharma. All other authors declare no competing interests. This communication reflects the author's view and that neither IMI nor the European Union, EFPIA, or any Associated Partners are responsible for any use that may be made of the information contained therein. This project ('*How to develop a Controlled Human Infection Model for Clostridioides difficile*') has received funding from the Innovative Medicines Initiative 2 Joint Undertaking under grant agreement No 101007799 (Inno4Vac). This Joint Undertaking receives support from the European Union's Horizon 2020 research and innovation programme and EFPIA.

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