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ORIGINAL ARTICLE

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

Wajid Ali[1] | **Zhen Hu**[2] | **Zhe-ren Tang**[3] | **Si-yi Liu**[1] | **Zaheer Ahmad Nasir**[4] | **Frederic Coulon**[4] | **Peng Liu**[1] | **Cheng Yan**[1]

[1]School of Environmental Studies, China University of Geosciences, Wuhan, P. R. China

[2]Wuhan Huzhenyu Environmental Technology Co., Ltd, Wuhan, P. R. China

[3]National Engineering Research Center of Eco-Environment in the Yangtze River Economic Belt, China Three Gorges Corporation, Wuhan, P. R. China

[4]School of Water, Energy and Environment, Cranfield University, Bedfordshire, UK

Correspondence
Cheng Yan, School of Environmental Studies, China University of Geosciences, 388 Lumo Road, Wuhan, P. R. China.
Email: cheng_yan@cug.edu.cn

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Abstract

Bioaerosol emissions during toilet flushing are an often-overlooked source of potential health risks in shared public facilities. This study systematically investigated the emission characteristics of *Staphylococcus aureus* and *Escherichia coli* bioaerosols in washrooms with squat and bidet toilets under varying flushing conditions and ventilation scenarios. Using Monte Carlo simulation–based quantitative microbial risk assessment and sensitivity analysis, the study estimated the disease burden and identified key factors influencing risk. The results showed that squat toilets generated 1.7–2.6 times higher concentrations of *S. aureus* bioaerosols and 1.2–1.4 times higher concentrations of *E. coli* bioaerosols compared to bidet toilets. After the first flush, bioaerosol concentrations were 1.3–1.8 times (*S. aureus*) and 1.2–1.4 times (*E. coli*) lower than those observed after the second flush. The second flush released a higher proportion of fine bioaerosol particles (<4.7 μm), increasing inhalation risks. The disease health risk burden was consistently one order of magnitude lower after the first flush than the second one. Ventilation with a turned-on exhaust fan further reduced the risk by one order of magnitude. Sensitivity analysis identified exposure concentration as the most influential parameter, contributing up to 50% of the overall risk. This study highlights the importance of optimizing toilet design and ventilation systems to mitigate bioaerosol emissions and associated health risks. It provides actionable insights for improving public washroom hygiene and minimizing bioaerosol exposure.

KEYWORDS
bioaerosol, disease burden, Monte Carlo simulation, quantitative microbial risk assessment, sensitivity analysis, toilet

1 | INTRODUCTION

Individuals spend a substantial amount of time in indoor environments, such as homes, schools, and workplaces. As a result, indoor air quality and public awareness of its significance are critical for protecting human health (Chithra & Nagendra, 2012). In particular, the act of flushing in indoor washrooms, featuring diverse toilet designs, remains a notable contributor to bioaerosol contamination within indoor environments (Johnson, Mead et al., 2013; Usman et al., 2021). Bioaerosol refers to tiny particles of a pathogenic biological nature dispersed in the air, and inhalation is the main pathway of exposure to these emitted bioaerosols (Kim et al., 2018; Yang et al., 2019). Fine bioaerosol particles have an aerodynamic diameter of less than 5 μm, making it easy to penetrate deep into the alveoli and cause allergic alveolitis (Guzman, 2021; Stobnicka & Górny, 2015). Previous studies have documented bioaerosol emission and concentration upon flushing a toilet, leading to the dispersion of biological particles with a diameter of 3 μm or smaller into the surrounding indoor atmosphere (Johnson, Lynch et al., 2013; Nazaroff, 2016). These biological particles can induce distressing symptoms, including abdominal cramps, nausea, diarrhea, and vomiting (Matini et al., 2020).

Compared to private washrooms, public washrooms often see higher usage levels throughout the day, which raises the possibility and degree of contamination (Flores et al., 2011). Luo et al. (2023) and Vardoulakis et al. (2021) both stated that public toilets tend to be more contaminated due to overload and shared usage and then have a higher risk of transmission than private toilets because they are used more frequently and have a broader user base. In the

studies conducted by Johnson, Mead et al. (2017) and Abney et al. (2021), it was observed that the water in the toilet bowl remained contaminated even after multiple flushes. Matsui et al. (2023) stated that the SARS-CoV-2 hospitalized patient attended the toilet in a hospital private room. However, after flushing the toilet, the SARS-CoV-2 RNA was detected in the indoor air and toilet bowl water. Furthermore, Aithinne et al. (2019) reported the presence of *Clostridium difficile* in the toilet bowl water following several flushes. These findings demonstrate that flushing alone cannot entirely eliminate toilet contamination, and the resultant emission of pathogenic bacteria bioaerosols can pose detrimental effects on human health (Arena et al., 2021; Luo et al., 2023). In public washrooms, individuals commonly flush toilets before use to ensure proper functioning and remove any residual contaminants from previous users (Hartigan et al., 2020; Siu, 2006; Wan et al., 2021). However, due to limited awareness, they often overlook the potential health risks associated with bioaerosol emissions during flushing, exposing themselves to these hazards while using the toilet. *Staphylococcus aureus* and *Escherichia coli* bioaerosols are among the most prevalent airborne pathogenic bacteria detected in indoor washrooms and are commonly used as indicator bioaerosols for evaluating human health risks (Kozajda et al., 2019; Vardoulakis et al., 2021). Exposure to these pathogens poses significant health risks, including respiratory infections, pneumonia, gastrointestinal illnesses, and systemic infections (Tong et al., 2015). Monte Carlo simulation–based quantitative microbial risk assessment (QMRA) can provide comprehensive information to understand bioaerosol potential health effects (Buse et al., 2012). The classical QMRA works by following four steps: (1) hazardous identification, (2) exposure assessment, (3) dose–response analyses, and (4) risk characterization (Haas et al., 2014). It is broadly used to identify the disease health risk burden associated with exposure to bioaerosol under varying exposure scenarios (Havelaar et al., 2012, 2015). The World Health Organization (WHO) recommends using QMRA with Monte Carlo simulation to quantitatively assess health risk ranges and likelihood (Pasalari et al., 2019). The recommended and widely used health risk level for risk characterization is the WHO Benchmark ($\leq 10^{-6}$ disability-adjusted life years [DALYs] per person per year [pppy]) for disease health risk burden (Lim et al., 2015; Shi et al., 2018). Moreover, sensitivity analysis is a vital component of mathematical models commonly used to examine the relationship between stochastic input parameters and their impact on the corresponding output parameters (Saltelli, 2002; Saltelli et al., 2019). In the context of bioaerosol-related health risks, sensitivity analysis plays a crucial role in revealing the most influential input parameter in QMRA framework, shedding light on its quantified impact on the resultant health risk outcomes (Carducci et al., 2018; Mok & Hamilton, 2014). However, in the absence of research on sensitivity analysis of bioaerosol emissions in indoor washrooms, it is difficult to determine the most contributory factor influencing health risks associated with toilet usage.

In this study, a systematic investigation of the concentrations and size distribution of *S. aureus* and *E. coli* bioaerosols was carried out in two washrooms, each equipped with squat and bidet toilets. Different flushing conditions (the first without stool material and the second with stool material) and ventilation scenarios (the exhaust fan was turned off and turned on) were considered. QMRA was performed to quantitatively estimate the disease health risk burden for each exposure scenario. Furthermore, for the first time, sensitivity analysis was conducted on stochastic input parameters in each exposure scenario to identify the contribution of each input parameter to the variability and uncertainty of the calculated disease health risk burden. The current study offers innovative evidence about bioaerosol emission characteristics, consequent health risks, and related sensitivity analysis for the first flush before and the second flush after attending the toilet. This study offers novel insights into bioaerosol emissions and potential health risks in public washrooms that can inform the development of more effective ventilation strategies, and therefore reduce health risks for users and promote safer, healthier indoor environments.

2 | MATERIALS AND METHODS

2.1 | Washroom description

This study focused on an office washroom area, where two washrooms with distinct toilet designs (squat and bidet toilets) were situated at the building's corner (Figure 1). Both washrooms had an identical water flushing volume of 4.9 L and followed a standardized layout. The layout adhered to a typical configuration found in Chinese office spaces, with the washroom facing north and having dimensions of 264×180 cm^2 and a height of 300 cm. The washroom entrance is centrally positioned, measuring 210 cm in height and 36 cm in width. A dedicated mechanical extraction ventilation system, located at the center of the washroom, featured a ceiling exhaust fan without a filter. The ventilation system could be independently operated using an on/off button and maintained a fixed air volume rate of 180 m^3/h, resulting in 2.26 air exchanges per hour.

2.2 | Experimental setup and sampling procedure

In both washrooms, the indoor concentrations and size distributions of *S. aureus* and *E. coli* bioaerosols were determined using a six-stage Andersen impactor during toilet flushing (Kowalski et al., 2017). Details on the size distribution of the impactor are provided in the Supporting Information section. For convenience, potential sources of *S. aureus* bioaerosols, such as shedding from the mouth and nose, were excluded due to their typically much lower concentration levels (Nazaroff, 2016). Although toilet flushing under normal circumstances primarily disperses pathogens transmitted

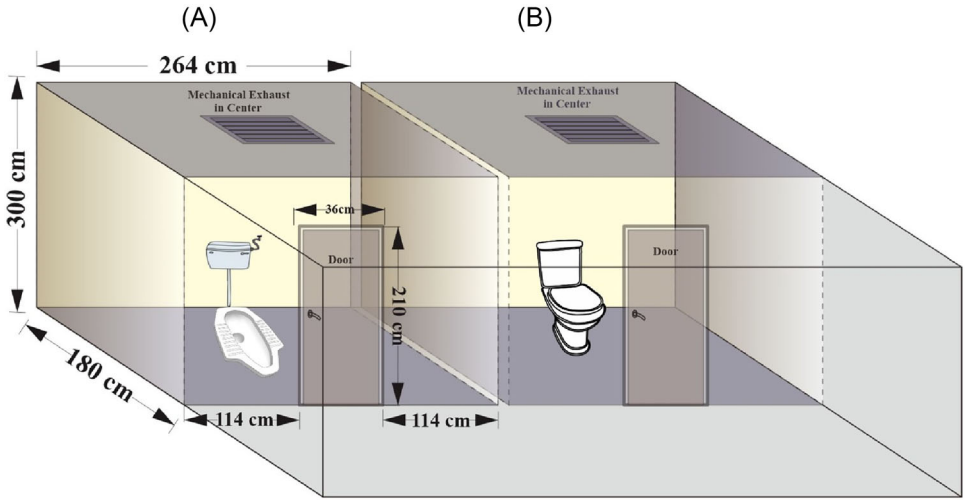

FIGURE 1 *Washroom* descriptions of (A) squat and (B) bidet toilets.

via the enteric route, this study assumes that all captured bioaerosols are capable of entering the respiratory tract (Lee et al., 2015).

The bioaerosol concentrations obtained from Stages 3–6 of the impactor were considered posing potential health effects (Ghosh et al., 2015). Therefore, the maximum respirable bioaerosol particle ingestion rate (AG) for *S. aureus* and *E. coli* was set as the ratio of Stages 3–6 to Stages 1–6 of the impactor (Table S1). Egg-yolk mannitol salt agar mediums and MacConkey agar medium were used to cultivate *S. aureus* and *E. coli* bioaerosols, respectively (Duan et al., 2008; Grzyb & Lenart-Boroń, 2019). Following the standardized sampling procedure, Petri dishes containing the medium were placed within each of the six stages of the Andersen impactor (Mohammed et al., 2020).

The study focused on defecation activities in both washrooms, considering two flushing conditions: the first without stool material and the second with stool material. The initial concentrations of *S. aureus* and *E. coli* in the stool samples were approximately 400 colony-forming unit (CFU)/g and 10^8 CFU/g, respectively, which are in agreement with previous studies (Leatham et al., 2009; Lindberg et al., 2000). In the human digestive system and in stool materials, *S. aureus* and *E. coli* are commonly identified as part of the normal flora in the human digestive system and stool (Gravet et al., 1999; Ling et al., 2019; Yoon et al., 2022). Therefore, actual human stool samples were used in this study for flushing and sampling. Details of the eight exposure scenarios considered are provided in Table 1. In each exposure scenario, samples were collected three times. The time duration for each sample collection was 5 min using the Andersen impactor at a flow rate of 28.3 L/min with sampling volume was 141.5 L (Kowalski et al., 2017). Prior to sample collection, the toilets were sterilized, and proper ventilation was ensured to eliminate uncontrolled factors. The sampling heights for the squat and bidet toilets were set at 0.5 and 0.8 m above the floor, respectively, based on the users' posture.

2.3 | Bioaerosol analysis

After sampling, the Petri dishes were immediately stored at 4°C in an ice box and then transferred to the laboratory within 1 h for incubation at 37°C for 48 h (Gorman et al., 2002). Using an automated colony enumeration instrument, the samples were measured as CFUs (Sieuwerts et al., 2008). The positive hole approach was used at each Petri dish stage to correct and obtain a certain number of colonies (Pongracic et al., 2010). Each bioaerosol concentration result was expressed in CFU per cubic meter of air (CFU/m^3) (Kalogerakis et al., 2005). Further information on the analysis of bioaerosol concentration is shown in the Supporting Information section.

2.4 | QMRA framework

This study focuses on hazard identification by considering *S. aureus* and *E. coli* bioaerosols, which are the most representative and prevalent airborne pathogenic bacteria associated with indoor toilet flushing (Kozajda et al., 2019; Vardoulakis et al., 2021). Different flushing conditions were found to produce varying levels of bioaerosol. An exponential dose–response model and β-Poisson dose–response model were employed to calculate the exposure to *S. aureus* and *E. coli* bioaerosols, respectively, and assess the annual probability of infection risk (Esfahanian et al., 2019; Shi et al., 2018). The disease health risk burden was quantified using DALYs and the annual probability of infection, which were employed to estimate the burden of *S. aureus* and *E. coli* bioaerosol exposure (Havelaar et al., 2012, 2015). DALYs is a standardized statistic used by the WHO to compare disease health risks across nations, populations, and time (Gibney et al., 2013). Monte Carlo simulation with over 10,000 iterations was employed to determine the probability distributions of disease health risk burden (Pasalari et al., 2019). The results

TABLE 1 Details of the exposure scenarios.

Items	Exposure scenarios
Ventilation scenarios	Turned-off exhaust fan
	Turned-on exhaust fan
Activity duration	Uniform distribution (min = 5 min/day, max = 10 min/day)
Exposure site	Two distinct types of washrooms: washroom with squat toilet; washroom with bidet toilet
	Activity: for defecation (squat toilet sampling height 0.5 m; bidet toilet sampling height 0.8 m)
Toilet flashing conditions	First flushing without stool material with the intention of ensuring proper functioning of toilet and eliminating any lingering contaminants from the previous flush
	Second flushing with stool material for clean excrement
Exposure frequency per year	365 days
Eight exposure scenarios	Squat toilet washroom: (i) first flushing without stool material and exhaust fan turned off, (ii) first flushing without stool material and exhaust fan turned on, (iii) second flushing with stool material and exhaust fan turned off, and (iv) second flushing with stool material and exhaust fan turned on
	Bidet toilet washroom: (i) first flushing without stool material and exhaust fan turned off, (ii) first flushing without stool material and exhaust fan turned on, (iii) second flushing with stool material and exhaust fan turned off, and (iv) second flushing with stool material and exhaust fan turned on

were then compared against a WHO-recommended acceptable burden benchmark ($\leq 10^{-6}$ DALYs pppy) (Lim et al., 2015; Shi et al., 2018). Disease health risk burdens above this benchmark were considered unacceptable, whereas values below the benchmark were deemed acceptable (Shi et al., 2018). Although this benchmark likely refers to gastroenteritis, it provides a scientifically established framework for assessing and managing public disease health risk burden of *S. aureus* and *E. coli* bioaerosols (Kataki et al., 2022). For more detailed information, refer to Tables 1 and 2 and the Supporting Information section, which provide comprehensive details on the steps, parameters, and features of the eight exposure scenarios analyzed in this QMRA.

2.5 | Monte Carlo simulation and sensitivity analysis

Monte Carlo simulation–based QMRA was used to analyze the probability distribution of the output parameter, disease health risk burden. The input parameters for the simulation, including exposure concentration (EC), exposure time (ET), bioaerosol ingestion rate (AG), and breathing rate (BR), were randomly assigned. Sensitivity analysis was performed to identify the input parameter with the greatest influence on the estimated output for each of the eight exposure scenarios involving different types of toilets and flushing conditions (Dias et al., 2019; Saltelli et al., 2019). The input parameters were assigned probability distributions: lognormal for EC and uniform (log scale) for BR, AG, and ET (Carducci et al., 2018). The details of each input parameter can be found in Tables 1 and 2 and the Supporting Information section. The correlation coefficient values were used to assess the influences of each input variable on the disease health risk burden. A high correlation coefficient indicated a significant impact on the variability of the disease health risk burden. The Spearman rank correlation coefficient was used to quantify the contribution of each input variable and calculate the sensitivity percentage ratio in Oracle Crystal Ball (Unice et al., 2019).

3 | RESULTS AND DISCUSSION

3.1 | Bioaerosol emission characteristics

Table 3 presents the concentrations of aerosolized *S. aureus* and *E. coli* in the two washrooms, considering different ventilation scenarios and flushing conditions. A comparison between the two toilet types revealed that the indoor washroom with a squat toilet had concentrations 1.7–2.6 times higher of *S. aureus* and 1.2–1.4 times higher of *E. coli* bioaerosol than the washroom with a bidet toilet. This was attributed to the influence of toilet design on bioaerosol emission (Farling et al., 2019), with the recorded concentrations also being significantly affected by the sampling height (Wagner et al., 2022). Squat toilets have smaller bowl walls and are installed at floor level, whereas bidet toilets have elevated bowl walls above the floor, which helps control bioaerosol emissions (Sun & Han, 2021). Furthermore, higher sampling heights typically result in lower bioaerosol concentrations due to the settling of bioaerosol particles (Farling et al., 2019). Therefore, the higher sampling height of the bidet toilet in our study was naturally disadvantageous for capturing bioaerosols in the impactor. This finding aligns with the

TABLE 2 Parameters for quantitative microbiological risk assessment calculation process.

Parameters	Unit	Values	References
EC: Exposure bioaerosol concentration	CFU/m^3	Lognormal distribution (Mean ± SD in Table 3)	–
BR: Breathing rate	m^3/day	Uniform distribution (min = 9.8; max = 13.0)	Duan (2013)
ET: Exposure time	h/day	Table 2	–
AG: Respirable bioaerosol particles ingestion rate	%	Uniform distribution (*Staphylococcus aureus* and *Escherichia coli* bioaerosol min = 0.10; max = shown in Table S1)	Shi et al. (2018)
Exposure dose $d = EC \times BR \times T \times AG$	CFU/day	Calculation	Pasalari et al. (2019)
Daily probability of infection	Unitless	$k = 8.05 \times 10^{-8}$	Esfahanian et al. (2019)
Exponential dose–response model for *Staphylococcus aureus* bioaerosol: $Pi\,(d) = 1 - e^{-dk}$			
β-Poisson dose–response model for *Escherichia coli* bioaerosol: $P_{(d)inf} = 1 - (1 + \frac{d}{\beta})^{-\alpha}$	Unitless	$\beta = \frac{D_{50}}{\frac{1}{2^{\alpha}}}$ $\alpha = 0.155$ $D_{50} = 2.11 \times 10^6$	Shi et al. (2018)
Annual probability of infection $Py = 1 - (1 - Pi\,(d))^n$	pppy	n means exposure frequency $n = 365$	Esfahanian et al. (2019)
Disease burden $DB = Py \times HB$	DALYs pppy	Health burden (HB) *Staphylococcus aureus*: 0.0026 *Escherichia coli*: 0.0455	Havelaar et al. (2012) Havelaar et al. (2015)

results of Lou et al. (2021), who reported that different toilet types can emit bioaerosols at varying concentrations during flushing. For the squat toilet, the bioaerosol concentrations of *S. aureus* and *E. coli* after the first flush (without stool material) were 1.8 and 1.4 times lower, respectively, than those observed after the second flush (with stool material) under both exhaust fan on and off scenarios. Similarly, for the bidet toilet, the bioaerosol concentrations of *S. aureus* and *E. coli* after the first flush were both 1.3 times lower than those after the second flush across both ventilation conditions. This suggests that although additional flushing can dilute contamination to some extent, residual stool material continues to aerosolize during subsequent flushes (Johnson, Lynch et al., 2013). Notably, flushing does not completely remove contamination from the toilet bowl water or surfaces (Gerba et al., 1975). This is corroborated by Verani et al. (2014), who found that potentially harmful bacterial pathogens can persist in the toilet bowl even after multiple flushes and may become aerosolized into the surrounding air. Furthermore, Barker and Jones (2005) highlighted the long-term presence of microbial contamination within the toilet bowl biofilm, underscoring the persistent risk of bioaerosol generation during flushing in public washrooms. With the exhaust fan turned on, bioaerosol concentrations of *S. aureus* and *E. coli* in both squat and bidet toilets across various flushing conditions were 1.6–2.2 and 1.3–1.7 times lower, respectively, compared to the turned-off exhaust fan scenario. This reduction is attributed to the effectiveness of the ventilation system continuously exchanging contaminated indoor air with fresh airflow, thereby diluting bioaerosols (Blocken et al., 2021). Previous studies by Stockwell et al. (2019) and Liu et al. (2020) have similarly highlighted the critical role of strong mechanical ventilation in minimizing airborne microbial contamination.

The bioaerosol concentrations in Stages 3–6 of the Andersen impactor showed consistent trends across the two washrooms with different toilet types and flushing conditions under various ventilation scenarios (Table 3). These stages primarily contained respirable fine particles (smaller than 4.7 μm) that can penetrate deep into the respiratory system, posing significant health risks (Akpeimeh et al., 2019).

Figure 2 shows the bioaerosol particle size distribution for *S. aureus* and *E. coli* in the two washrooms under different ventilation scenarios and flushing conditions. The results showed that the percentage of respirable *S. aureus* and *E. coli* fine bioaerosol particles (Stages 3–6 of Andersen six-stage impactor) in both washrooms (Figure 2A–D) with distinct types of toilets and toilet flushing conditions was as high as 55%–70% and 52%–62%, respectively. This high percentage

TABLE 3 Mean values of *Staphylococcus aureus* and *Escherichia coli* bioaerosol concentration (colony-forming unit [CFU]/m^3) and aerosol ingestion ratio (%) in squat and bidet toilets.

Items				Bioaerosol concentrations		
		Toilet flashing conditions	Two different ventilation scenarios about exhaust fan	1–6 stages of Andersen six-stage cascade impactor	3–6 stages of Andersen six-stage cascade impactor	Ingestion ratio of respirable bioaerosol particles (%)
Staphylococcus aureus	Squat toilet	First flushing without stool material	Turned-off	1.07E + 03	6.87E + 02	0.64
			Turned-on	5.69E + 02	3.17E + 02	0.56
		Second flushing with stool material	Turned-off	1.88E + 03	1.33E + 03	0.71
			Turned-on	8.47E + 02	5.25E + 02	0.62
	Bidet toilet	First flushing without stool material	Turned-off	5.38E + 02	3.54E + 02	0.66
			Turned-on	3.33E + 02	1.84E + 02	0.55
		Second flushing with stool material	Turned-off	7.14E + 02	4.95E + 02	0.69
			Turned-on	4.17E + 02	2.61E + 02	0.63
Escherichia coli	Squat toilet	First flushing without stool material	Turned-off	3.38E + 02	2.01E + 02	0.60
			Turned-on	2.62E + 02	1.40E + 02	0.53
		Second flushing with stool material	Turned-off	4.57E + 02	2.84E + 02	0.62
			Turned-on	2.94E + 02	1.64E + 02	0.56
	Bidet toilet	First flushing without stool material	Turned-off	3.24E + 02	1.82E + 02	0.56
			Turned-on	1.90E + 02	9.89E + 01	0.52
		Second flushing with stool material	Turned-off	3.87E + 02	2.28E + 02	0.59
			Turned-on	2.52E + 02	1.34E + 02	0.53

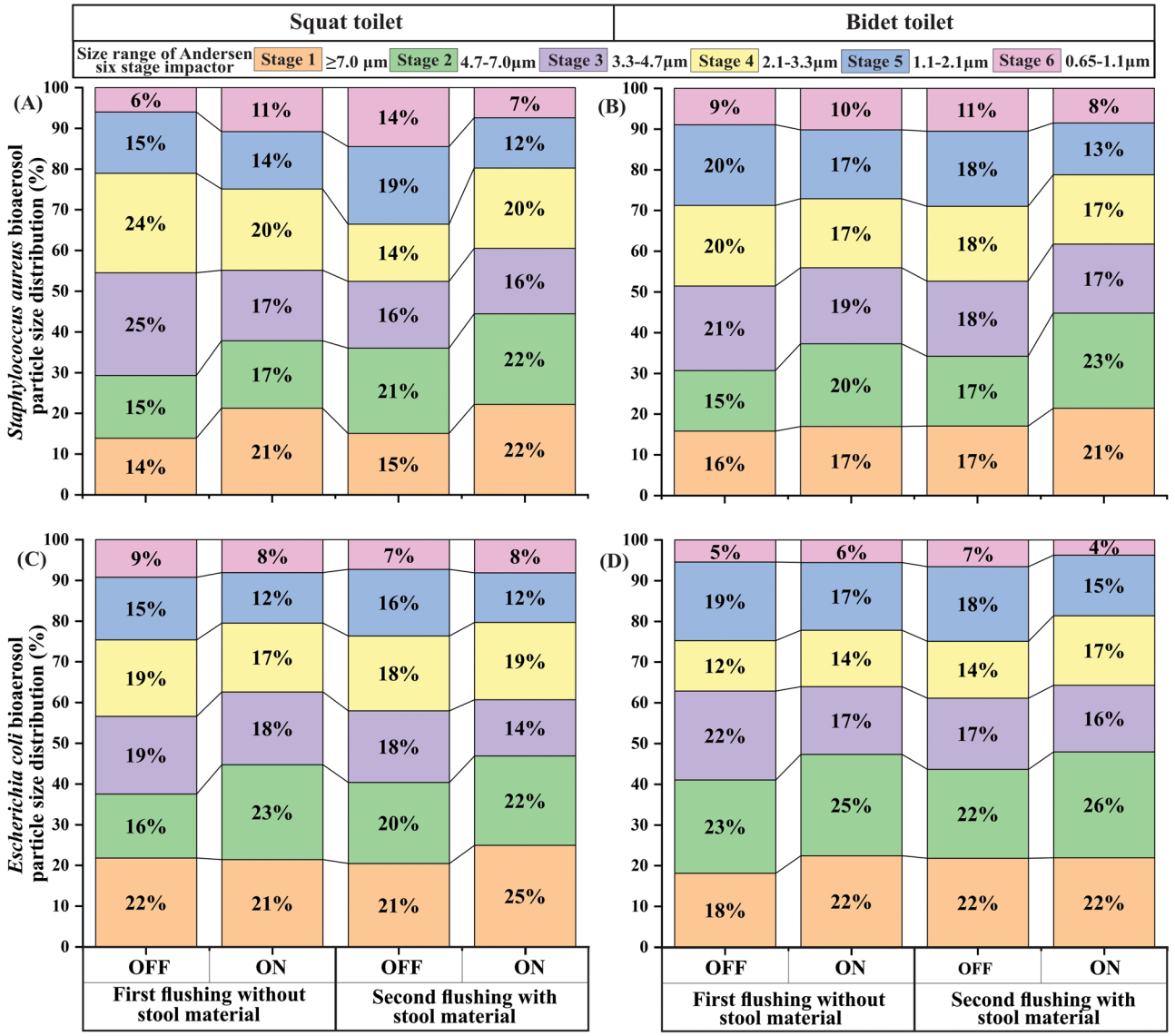

FIGURE 2 *Proportion* of particle size distribution of *Staphylococcus aureus* and *Escherichia coli* bioaerosols in (A) *Staphylococcus aureus* bioaerosol particles size distribution in squat toilet (B) *Staphylococcus aureus* bioaerosol particles size distribution in bidet toilets (C) *Escherichia coli* bioaerosol particles size distribution in squat toilet (D) *Escherichia coli* bioaerosol particles size distribution in bidet toilet, considering different flushing conditions and ventilation scenarios. OFF, turned-off exhaust fan; ON, turned-on exhaust fan.

was attributed to the interaction between liquid and air during toilet flushing, along with the siphon phenomenon created by the high water flow velocity, which predominantly released fine bioaerosol particles (smaller than 4.7 μm) (Ali et al., 2021). Furthermore, studies by Lou et al. (2021) and Johnson, Lynch et al. (2013) supported the notion that toilet flushing activities primarily emit fine bioaerosol particles due to turbulence and fluctuations.

Flushing conditions also influenced the distribution of fine bioaerosols. In both squat and bidet toilets, the second flush (with stool material) produced higher proportions of respirable fine particles, ranging from 62% to 70% for *S. aureus* and 53% to 62% for *E. coli*. In contrast, the first flush (without stool material) yielded lower levels, at 55%–66% and 52%–59%, respectively. The increased emission during the second flush can be attributed to stool material introducing pathogenic bacteria into the toilet bowl, which are agitated during flushing due to swirling, splashing, and bubbling water dynamics (Obayori, 2023). This observation is consistent with the findings of Ali et al. (2021), who highlighted the role of stool material in elevating bioaerosol emissions. The ventilation scenario also played a significant role in reducing fine bioaerosol concentrations. Under the turned-on exhaust fan condition, the percentage of fine bioaerosols was 6%–10% lower for *S. aureus* and 4%–7% lower for *E. coli* compared to the turned-off scenario (Figure 2). This reduction is attributed to the ability of mechanical ventilation systems to dilute contaminated air, enhance directional airflow, and increase the air exchange rate, thereby mitigating bioaerosol concentrations (Bueno de Mesquita et al., 2022). Similar findings by

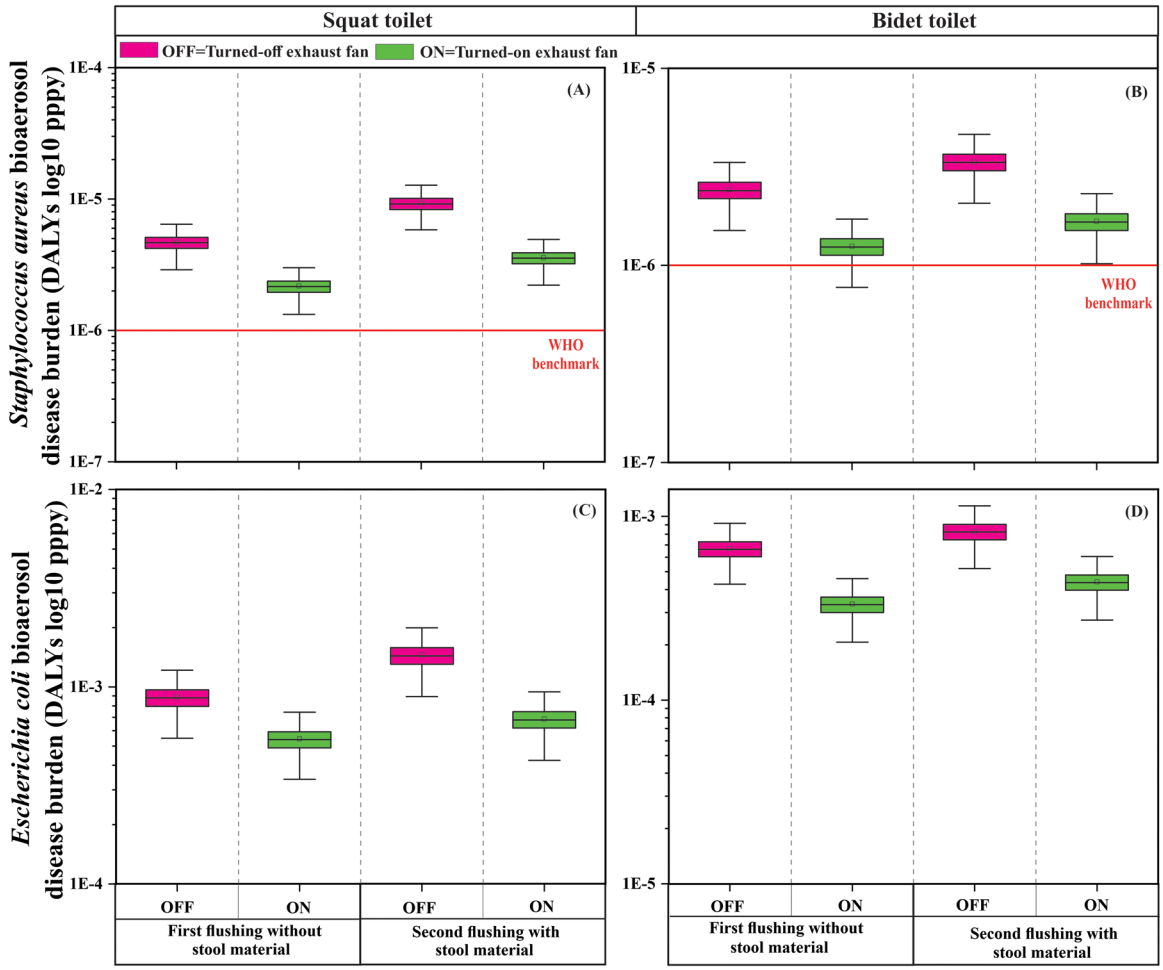

FIGURE 3 Disease *health* risk burden (disability-adjusted life years [DALYs] per person per year [pppy]) of *Staphylococcus aureus* and *Escherichia coli* bioaerosols in (A) *Staphylococcus aureus* bioaerosol disease health risk burden in squat toilet (B) *Staphylococcus aureus* bioaerosol disease health risk burden in bidet toilets (C) *Escherichia coli* bioaerosol disease health risk burden in squat toilet (D) *Escherichia coli* bioaerosol disease health risk burden in bidet toilet, considering different flushing conditions and ventilation scenarios. The bottom and top of the box represent the first and third quartiles (25th and 75th percentiles), respectively. The band inside the box denotes the second quartile (median), and the tetragon inside refers to the mean value (general condition). The whiskers show the 2.5th percentile (optimistic estimate at the best situation) and 97.5th percentile (conservative estimate at the worst situation) from each end of the box.

Zhao and Liu (2020) demonstrated that mechanical ventilation could reduce fine particle concentrations by as much as 65%, highlighting its effectiveness in maintaining indoor air quality.

3.2 | Disease health risk burden

The majority of disease health risk burden values for *S. aureus* and *E. coli* bioaerosols exceeded the WHO-recommended acceptable benchmark ($\leq 10^{-6}$ DALYs pppy) in both squat and bidet toilets across all flushing conditions (Figure 3A–D). This can be attributed to the high bioaerosol concentrations, which significantly influence the exposure dose and subsequently impact the disease health risk burden (Madureira et al., 2018). Elevated bioaerosol levels are linked to adverse health outcomes, as documented by Bolookat et al. (2018) and highlighted in the WHO manual on indoor air quality (Bluyssen, 2009).

The results further demonstrate significant variability in health risk burden between the two flushing conditions. The first flush (without stool material) consistently resulted in disease health risk burdens one order of magnitude lower than the second flush (with stool material). This reduction is likely due to the lower bioaerosol concentrations and smaller proportion of fine particles associated with the absence of stool material, which reduces the exposure dose and mitigates health risks (Jahne et al., 2015). Similar findings have been reported by Zoran et al. (2020), who identified a strong correlation among high bioaerosol exposure, increased fine particle distribution, and elevated morbidity and mortality rates. The ventilation scenario also played a critical role in mitigating disease health risks. Turning on the exhaust fan reduced the disease health risk burden of *S. aureus* and *E. coli* bioaerosols by one order of magnitude compared to the turned-off scenario (Figure 3). However, most values still exceeded the benchmark, except for *S. aureus* in the bidet toilet after the first flush, where the health risk bur-

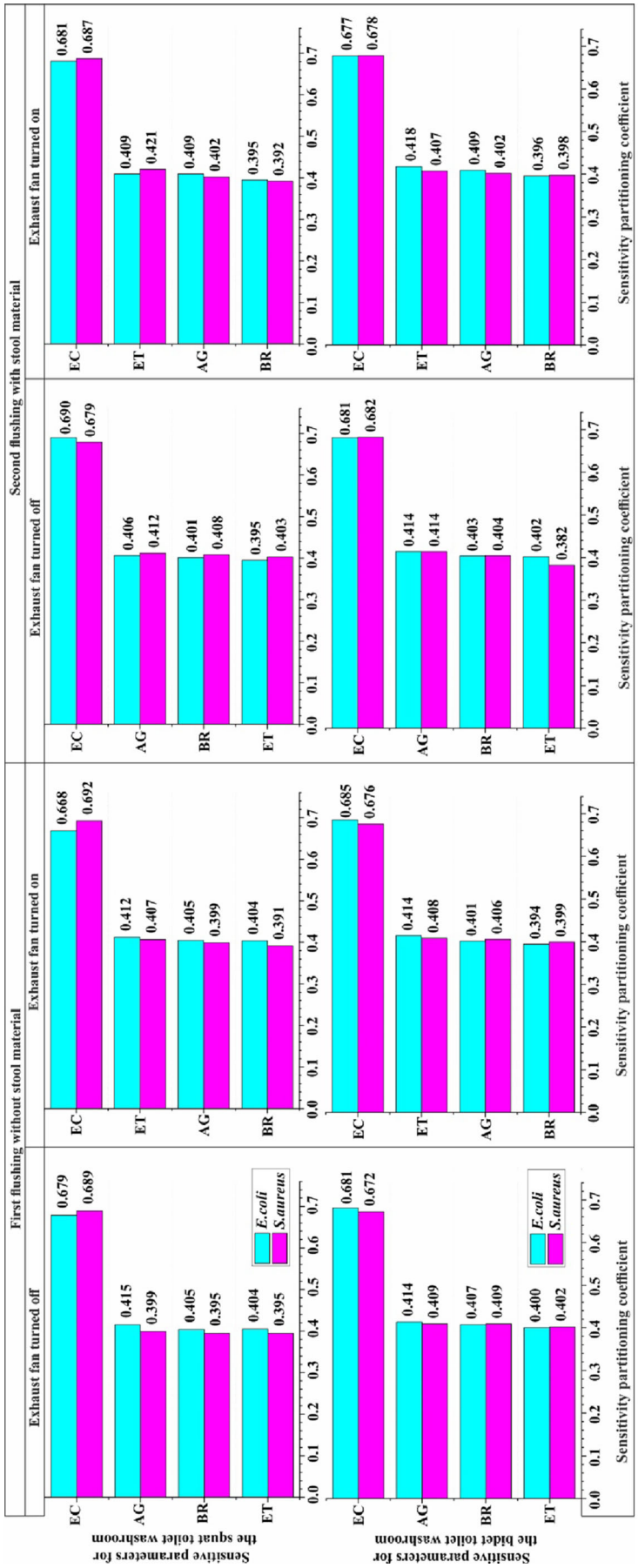

FIGURE 4 Ranking of sensitivity partitioning coefficient of each input sensitivity parameter that affects the output value for expose to *Staphylococcus aureus* and *Escherichia coli* bioaerosol in two washrooms. AG, aerosol ingestion rate; BR, breathing rate; EC, exposure bioaerosol concentration; ET, exposure time.

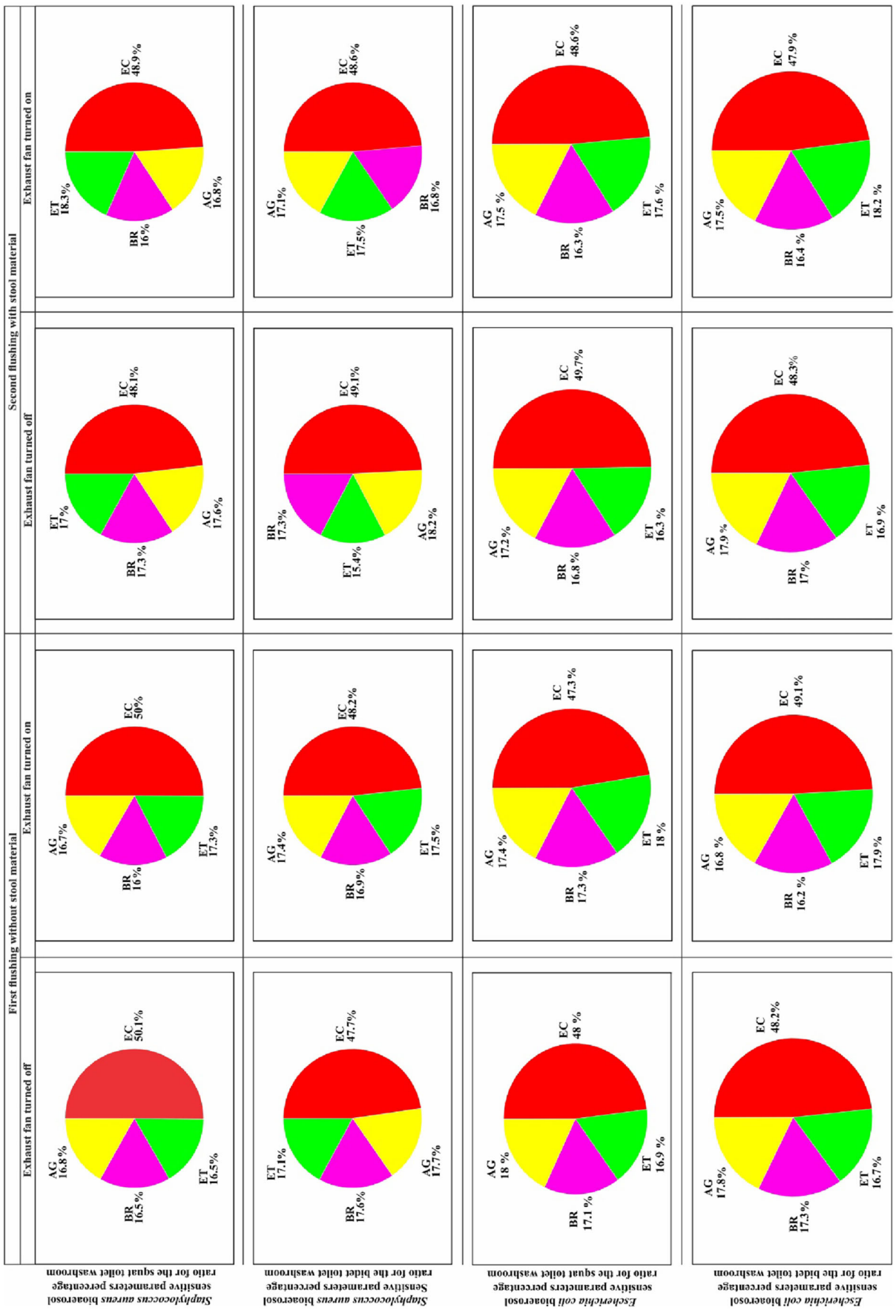

FIGURE 5 Specific contributions of the sensitivity percentage ratio of each input sensitivity parameter that affect the output value for exposed to *Staphylococcus aureus* and *Escherichia coli* bioaerosol in two washrooms. AG, aerosol ingestion rate; BR, breathing rate; EC, exposure bioaerosol concentration; ET, exposure time.

den (9.81×10^{-7}) was within the acceptable range. Notably, for the second flush with stool material in the bidet toilet under the turned-on exhaust fan condition, the disease health risk burden (1.41×10^{-6}) was close to the benchmark (Figure 3B). These findings demonstrate the importance of ventilation in reducing indoor environmental health risks. Previous studies have demonstrated the effectiveness of mechanical ventilation in mitigating bioaerosol-associated health risks by removing airborne particles and improving air circulation (Qian & Zheng, 2018; Stockwell et al., 2019; Zhao et al., 2018), reinforcing the need for ventilation optimization as a key mitigation strategy in public washrooms.

3.3 | Sensitivity analysis of the results of disease health risk burden

The sensitivity analysis consistently showed that the EC was the most dominant input parameter, contributing up to 50% to the estimated disease health risk burden for both *S. aureus* and *E. coli* bioaerosols in all scenarios (Figures 4 and 5). This result was attributed to the higher dispersion levels of the bioaerosol concentration compared to other input parameters.

The sensitivity analysis revealed consistent trends for the remaining input parameters, including AG, BR, and ET, across all ventilation scenarios in assessing the disease health risk burden of *S. aureus* and *E. coli* bioaerosols. This consistency was attributed to the uniform distribution patterns of these parameters in the QMRA calculation and sensitivity analysis, where each possible health risk outcome had an equal chance of occurrence (Nag et al., 2021). Under the turned-off exhaust fan scenario, the sensitivity rankings for *S. aureus* and *E. coli* bioaerosols in both squat and bidet toilets were AG > BR > ET. When the exhaust fan was turned on, the rankings shifted to ET > AG > BR (Figure 4). This variation highlights the role of ventilation in redistributing the relative importance of these parameters. Additionally, the specific contribution percentages of AG, BR, and ET varied based on the ventilation scenario and flushing condition (Figure 5). The specific contribution of AG, BR, and ET for *S. aureus* and *E. coli* bioaerosols in both squat and bidet toilets ranged between 15% and 18%, with minimal variation based on the ventilation scenario and flushing condition (Figure 5).

Similar trends were observed in bidet toilets, where ventilation also influenced the rankings and specific contributions of these parameters (Figures 4 and 5). The turned-on exhaust fan scenario, known for its efficiency in improving air quality and reducing fine bioaerosol particles, altered the sensitivity of AG, BR, and ET. AG demonstrated higher sensitivity in the turned-off scenario and lower sensitivity when ventilation was active, reflecting the mitigating effect of airflow on AG (Blocken et al., 2021; Shiue et al., 2019).

ET emerged as a critical parameter, particularly under the turned-on exhaust fan scenario, due to its direct influence on disease health risk burden. Prolonged exposure to bioaerosols is strongly correlated with elevated health risks, including premature mortality (Stafoggia et al., 2022). The substantial contribution of ET, AG, and BR to the disease health risk burden underscores the importance of these parameters in health risk modeling (Duan, 2013; Lim et al., 2012). These findings highlight the critical role of ventilation strategies in reducing bioaerosol exposure and altering the relative importance of input parameters. Mechanical ventilation, through enhanced air exchange and reduced aerosol concentrations, proves effective in mitigating health risks, particularly for AG and ET, as documented in prior research (Bist & Chai, 2022; Blocken et al., 2021).

4 | CONCLUSION

This study provides critical insights into bioaerosol emissions, their particle size distribution, and associated health risks in public washrooms under varying ventilation scenarios, toilet types, and flushing conditions. Key findings reveal that second flushes containing stool material release significantly higher levels of fine, respirable bioaerosol particles (<4.7 μm) compared to initial flushes without stool material. These fine particles are critical contributors to the disease health risk burden, with most calculated risks exceeding the WHO-recommended benchmark. Such findings underscore the substantial health risks posed by bioaerosol exposure in public washrooms. Ventilation was shown to play a key role in mitigating these risks. Scenarios involving turned-on exhaust fans consistently reduced bioaerosol concentrations and associated health risks compared to turned-off scenarios. However, even under optimal ventilation conditions, health risks occasionally remained above acceptable thresholds, emphasizing the need for further interventions to address residual exposure risks. Sensitivity analysis identified EC followed by ET, AG, and BR as key input parameters influencing the disease health risk burden. The rankings and contributions of these parameters varied with ventilation scenarios, highlighting the importance of mechanical airflow in influencing exposure outcomes. These findings highlight key opportunities for improvement across engineering, design, and public health. Enhancing ventilation systems by optimizing exhaust fan efficiency and air exchange rates can effectively reduce bioaerosol concentrations and exposure risks. In terms of design, developing advanced toilet technologies that limit bioaerosol emissions during flushing, especially in the presence of stool material, is critical. For public health policymakers, the results provide evidence-based recommendations to define safe bioaerosol exposure limits and promote hygiene practices in public washrooms, ensuring healthier and safer indoor environments.

AUTHOR CONTRIBUTIONS

Wajid Ali: Conceptualization; methodology; data curation; software; writing—original draft; writing—review and editing. **Zhen Hu**: Investigation; funding acquisition; resources. **Zhe-ren Tang**: Investigation; funding acquisition; resources. **Si-yi Liu**: Project administration; methodology; validation;

visualization; software. **Zaheer Ahmad Nasir**: Writing—review and editing; resources. **Frederic Coulon**: Writing—review and editing; resources. **Peng Liu**: Data curation; writing—review and editing; visualization; software. **Cheng Yan**: Conceptualization; data curation; writing—original draft; writing—review and editing; visualization; software; investigation; funding acquisition; resources; supervision and validation.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The author has provided the required data availability statement unless the article type is exempt and, if applicable, included functional and accurate links to said data therein.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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