SUPPLEMENTARY MATERLS

SUPPLEMENTARY MATERIALS TABLE 1 Aerosol ingestion rate of *Staphylococcus aureus* and *Escherichia coli* bioaerosols in squat and bidet toilets

	Items	Toilet flashing conditions	Two different Ventilation scenarios about exhaust fan	Ingestion rate of respirable bioerosol (AG)* Uniform distribution
Staphylococcus aureus	Squat toilet	First flushing without stool material	Turned-off	Min=0.10; Max=0.64
			Turned-on	Min=0.10; Max=0.56
		Second flushing without stool material	Turned-off	Min=0.10; Max=0.71
			Turned-on	Min=0.10; Max=0.62
	Bidet toilet	First flushing without stool material	Turned-off	Min=0.10; Max=0.66
			Turned-on	Min=0.10; Max=0.55
		Second flushing with stool material	Turned-off	Min=0.10; Max=0.69
			Turned-on	Min=0.10; Max=0.63
Escherichia coli	Squat toilet	First flushing without stool material	Turned-off	Min=0.10; Max=0.60
			Turned-on	Min=0.10; Max=0.53
		Second flushing with stool material	Turned-off	Min=0.10; Max=0.62
			Turned-on	Min=0.10; Max=0.56
	Bidet toilet	First flushing without stool material	Turned-off	Min=0.10; Max=0.56
			Turned-on	Min=0.10; Max=0.52
		Second flushing with stool material	Turned-off	Min=0.10; Max=0.59
			Turned-on	Min=0.10; Max=0.53

*The maximum value was set as the proportion of 3–6 stages of the Andersen six-stage cascade impactor (bioaerosol particle size less than 4.7 μm); and the minimum value was set as 0.10 (Shi, Wang, & Jiang, 2018b).

DETAILS ABOUT QMRA CALCULATION AND SENSITIVITY ANALYSES

1 BIOAEROSOL ANALYSIS

A six-stage Andersen impactor (FA-1 Hongchangxinlnc, Beijing, China) was used to measure the *Staphylococcus aureus* and *Escherichia coli* bioaerosol concentration and size distribution (Jahne, Rogers, Holsen, Grimberg, & Ramler, 2015; Pasalari, Ataei-Pirkooh, Aminikhah, Jafari, & Farzadkia, 2019). The size ranges of the impactor can be classified into six different aerodynamic diameters of bioaerosol: stage 1 (≥ 7.0 μm), stage 2 (4.7~7.0 μm), stage 3 (3.3~4.7 μm), stage 4 (2.1~3.3 μm), stage 5 (1.1~2.1 μm), and stage 6 (0.65~1.1 μm) (Brągoszewska, Mainka, & Pastuszka, 2017).

After sampling, the petri dish was transported to the laboratory using a cold storage box, and the samples were incubated for 48 hours at 37°C (Yanpeng, Lu, Li, Xie, & Song, 2017). After cultivation, the colonies were counted with the help of an automated colony counter, which resulted in colony-forming units (CFU) (Needs, Osborn, & Edwards, 2021). To correct and obtain the actual numbers of colonies at each stage of the petri dish, the positive hole method Eq. (1) was employed (Macher, 1989; Yan, Leng, & Wu, 2021)

$$Pr = N \cdot \left[\frac{1}{N} + \frac{1}{N-1} + \frac{1}{N-2} + \cdots + \frac{1}{N-r-1}\right] \quad (1)$$

where *Pr*, *r*, and *N* represent the modified colonies, sampling colonies, and number of sieve pore at each stage of the Anderson six-stage cassette impactor, respectively.

The bioaerosol concentration was then evaluated and frequently represented as total colony-forming units (CFU/m^3). The following Eq. (2) was used for calculation (Akpeimeh, Fletcher, & Evans, 2019).

$$C = \frac{Pr \times 1000}{t \times F} \quad (2)$$

where C denotes the concentration of bioaerosol (CFU/m^3), F is the sampling flow rate (L/min), and t is the sampling time (min).

2 QUANTITATIVE MICROBIAL RISK ASSESSMENT (QMRA)

Quantitative microbial risk assessment (QMRA) was carried out following the classical four-step framework, which consists of the following: (i) hazard identification, (ii) exposure assessment, (iii) dose-response assessment, and (iv) risk characterization (Haas, 2015; Haas, Rycroft, Bibby, & Casson, 2017). The specific values for the related parameters in the equations are shown in Table 2.

2.1 Hazard identification

The pathogens of concern in this study was *Staphylococcus aureus* and *Escherichia coli* bioaerosol, which originated due to toilet flushing in the indoor washrooms. *Staphylococcus aureus* and *Escherichia coli* are the most prevalent airborne pathogenic bacteria found in indoor washroom environments (Levy et al., 2021).

2.2 Exposure assessment

Bioaerosol concentration and size distribution were analyzed in two washrooms, each equipped with squat and bidet toilets, under two ventilation scenarios: exhaust fan turned

47 off and exhaust fan turned on. The study focused on defecation activities in both
48 washrooms, considering two flushing conditions: the first without stool material and the
49 second with stool material. Eight exposure scenarios were considered in total. For the squat
50 toilet washroom: (i) first flushing without stool material and exhaust fan turned off, (ii)
51 first flushing without stool material and exhaust fan turned on, (iii) second flushing with
52 stool material and exhaust fan turned off, and (iv) second flushing with stool material and
53 exhaust fan turned on. For the bidet toilet washroom: (v) first flushing without stool
54 material and exhaust fan turned off, (vi) first flushing without stool material and exhaust
55 fan turned on, (vii) second flushing with stool material and exhaust fan turned off, and (viii)
56 second flushing with stool material and exhaust fan turned on.

57 All the exposure assessment parameters of the QMRA calculation frameworks and
58 details of the exposure scenarios in two types of washrooms are shown in Tables 1 and 2.

59 The exposure dose of pathogens per person per day was calculated in Eq. (3) (Brooks,
60 McLaughlin, Gerba, & Pepper, 2012).

61 $$d = C \times BR \times T \times AG \qquad (3)$$

62 where d represents daily exposure dose, C is bioaerosol concentration (CFU/ m^3), BR is
63 exposed person respiration rate (h/d), ET is per day exposure time (min/day), and AG is
64 ingestion rate of inhalable bioaerosol (%).

65 In this study, we assumed a fixed exposure time when a person used the toilet. Its value
66 was set following a uniform distribution (Min = 5 min/day, Max = 10 min/day). Moreover,
67 the maximum value of *Staphylococcus aureus* and *Escherichia coli* respirable bioaerosol
68 AG is set as the proportion of 3–6 stages of the Andersen six-stage cascade impactor

(bioaerosol particle size less than 4.7 μm) and the minimum value of AG is set as 0.10 (Shi et al., 2018), shown in Table 3 and supplementary Materials in Tables 1. In this study, an assumption was made that all the trapped bioaerosols were cleared to enter the respiratory tract (Fry & Black, 1973; Shi et al., 2018; Stuart, 1984); however, only concentrations from the 3–6 stages of the impactor were assumed to have the capability to cause health effects (Ghosh, Lal, & Srivastava, 2015; Yong Liu, Hu, Zhou, Jiang, & Yang, 2016; Morakinyo, Mokgobu, Mukhola, & Hunter, 2016). Thus, the maximum value of AG was set at the ratio of 3–6 stages to 1–6 stages of the impactor.

2.3 Dose-response models

Staphylococcus aureus bioaerosol uses the exponential dose-response model as the dose-infection model (Esfahanian, Adhikari, Dolan, & Mitchell, 2019; Haas, 2015). Daily health infection risk was calculated in Eq. (4).

$$P_i(d) = 1 - e^{-dk} \tag{4}$$

where $P_i(d)$ represents the daily health infection risk, k is the exponential dose-response value model parameter (Table 1), and d represents the daily exposure dose (CFU/day).

For the exponential dose-response model of *Staphylococcus aureus* bioaerosol, several significant kinds of literature reported that airborne transmission (i.e., respirable bioaerosol) and direct transmission (i.e., transmission by mouth or hand) are equally crucial for public health (Bos et al., 2016; Chang & Lin, 2018; Kozajda, Jeżak, & Kapsa, 2019). However, this study mainly focuses on humans being exposed to emitted bioaerosol in indoor washrooms. This is because the anti-epidemic COVID-19 guideline of WHO clearly

states that bioaerosol from indoor toilet flushing significantly impacts human health (Hu, Yang, Wei, & Luo, 2021; Zhen et al., 2022). So, direct transmission, including skin infection, is not considered in the exposure scenarios of the current study; inhalation is the sole exposure route in this study for convenience.

Escherichia coli bioaerosol uses the beta-Poisson dose-response model as a dose-infection model. Daily health infection risk was calculated in Eq. (5) (Shi et al., 2018).

$$P_{(d)inf} = 1 - (1 + \frac{d}{\beta})^{-\alpha}$$

where $P_{(d)inf}$ is the daily health infection risk, α and β are the model parameters (Table 2).

2.4 Risk characterization

Risk characterization was performed on the basis of the dose-response models to assess the health risks associated with bioaerosols. The benchmark ($\leq 10^{-6}$ DALYs pppy) recommended by WHO (2008) was used to evaluate human health risks (Kongprajug, Denpetkul, Chyerochana, Mongkolsuk, & Sirikanchana, 2021). The annual probability of health infection per person per year (pppy) was calculated using Eq. (5) (Dungan, 2014).

$$P_y = 1 - (1 - P_i(d))^n \quad (5)$$

where P_y represents the annual probability of health infection risk (pppy), while n is the annual frequency of each exposure scenario per year (total days per year).

The specific potential disease health risk burden (DALYs pppy) illness caused by exposure to *Staphylococcus aureus* and *Escherichia coli* bioaerosol was calculated using Eq. (6) (Havelaar et al., 2015; Shi, Wang, & Jiang, 2018a).

$$DB = P_y \times HB \quad (6)$$

where *DB* represents disease health risk burdens (DALYs pppy), and *HB* represents health burden (DALYs/case) shown in (Table 2).

2.5 Monte Carlo simulation

Monte Carlo simulation was utilized to create a probabilistic-based risk model (Islam et al., 2021). This research completed ten thousand trials by using Crystal Ball and Microsoft Excel 2016 (Li et al., 2021). All input parameters (exposure concentration, exposure time, aerosol ingestion rate, and breathing rate) were randomly chosen from their probability distributions. The assumptions were as follows: exposure concentration obeyed lognormal distribution; exposure time, bioaerosol ingestion rate, and breathing rate obeyed uniform distribution (Pasalari et al., 2019). Over 10,000 iterations, output parameters (disease health risk burdens) were calculated so that the distributions could reach a stable state (Yanjun Liu, Lu, Wang, Gao, & Huang, 2019). The Monte Carlo simulation results were displayed using a box and whiskers chart. The lower whisker in the box chart represented the 2.5th percentile quartiles (optimistic estimate), and the upper whisker of the box chart presented the 97.5^{th} percentile quintiles (conservative estimate). The middle of the box represented average and tetragon inside the box medium value.

3 SENSITIVITY ANALYSIS

The sensitivity analysis was used to quantify the contributions of all the QMRA inputted variable parameters (exposure concentration, exposure time, ingestion rate of inhalable bioaerosol, and breathing rate) to the uncertainty in the health risk output (disease health risk burdens) (Dias, Ebdon, & Taylor, 2019; Saltelli, 2002; Saltelli et al., 2019). The quantification contribution, that is, the sensitivity partitioning coefficient, was calculated by using the Spearman rank correlation coefficient in Oracle Crystal Ball, and the rankings were visualized in tornado plots (Dean & Mitchell, 2020; Pouillot & Delignette-Muller, 2010; Vásquez, Busschaert, Haberbeck, Uyttendaele, & Geeraerd, 2014). The Spearman rank correlation coefficient values ranged from ‐ 1 to 1. For the positive correlation, a high correlation coefficient corresponds to the great contribution of a parameter to the health risk. The negative correlation implies the contrary (Bhat & Kumar, 2008; Vásquez et al., 2014). All calculations were conducted using Oracle Crystal Ball version 11.1.24 in Excel in Windows (Microsoft Office Excel 2013), and all figures were generated with Origin version 2018 software.

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Quantitative microbial risk assessment of bioaerosol emissions from squat and bidet toilets during flushing

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2025-08
Wiley
https://doi.org/10.1111/risa.70000
Ali W, Hu Z, Tang Z, et al., (2025) Quantitative microbial risk assessment of bioaerosol emissions from squat and bidet toilets during flushing. Risk Analysis, Volume 45, Issue 8, August 2025, pp. 2083-2096

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