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# Evaluating the risk of *Clostridioides difficile* infection from toilet flushing: a quantitative microbial risk assessment and implications for infection control

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SUMMARY

***Background:*** Despite stringent infection control measures, *Clostridioides difficile* infection (CDI) remains a challenge in healthcare settings, partly due to overlooked transmission vectors such as toilet plume bioaerosols.

***Aim:*** To systematically quantify the risks associated with CDI transmission via toilet flushing and provide critical insights to inform CDI preventive strategies.

***Methods:*** Impaction sampling was used to quantify airborne *C. difficile* post-flush and high-contact surfaces were swabbed to assess contamination levels, in a controlled toilet environment. A quantitative microbial risk assessment (QMRA) approach was then used to estimate the risk to subsequent users from contamination by a previously colonized individual.

***Findings:*** A single flush can release *C. difficile* into the air, with bioaerosol concentrations up to $29.50 \pm 10.52$ cfu/m$^3$ and deposit about 8–11 cfu on immediate surfaces. Despite a 4.4 log reduction in bacterial concentration within the toilet bowl post-flush, bacteria persist on its inner walls. Relative humidity increases by approximately 31.28% within the first 10 min post-flush, potentially enhancing the viability and transmission of aerosolized *C. difficile*. The flush button contact and inhalation-followed-by-ingestion in frequent-use hospital settings present the highest risks and exceed US EPA and WHO acceptable infection risk thresholds.

***Conclusion:*** The findings of this study necessitate a review of current toilet designs, public health policies and facility management practices to mitigate the overlooked risks of CDI transmission through toilet plume bioaerosols in healthcare settings. Additionally, this study lays a foundation for developing evidence-based interventions aimed at achieving substantial behavioural and infrastructural changes in infection control practices.



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## Introduction

*Clostridioides difficile* infection (CDI) remains a critical challenge in infectious disease management and is significantly impacting global public health. Characterized by its severe gastroenteritis and colitis, CDI imposes a significant financial burden on healthcare systems, costing approximately $796 million annually in the USA and €300 million in the European Union [1,2]. Despite ongoing efforts to mitigate its impact, CDI cases continue to surge, with the USA reporting 223,900 cases and 12,800 associated deaths in 2017 alone [3]. CDI is often linked to the use of antimicrobials, with a 60% increased risk of CDI among individuals exposed to antimicrobials [4]. However, recent studies have highlighted a demographic expansion in CDI impact, with a growing number of cases among those without prior antibiotic exposure and younger individuals [6] and has thus, broadened the scope of the population at risk beyond the traditionally recognized high-risk groups such as the elderly and hospitalized patients. This evolving understanding of CDI reinforces the need for vigilant monitoring and ongoing research to continually refine the understanding of transmission dynamics and to develop targeted interventions.

Transmission of CDI is multi-faceted, occurring not only through contaminated food, water, or fomites but also via indoor air [6–8], and this poses unique challenges for infection control. The bacterium's ability to form spores, which can remain airborne for extended periods and resist environmental stressors such as heat and hospital-grade disinfectants [8–10], further makes efforts to stop its spread even more difficult. One critical vector for *C. difficile* airborne dispersion is toilet flushing [11–13]. When an individual with CDI uses and flushes a toilet, *C. difficile* bioaerosols can be dispersed on to nearby surfaces and into the air in the toilet environment. These *C. difficile*-laden bioaerosols can also remain suspended in the air and be transported by air currents, thereby exposing subsequent toilet users. While many hospitals implement isolation protocols for known colonized patients, not all colonized patients are promptly isolated and asymptomatic carriers using shared toilets will continue to increase the potential for environmental contamination and *C. difficile* transmission. Thus, toilet flushing presents two risks of CDI transmission for the next susceptible user: direct contact with surfaces contaminated by *C. difficile* and the inhalation and subsequent ingestion of *C. difficile* bioaerosols [14]. The pathway of inhalation followed by ingestion is a recognized route for the transmission of infectious diseases, facilitated when particles are inhaled into the upper respiratory tract, particularly the nasal passages and throat, and subsequently swallowed with mucus, propelled by the mucociliary clearance mechanism of the respiratory tract [15–18].

To date, the two CDI risks posed by toilet flushing have not been systematically assessed, leaving uncertainties about the potential for transmission within toilet environments and identifying effective interventions to mitigate these risks. Recognizing the gap, i.e., the lack of systematic assessment of CDI risks from toilet flushing, this study applies a quantitative microbial risk assessment (QMRA) to assess CDI risks from toilet plume for the first time, pioneering a methodological approach that quantifies these risks with precision. QMRA, with its structured approach comprising hazard identification, exposure assessment, dose–response assessment, and risk characterization, provides a comprehensive framework for evaluating the infection risks associated with pathogens or exposure sources [6]. Additionally, this study monitors the relative humidity post-flush, offering a holistic view of the environmental conditions under which *C. difficile* transmission may occur in toilet environments.

This study is particularly relevant to a diverse group of stakeholders including healthcare workers, policymakers and facility managers aiming to achieve substantial behavioural and infrastructural changes in infection control practices. The potential for healthcare workers to accidentally spread CDI infection to immunocompromised patients amplifies the necessity for improved prevention strategies. For immunocompromised individuals such as the elderly and ICU patients, contracting CDI can worsen existing infection symptoms, disrupt treatment regimes, and potentially lead to the development of antimicrobial resistance [19].

The findings of this study could reform infection control practices, inform toilet design, and influence public health policies to better prevent the spread of *C. difficile* and other pathogens in toilets in diverse settings, including hospitals. Ultimately, this study supports the sustainable development goal of reducing communicable diseases.

## Methods

### *Experimental set-up and preparation of* C. difficile *spore suspensions*

A controlled indoor toilet cubicle was constructed in a biological laboratory to replicate a toilet in a healthcare setting. A 6-litre dual flush toilet (Screwfix, Model SXPTP0056) was installed, and the cubicle was ventilated using a mechanical extraction system and a HEPA-filtered air purifier. To replicate the consistency of diarrhoeal faeces, non-toxigenic *C. difficile* spore suspensions were prepared to a concentration of $10^7$ cfu/mL based on the procedure described by Best *et al.* [12] in their previous study on the airborne dissemination of *C. difficile*. Detailed set-up, preparation of spore suspensions and ventilation rationale are available in the Supplementary material.

### *Bioaerosol sampling, surface contamination assessment and relative humidity monitoring*

Over a 14-day period, the toilet was inoculated with $10^7$ cfu/mL *C. difficile* spore suspension and flushed once daily. Bioaerosols released post-flush were sampled using a 400-hole Micro Bio MB1 Sampler at a flow rate of 100 L/min. Concurrently, four high-contact surfaces as identified by previous studies [20,21] – the flush button, toilet seat, lid and floor – were swabbed (Supplementary Figure A2), along with collected residual bowl water, totalling 98 samples (14 air, 14 bowl water, and 14 per surface) for microbial analysis. Control samples were also collected prior to flushing to confirm that post-flush presence of *C. difficile* was solely attributable to the flushing activity.

To evaluate the impact of flushing on air quality and assess the environmental conditions that may influence the viability and transmission of aerosolized *C. difficile*, relative humidity

(RH) levels within the toilet environment were monitored daily. This was carried out using an RS PRO DT802D air quality monitor (RS Components Ltd., UK), placed centrally at a height of 1 m above the floor to ensure that the measurements represented a well-mixed air sample. The device recorded RH levels before and after each flush, and could detect values from 0% to 90% with an accuracy of ± 5% and a precision of 0.1%.

The detailed procedure for bioaerosol sampling and microbial analysis are provided in the Supplementary material. Toilet flushing in this study was performed by the first author. This study did not investigate or collect data on the impacts of toilet flushing on a human operator.

## QMRA

### *Hazard identification and exposure assessment*

*C. difficile* spores released from flushing were identified as the hazard. Two exposure pathways (exposure scenarios) were assessed (Figure 1): (1) fomite-to-hand-to-mouth – spores deposited on surfaces are transferred to hands upon contact and subsequently ingested; (2) inhalation-followed-by-ingestion – airborne spores are inhaled into the respiratory tract, trapped in mucus and then swallowed.

The effective infective dose for the exposure scenarios was calculated using equations and exposure parameters (Supplementary Table A1) detailed in the Supplementary material.

### *Dose–response assessment*

The CDI risk was calculated using the Beta Poisson model [6] with optimized parameters from fitting *C. difficile* dose–response data from Chen *et al.* [22]. A Monte Carlo simulation was conducted in R, randomly sampling parameter values based on their means and standard deviations/errors provided in Supplementary Table A1, over 10,000 iterations. The CDI risk was recalculated for each iteration, with the median and mean infection risks across all iterations providing an estimate that reflects the inherent uncertainty and variability. To determine the appropriateness of using median or mean infection risk for risk characterization, the distributions of mean and median risks of infection for both fomite and

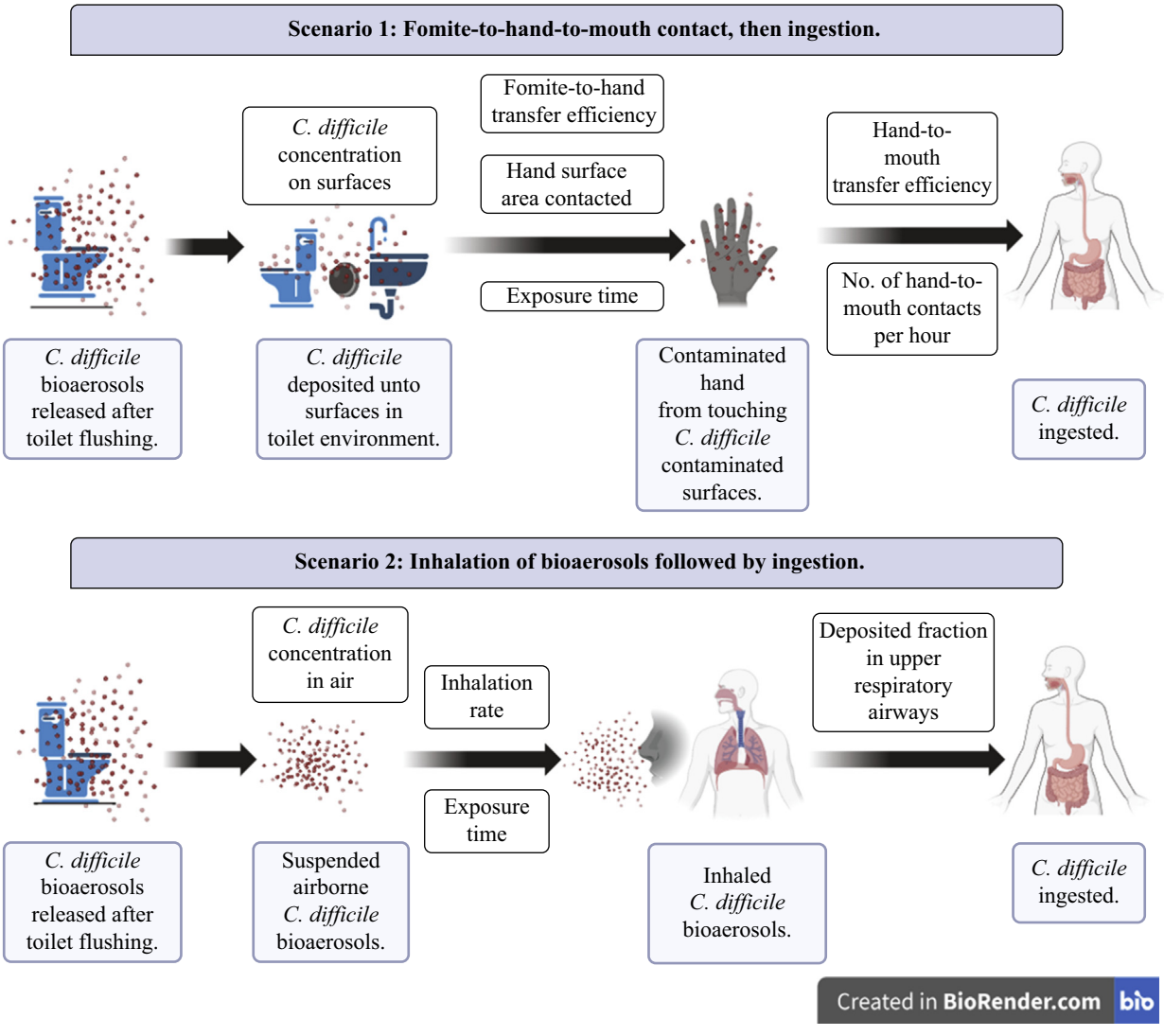


**Figure 1.** Exposure scenarios for *Clostridioides difficile* transmission via toilet plume bioaerosols.

inhalation scenarios were examined for skewness via histograms and density plots as detailed in the Supplementary material.

*Risk characterization*

The daily and annual CDI risks for each exposure scenario were calculated for two distinct cases in settings where shared toilets are utilized by multiple individuals, including *C. difficile* colonized individuals who are not yet isolated due asymptomatic carriage. Case 1 assumed an exposed individual uses the toilet once daily but only on a single day within the year, applicable to healthcare facility visitors/outpatients. Case 2 considered an exposed individual using the toilet three times daily over 8.3 days annually, reflecting the scenario for hospitalized patients. The duration of 8.3 days was selected based on the average annual hospitalization duration, incorporating elective and emergency admissions [23].

The annual CDI risks for Case 1 and 2 were evaluated against the annual health risk benchmarks set by the United States Environmental Protection Agency (US EPA) (2005) and the foodborne disability adjusted life years (DALYs) for diarrhoeal disease bacteria as recommended by World Health Organization (WHO) (2008) [24]. This comparison aimed to contextualize the magnitude of CDI risk in relation to established health standards, providing insight into how the study's findings align with or exceed these international benchmarks. Acceptable levels are defined as $\leq 10^{-4}$ pppy for US EPA annual infection risk and $\leq 10^{-6}$ DALYs pppy for WHO disease burden [25].

Equations for estimating the daily CDI risks, annual CDI risks and disease burdens are detailed in the Supplementary material.

### Statistical analyses

Analysis of variance (ANOVA) and post-hoc Tukey tests identified significant differences in *C. difficile* concentrations across surfaces ($P<0.05$). Monte Carlo simulations, sensitivity analyses, and all statistical tests, including the generation of plots and graphs, were conducted in R (v4.3.2) [26]. All assumptions made in this QMRA, and detailed statistical analyses are provided in the Supplementary material.

## Results

### Quantified bacteria in air and on surfaces

The average *C. difficile* bioaerosol concentration measured from a single flush was 29.50 ± 10.52 cfu/m$^3$. Zero bioaerosol concentrations were recorded for control samples, which sampled air before flushing, indicating that the presence of *C difficile* in air was due to the flushing activity. The average cfu counts recovered from swabbing the surfaces in the toilet considered in this study are presented in Table I.

The ANOVA results (Supplementary Table A2) indicated a high level of significance ($P<0.001$) and confirmed statistically significant differences in the mean concentrations of *C. difficile* across the experimentally examined surfaces (toilet floor, flush button, toilet seat and toilet lid). Further analysis using post-hoc Tukey's tests (Supplementary Table A3 and Supplementary Figure A3) revealed highly significant differences in *C. difficile* counts between the toilet seat, the flush button and the toilet floor, and between the toilet floor and the flush button. This observation is confirmed by box plots illustrating *C. difficile* counts by surface (Supplementary Figure A4), highlighting the floor and toilet seat as the most highly contaminated surfaces. Although floor–hand–mouth risks are low, this pathway was included to capture accidental contacts during cleaning, maintenance, or by individuals with limited mobility.

**Table I**
Post-flush microbial counts on sampled toilet surfaces

| Description of sampling point | Area sampled (m$^2$) | Average counts after a single flush (cfu) | Standard deviation (cfu) |
|---|---|---|---|
| Toilet floor | $2.25 \times 10^{-2}$ | 8.5 | 2.68 |
| Flush button | $0.20 \times 10^{-2}$ | 3.14 | 1.56 |
| Toilet seat | $7.60 \times 10^{-2}$ | 11.29 | 2.02 |
| Toilet lid | $1249 \times 10^{-2}$ | 5.43 | 1.55 |

The average bowl water concentration measured after a single flush was 394 ± 51 cfu/mL, representing a 4.4 log reduction in bacterial concentration after a single flush. The average count recovered from swabbing the interior sidewalls of the toilet bowl was 28 ± 6 cfu. All control swab samples collected from surfaces before flushing showed no growth and thus zero bacterial count. This confirmed that the presence of bacteria identified on the surfaces post-flush was directly attributable to the act of toilet flushing. The average RH recorded before and after flushing experiments were 41.89 ± 0.94% and 54.99 ± 3.59%, respectively. This corresponded to an average increase of about 31.28% within the first 10 min of flushing and then a gradual decline afterwards (Supplementary Figure A5).

### QMRA

*Estimated daily and annual CDI risks*

Supplementary Table A7 shows the estimated daily and annual CDI risks across the exposure scenarios considered in this study, using the estimated mean and median CDI risks from the Monte Carlo simulation (Supplementary Table A4). In Case 1, where an individual uses the toilet once daily for a single day within a year, the daily infection risk is equivalent to the annual, because the exposure occurs only once throughout the year. In contrast, Case 2 involves an individual hospitalized and using the toilet three times daily for 8.3 days annually. This increased exposure frequency escalates the daily risk compared with Case 1. As a result, the annual risks in Case 2 were markedly higher, incorporating the cumulative effect of multiple exposures across the 8.3 days, significantly elevating the infection risk compared with a single day of exposure. As shown in Figure 2, the flush button fomite exposure and inhalation scenarios consistently presented the highest daily and annual infection risks. Risks from the floor, seat, and lid scenarios were comparatively lower, remaining below a 1% threshold.

*Disease health burden and annual infection risk per person per year*

Supplementary Table A8 compares the estimated disease health burdens and the annual risk of CDI alongside the thresholds set by the US EPA and WHO. The table shows that the

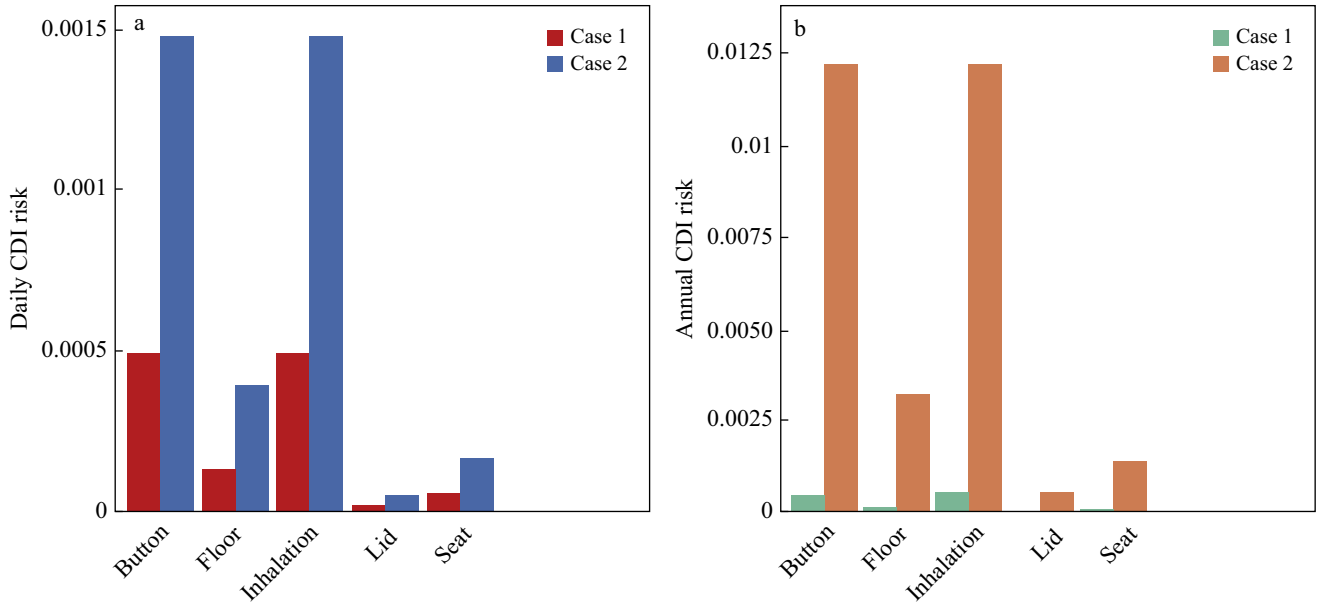


**Figure 2.** Daily and annual *Clostridioides difficile* infection (CDI) risks for the different exposure scenarios and cases.

estimated disease burden and annual risk are significantly higher in Case 2 compared with Case 1. This reinforces the cumulative effect of repeated exposures and highlights the increased risk hospitalized patients face. For the estimated disease burdens, all scenarios, except the lid contact in Case 1, exceeded the WHO's acceptable threshold for disease burden ($\leq 10^{-6}$ DALYs pppy). The flush button and inhalation scenarios in Case 2 stood out, showing the highest disease burdens (Figure 3). Similarly, for the estimated annual CDI risks, all scenarios except seat and lid contacts in Case 2, exceeded the US EPA's acceptable annual infection risk threshold ($\leq 10^{-}4$ pppy), with the flush button in Case 2 posing the highest risk (Figure 3). Similarly, the inhalation scenario mirrored this high risk, comparable to the Flush Button Case 2, due to the frequency of exposure.

## Discussion

The quantified bacterial presence in the air and on surfaces post-flush confirms that toilet flushing facilitates the spread of *C. difficile* through surface contamination and airborne dispersion [11,13,27]. Essentially, the reported measured concentrations in each exposure scenario imply that toilet flushing

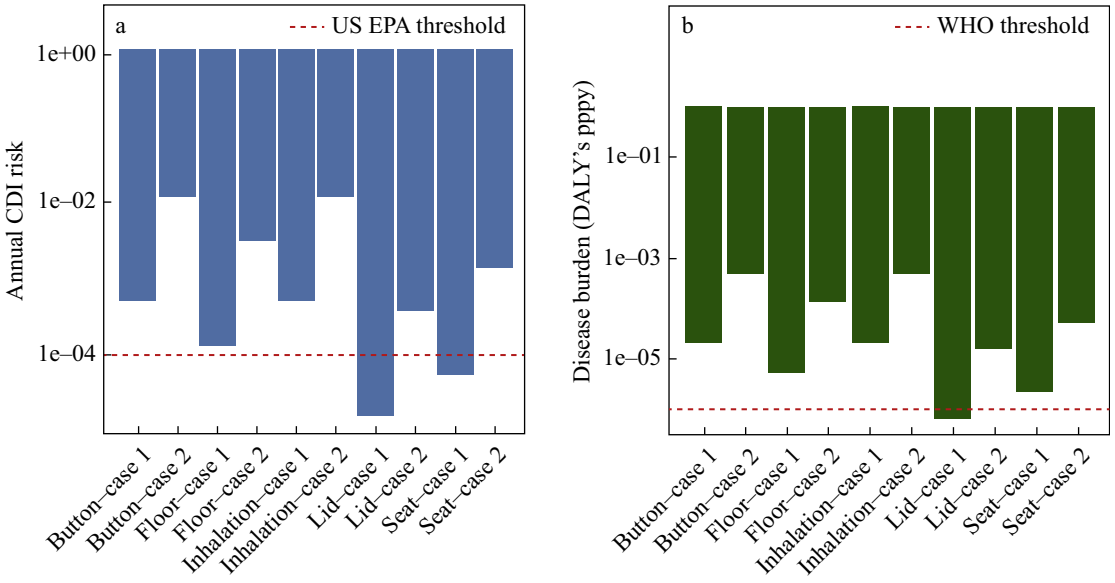


**Figure 3.** Annual *Clostridioides difficile* infection (CDI) risks and disease burdens across exposure scenarios compared with the US EPA and WHO thresholds. DALYs, disability adjusted life years.

after an individual with CDI uses the toilet leaves behind residual contamination, posing risks to subsequent users. Notably, surfaces such as toilet seats and floors exhibited the highest contamination levels, aligning with prior research [12,28,29] and suggesting that, these areas are major hotspots requiring targeted cleaning protocols to mitigate *C. difficile* transmission. Despite achieving a 4.4 log reduction in bacterial concentration within the toilet bowl post-flush, residual contamination persisted, demonstrating the resilience of *C. difficile* spores [13,30]. This persistence indicates that high-traffic hospital toilets frequented by patients can act as reservoirs for infectious bioaerosol generation. Current surface sampling methods probably underestimate actual contamination levels, as contact plates and swabs recover only 19—32% and 76—94% of bacteria, respectively [20]. Therefore, the true extent of contamination may be greater, necessitating more effective sampling and cleaning strategies.

Toilet flushing induced a 31% increase in RH within the first 10 min, altering indoor air quality in a manner that supports pathogen survival and transmission. Elevated RH enhances the viability of aerosolized pathogens and increases the transfer efficiency of *C. difficile* from non-porous surfaces, thereby escalating the risk of fomite-mediated transmission [31,32]. Poor ventilation further exacerbates these risks by prolonging the airborne persistence of pathogens. To mitigate these risks, the installation of efficient ventilation systems that can rapidly normalize RH levels post-flush is recommended [33]. Such interventions would reduce the window during which pathogens remain viable and transmissible, thereby enhancing the overall safety of toilet environments.

QMRA revealed that annual CDI risks exceed both the WHO and US EPA thresholds in most exposure scenarios, particularly involving flush button contact and repeated exposures. Specifically, the annual risk of contracting CDI through fomite-to-hand-to-mouth contact from touching a contaminated flush button once daily is approximately 49 per 100,000 individuals. This risk escalates to about 1220 per 100,000 individuals in scenarios involving three daily exposures over 8.3 days, reflecting typical hospitalization durations [34]. The physical dimensions of *C. difficile* spores (1—1.5 μm in length and 0.5—0.7 μm in diameter) facilitate their suspension in air and inhalation into the respiratory tract, where they can be deposited and subsequently ingested [6,10]. Given their ability to remain airborne for 7—28 h under undisturbed conditions, inhalation-followed-by-ingestion represents a significant transmission pathway that must be addressed in infection control strategies. This study estimated that in scenarios where *C. difficile* aerosols are present in a toilet that was used and flushed by an infected individual the estimated annual risk of CDI through this route is approximately 49 per 100,000 for once-daily toilet use. If the frequency increases to three times daily over 8.3 days annually, the risk significantly escalates to approximately 1220 per 100,000 individuals.

These findings reinforce the urgent need for enhanced CDI prevention and control measures to maintain a hygienic environment for subsequent toilet users, especially in high-risk settings such as hospitals. Key recommendations should be multi-faceted and include the establishment of stringent cleaning routines for high-contact surfaces such as flush buttons, toilet seats, and floors, adoption of touchless flushing mechanisms to minimize direct hand contact with contaminated surfaces, incorporation of antimicrobial surfaces, utilization of air sanitization systems and the optimization of ventilation systems to effectively reduce bioaerosol persistence and normalize RH swiftly post-flush.

The study's findings highlight the importance of addressing all potential transmission pathways to comprehensively reduce CDI risks and call for a comprehensive re-evaluation of current guidelines and policies related to CDI management in healthcare settings. This study's risk assessment, based on measurements from a single toilet flush, probably underestimate the true CDI risk, particularly in high-traffic toilets where frequent use reduces intervals between exposures. Additionally, the study focused on non-toxigenic *C. difficile* strains, suggesting that toxigenic strains could pose even greater infection risks. Particularly concerning is the elevated risk for patients and hospital staff, who experience continuous exposure compared with outpatients and visitors. Despite the known efficacy of handwashing in mitigating infection risks, adherence to recommended handwashing practices remains low even among healthcare workers, with only 26.2% of global toilet visits involving handwashing with soap [35—37]. This low compliance, coupled with the limited effectiveness of singular interventions, such as closing the toilet lid or basic cleaning, highlights the necessity for a multi-faceted approach to managing CDI risks and other infections such as Legionella, where similar toilet plume transmission dynamics have been implied [38].

This study has several limitations. A single, standardized toilet mock-up was used, and a consistent flush order was assumed. Future research should incorporate diverse toilet designs, flushing postures, varied usage patterns, *C. difficile*-specific transfer efficiencies and time-resolved sampling to better capture *C. difficile* spore exposure, dispersal dynamics and airborne persistence. These improvements will enhance the accuracy and generalizability of risk assessments and inform more effective prevention and control strategies across diverse healthcare settings globally. Exploring the effectiveness of intervention measures, such as antimicrobial surface coatings and automated flushing systems, can offer practical solutions for mitigating CDI transmission via toilet flushing. Interdisciplinary collaborations among microbiologists, engineers, and healthcare professionals will be essential for developing comprehensive CDI prevention strategies. Furthermore, adherence to standardized sanitation guidelines, such as those outlined in Health Building Note 00—02 [39], can help mitigate the variability in bioaerosol dispersal and reduce CDI transmission risks.

This study illuminates the under-recognized risks of toilet plume bioaerosols in CDI transmission, utilizing a QMRA approach to quantify the threat they pose. The findings reveal that contact with the flush button and inhalation of bioaerosols followed by ingestion present the highest risks for spreading *C. difficile* spores, particularly in scenarios involving multiple exposures. These insights advocate for reassessing current sanitation and hygiene strategies, urging the implementation of comprehensive measures that address inhalation pathways, high-risk direct contact areas, and environmental factors contributing to the transmission of *C. difficile*. By establishing a foundational QMRA framework, this study paves the way for more detailed investigations into CDI transmission dynamics and serves as a pivotal guide for developing targeted, evidence-based interventions to mitigate CDI spread,

ultimately supporting the United Nations Sustainable Development Goals related to health and enhancing safety within healthcare settings.

**Conflict of interest statement**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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**Appendix A. Supplementary data**

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhin.2025.02.012.

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