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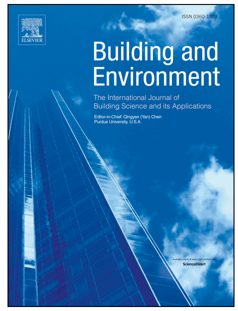

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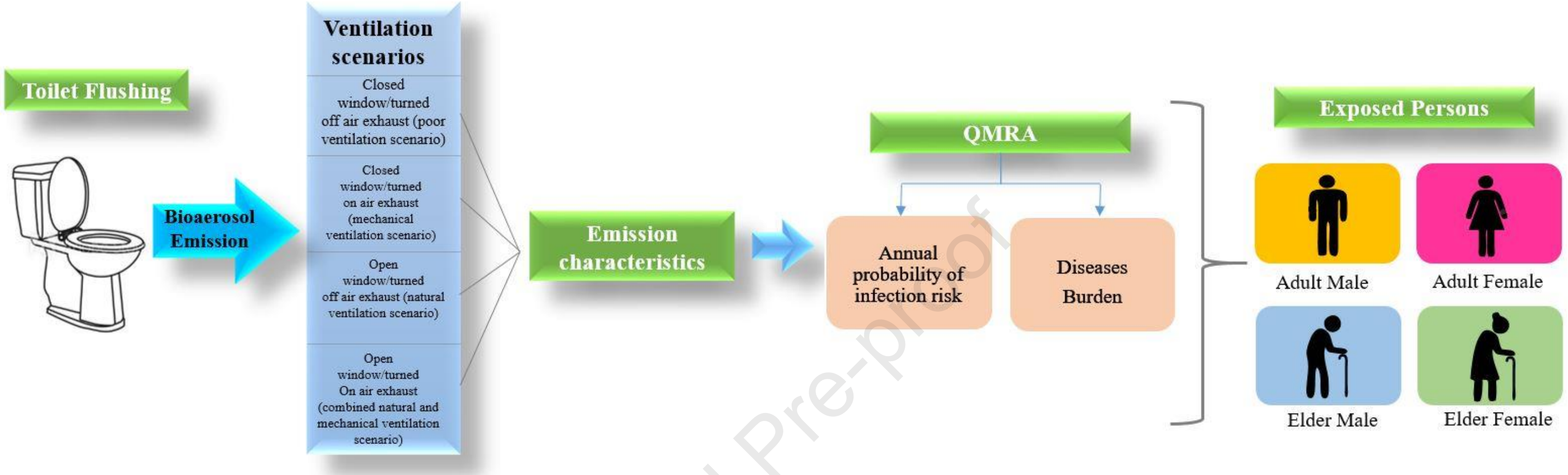

Graphic abstract

Emission characteristics and quantitative health risk assessment of bioaerosols in an indoor toilet after flushing under various ventilation scenarios

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ABSTRACT

In the indoor environment, toilet is one of the primary sources of bioaerosol because flushing events can disturb stool materials. Bioaerosol exposure has a significant impact on human health. Therefore, this research focused on systematical investigation of *Staphylococcus aureus* bioaerosol emission characteristics in an indoor toilet after flushing with time. Then, annual probability of infection and disease burden with time under various ventilation scenarios were determined using a Monte Carlo simulation-based quantitative microbial risk assessment. The results showed that at the initial phase, the highest and lowest bioaerosol concentrations were found in poor and combined ventilation scenarios, respectively. The bioaerosol concentration in natural ventilation scenario was 1.1 times higher than that in mechanical ventilation scenario. However, a decreasing trend was observed after flushing. The adult male's health risks were consistently higher than those of all other exposed persons. However, the maximum and minimum health risks were observed in the poor and combined ventilation scenario, respectively. The health risks in the mechanical ventilation scenario were lower than those in the natural ventilation scenario. However, the health infection risk varied with time: it was unbearable to the U.S. Environmental Protection Agency benchmark at 0 min to 15 min after flushing, but it was tolerable after flushing 35 min. Moreover, the disease health burdens were below the World Health Organization benchmark after flushing 20 min to 35 min. This research delivered novel data and provide a guideline for controlling the essential health threats from bioaerosol emissions in various toilet usage scenarios.

Key words

Quantitative microbial risk assessment; Annual probability of infection; Diseases burden; Size distribution; Concentration; Monte Carlo simulation

1. Introduction

In the indoor environment, toilet is one of the primary sources of bioaerosol due to flushing events [1, 2]. The flow of toilet water can aerosolize stool materials (e.g., bubbling, swirling, and splashing) [3, 4]. Given the turbulence and fluctuation of toilet water, toilet flushing releases a significant amount of bioaerosols [5] that can contaminate the indoor air and affect human health [6, 7]. In the 1950s, Jessen reported for the first time the bioaerosol emission during toilet flushing when he detected bacteria seeded around the toilet after flushing [3]. The emission characteristics of bioaerosol in hospital toilets were measured by Knowlton et al. [8] under three different scenarios. In addition, Aithinne et al. [9] examined the survival of *Clostridioides difficile spores*, which originated from the bioaerosol that settled down, in contaminated indoor environments nearby and distant from the toilet seat. However, the research about bioaerosols emission characteristics and its exposure health risk assessment with the time passage is comparatively limited.

Bioaerosols are particles of a pathogenic biological nature dispersed in the air [10]. Thus, bioaerosol exposure has a significant impact on human health. Inhalation is the main pathway of bioaerosol exposure [11, 12]. Bioaerosols with an aerodynamic diameter of 5 μm to 10 μm are often trapped in the upper respiratory system and can cause allergic symptoms. Meanwhile, bioaerosols with a diameter of less than 5 μm are also known as respirable particles. They can penetrate deep into the alveoli and cause allergic alveolitis [13]. After toilet flushing, the bioaerosol concentration increases; the bioaerosol particles are 3 μm in diameter or less [14, 15]. *Staphylococcus aureus* bioaerosol is one of the most prevalent airborne pathogenic bacteria in the indoor toilet environment, and it exhibits a hygiene-related biological activity as strong as *E. coli* [16]. The *E. coli* and *Staphylococcus aureus* bioaerosol are both frequently identified and utilized as indicator bioaerosol [17, 18], even though a minor influence of human skin normal flora of *Staphylococcus aureus* exists [19, 20]. This bioaerosol can enter the human body in various ways, including the digestive system through respiration [21, 22], which can cause lower respiratory tract infection, pneumonia, and bacteremia [23]. Understanding how bioaerosol emission characteristics fluctuate quantitatively over time can be used to better describe the bioaerosol exposure assessment and risk characterization [24].

Specific methods have been developed for the investigation of bioaerosol emission characteristics and their health risks on humans. Quantitative microbial risk assessment (QMRA) is broadly used to identify the health risks (annual probability of infection ($P_{(a)inf}$) and disease burden (DB)) associated with exposure to a bioaerosol environment [25, 26]. World Health Organization (WHO) recommends using QMRA with Monte Carlo simulation to assess the range and likelihood of health risk quantitatively [27, 28]. The two most widely used health risks benchmarks for risk characterization are U.S. Environmental Protection Agency (EPA) (≤E-4 pppy) for $P_{(a)inf}$ and WHO (≤E-6 disability-adjusted life year (DALYs) pppy) for DB [29, 30].

The emission of bioaerosol concentration due to toilet flushing is one of the reasons for disease transmission by a medium [31]. As a result, daily toilet users may inhale bioaerosol. According to Widdowson et al. [32] that, several passengers become infected with norovirus in flights from London to the Philippines after using the plane toilet [33]. During the diarrhea of patients, bioaerosol may be efficient in spreading pathogenic microorganisms through the moving air [34]. Therefore, temperature, relative humidity, and ventilation systems significantly affect bioaerosol concentration and health risk assessment in the indoor air [35]. However, systematic research about emission characteristics and quantitative health risk assessment of toilet flushing bioaerosols under various ventilation scenarios is insufficient.

Therefore, in this research, the bioaerosol emission characteristics (size distribution and concentration) of *Staphylococcus aureus* were systematically investigated in an indoor toilet after flushing with time. An Andersen impactor was used for the field measurements. Then, this work focused on the quantitative health risk assessment regarding the $P_{(a)inf}$ and DB of the exposed persons with time under various ventilation scenarios by performing a Monte Carlo simulation-based QMRA modelling. The current research delivers novel data about the emission characteristics of bioaerosol and its health risks quantitatively over time and bridges the knowledge gap between the emission characteristics and the assessment of risk characterization for exposed persons after toilet flushing. The results can provide a guideline for controlling essential health threats from bioaerosol emissions in various toilet usage scenarios.

2. Materials and Methods

2.1. Indoor toilet description

An indoor bidet toilet (4.9 L water volume per flush) was selected for this study. The toilet door is at the lower right corner (size: 210 × 75 cm^2). The orientation of the toilet room is face north, situated in the corner of the apartment, having an indoor area of 264 × 180 cm^2 and a height of 300 cm as a typical floor plan (Fig. 1). A window, which can fully open up to 90°, was at the lower left corner; its height was above 150 cm from the ground surface, and its size was 55 × 55 cm^2. A mechanical extraction ventilation system (ceiling exhaust fan is equipped without filters) was switched independently at the center of the toilet ceiling. It has a default setting with a fixed air volume of 180 m^3/h. A basin was located at the top corner of the indoor toilet.

[Fig. 1 inserts here]

2.2. Sampling procedure and bioaerosol analysis

In order to avoid the contaminant influence, which may affect the concentration of bioaerosols, the toilet was closed for 6 hours and then conducted ventilation for 1 hour before sampling. A six-stage Andersen impactor (FA-1 Hongchangxinlnc, Beijing, China) was used to measure the concentrations of *Staphylococcus aureus* bioaerosol after toilet flushing. The size ranges of the impactor are shown in the Supplementary Materials.

Following the standard operative procedures, the egg-yolk mannitol salt agar petri dish was placed in a sampler each stage for *Staphylococcus aureus* bioaerosol sampling [36]. The height of the sample rack was 0.8 m due to the sitting posture of the bidet toilet. The samples were collected for 5 min by the Andersen impactor at a flow rate of 28.3 min/L [37].

Table 1 shows the 4 types of ventilation scenarios, exposure time, exposed position, exposure site, exposed persons and exposure frequency. We set these ventilation scenarios to open or closed window with turned on or off air exhaust before sampling. The impactor was cleaned with a 75% alcohol cotton slice before and after each sampling repetition. The sampler had been set on the exposure site while the subject was attending the toilet. After attending the toilet, the subject flushed the toilet one time with an open lid to remove the stool materials and immediately started taking samples. After one time flushing, the 8 different time sampling periods were set as follows: 0 min (the moment of pressing the flushing button), 5, 10, 15, 20, 25, 30, and 35 min. Thus the sampling was conducted every 5 min after pressing the flushing button to get results with the passage of time and compared with each other. One time sampling with one time flushing was completed in a single day. At each ventilation scenario, 3 times sampling were conducted for the whole 8 different time sampling periods. Then, 96 samples were obtained. In addition, a background sampling was also conducted 5 min before attending the toilet. Temperature and relative humidity were recorded on each sampling day, and their mean value for each ventilation scenario in 3 sampling days was shown in the Table 2. The temperature and the relative humidity were measured on site in the middle of the toilet room using a Testo-610 meter. Their measuring range, accuracy and resolution are shown in the Supplementary Materials Table 1. After sampling, the Petri dish was transported to the laboratory for analysis. The positive hole method was used to correct the actual numbers of colonies at each petri dish stage. The bioaerosol concentration was then evaluated. The details of the laboratory analysis are shown in the Supplementary Materials.

[Table 1 inserts here]

[Table 2 inserts here]

2.3. QMRA

The pathogen of concern in this study was *Staphylococcus aureus* bioaerosol, which is the most prevalent airborne pathogenic bacterium in the indoor toilet environment. The highest levels of bioaerosol occur after toilet flushing. This condition can induce intestinal flora dysbiosis, whose symptoms include vomiting, fever, and diarrhea [38].

Table 3 shows the parameters for QMRA calculation. An exponential dose-response model was utilized as a dose infection model for QMRA [39, 40]. The risk characterization is based on the dose response model. The annual infection health risk level recommended by the U.S. EPA (2005) and the DALYs recommended by the WHO (2008) were used to assess the health risks [41]. Monte Carlo simulation was utilized to create a probabilistic based risk model [42]. With over 10,000 iterations, output parameters ($P_{(a)inf}$ and DB) were calculated such that the distributions can reach a stable state [29, 43]. The details of the QMRA calculation process and the Monte Carlo Simulation analysis are shown in the Supplementary Materials.

[Table 3 inserts here]

3. Results and discussion

3.1. Bioaerosol concentration

Table 4 shows the *Staphylococcus aureus* bioaerosol concentrations under various ventilation scenarios from 0 min to 35 min after flushing. The bioaerosol concentrations significantly increased after pressing the flush button in all ventilation scenarios. At the initial phase (0 min), the maximum concentration of bioaerosol (855.15±84.81 CFU/m^3) was observed in the poor ventilation scenario given the poor airflow condition [44, 45]. This outcome was affected by the high average relative humidity in the poor ventilation scenario (Table 2), which may contribute to the retarded bioaerosol die-off [46]. Meanwhile, the minimum concentration (466.43±49.47 CFU/m^3) was found in the combined natural and mechanical ventilation scenario due to the combined effect of natural and mechanical ventilations, which can promote a strong airflow condition [47, 48]. The high airflow can decrease the relative humidity, which may affect the survivability of bioaerosol bacteria [49].

Furthermore, the bioaerosol concentration in the natural ventilation scenario was comparably 1.1 times higher than that in the mechanical ventilation scenario at the initial phase. This finding indicates that a mechanical ventilation scenario can ensure a specified level of air exchange, which affects the indoor relative humidity by employing fan-forced airflow diffusion via a duck work and dilute the contaminated air [50].

A decreasing trend in the concentration of bioaerosol was generally observed in all ventilation scenarios over time. After flushing, the concentration of bioaerosol in the poor, mechanical, natural, and combined natural and mechanical ventilation scenarios was considerably reduced by 90.08%, 89.89%, 89.90%, and 89.39%, respectively (Supplement Materials Fig. 1). These observations are attributed to surface evaporation, inertia-gravitational settling, and the natural decay rate of bioaerosol related to temperature and relative humidity and affected by the high flow of ventilation scenarios and time passage for air dilution [51]. Moreover, the high airflow and time passage could affect more on air dilution, which affecting the survivability of bioaerosol bacteria. In addition, the indoor toilet environment may also be affected by the outside environment. However, in this study as a limitation, we assumed that the humid air only comes from the activity of toilet flushing for convenience.

In the comparison of the decreases in bioaerosol concentration ratio over time, the mechanical ventilation scenario showed a higher decreasing ratio than the natural ventilation scenario (Supplement Materials Fig. 1). This result was due to the low relative humidity caused by mechanical ventilation in the indoor toilet (Table 2). Which can reduce surface evaporation, increase the natural decay rate [52], and remove or dilute the bioaerosol with a constant airflow ventilation rate [53]. Meanwhile, natural ventilation, which has an unstable airflow, is based on minor variations between pressures or humidity within and outside the indoor bidet toilet [54]. Thus, mechanical ventilation can disturb the airflow much more potently than natural ventilation [55].

Furthermore, the bioaerosol-concentration decrease ratio in the poor ventilation scenario was lower than that in the combined natural and mechanical ventilation scenario (Supplement Materials Fig. 1). This result was due to the nearly constant temperature. The maximum relative humidity was observed in the poor ventilation scenario and the minimum relative humidity in the combined ventilation scenario (Table 2). The combined ventilation scenario may ventilate and eliminate humidity [56]. However, it also depends on the settings of mechanical ventilation and window openings. While, in the poor airflow conditions, the high relative humidity level in poor ventilation scenario led to a low bioaerosol-concentration decrease ratio (Supplement Materials Fig. 1). A high relative humidity can reduce the bioaerosol decay rate and protect bioaerosol survival for an extended period [57].

[Table 4 inserts here]

3.2. Size distribution of bioaerosol particles

Fig. 2 demonstrates the size distribution of *Staphylococcus aureus* bioaerosol. For all evaluated ventilation scenarios (Fig. 2), the particle size distribution results indicated that the peak proportion of bioaerosol particle size distribution was generally in the size range of respirable particle stages 3 (3.3–4.7 μm), 4 (2.1–3.3 μm), and 5 (1.1–2.1 μm). The respirable bioaerosol particles can be inhaled and deposited in the respiratory tract and deeply deposited in the lungs, which are the most common routes of exposure to bioaerosol particles [58]. Furthermore, at 0 min after flushing, the particle size percentage of respirable particles approximately increased for all ventilation scenarios (Fig. 2). A similar study reported that flushing toilets releases bioaerosols, with a significant proportion of the particles being less than 3 μm in diameter [59]. In addition, the bioaerosol particles can disintegrate into smaller fractions, either due to the release mechanisms of a toilet flushing or during the sampling of the six-stage Andersen impactor [60].

A maximum respirable particle size percentage was observed in the poor ventilation scenario (Fig. 2a) due to poor airflow condition and high relative humidity [61]. The high relative humidity proved a well hospitable environment for the survival of respirable bioaerosol particles [62]. At 0–20 min after flushing, the percentage of respirable size particles was as high as 73% in the poor ventilation scenario (Fig. 2a). However, after flushing 20–35 min, a decreasing trend was noticed probably because of the air dilution effects of the accelerated settling down with inertia-gravity or with the passage of time [14]. By contrast, the minimum respirable particle size percentage was recorded in the combined natural and mechanical ventilation scenario (Fig. 2d). After flushing 0–35 min, a decreasing trend was observed. Given the combined effect of natural and mechanical ventilation, a strong airflow infiltrated the respirable particles and decreased the relative humidity which may inactivate bioaerosol and increase its natural decay rate [63].

In the comparison of the two types of ventilation, the percentage of the respirable particles of bioaerosol in the natural ventilation scenario (Fig. 2c) was higher than that in the mechanical ventilation scenario (Fig. 2b). For the mechanical ventilation scenario (Fig. 2b), 0–15 min after flushing, the percentage of respirable particles of bioaerosol showed a minor variation, whereas at 15–35 min after flushing, a remarkable reduction in rate of respirable particles was perceived. However, in the natural ventilation scenario (Fig. 2c), at 0–25 min after flushing, the respirable bioaerosol particle percentage remained high and was nearly constant but after flushing 30–35 min, a decreasing rate was observed.

[Fig. 2 inserts here]

3.3. Annual probability of infection

The $P_{(a)inf}$ for the health risks of bioaerosol after flushing 0–35 min under different ventilation scenarios are shown in Fig. 3 and Supplementary Materials Tables 2 and 3. The health infection risk of an adult male was consistently higher in each exposure ventilation scenario than that of the remaining exposed persons (adult female, elder male, and elder female). Breathing rate is one of the core aspects that distinctly affect the health risk [28, 64], and the adult male inhaled breathing rate was significantly higher than that of the other exposed persons [65] shown in Table 3.

The health infection risk varied with time for all exposed persons after flushing 0–15 min (Fig. 3 and Supplementary Materials Table 2 and 3); the infection risk in all ventilation scenarios was above the U.S. EPA benchmark and unacceptable. However, at 35 min after flushing, the infection risk of all exposed persons in all ventilation scenarios satisfied the benchmark and was tolerable. The exception was for the adult male under conservative estimate in the poor ventilation scenario (Fig. 3a). These results were obtained because the health risk assessment is primarily dependent on the concentration of bioaerosols [66, 67], and the concentrations largely decreased after flushing 35 min (Table 4). However, for the exception condition, a poor ventilation scenario will not inactivate the bioaerosol concentration because the window is closed, and the air exhaust is turned off [68]; thus, the health infection risk of an adult male still exceeded the benchmark under the worst case scenario (Fig. 3a).

Comparing the health infection risks in various ventilation scenarios, a high health infection risk was observed for all exposed persons in the poor ventilation scenario (Fig. 3a), and a low value was recorded in the combined natural and mechanical ventilation scenario (Fig. 3d). Therefore, well ventilation (e.g. open window or turn on air exhaust) should be used as an appropriate control strategy for lowering the health infection risk to an acceptable level [69]. After flushing 20–25 min in a poor ventilation scenario (Fig. 3a), the health infection risk for all exposed persons was intolerable. However, at 30 min after flushing, the health infection risk of the adult female, elder male, and elder female was tolerable under the optimistic estimate. In addition, the health infection risk of the adult male was still over the benchmark. By contrast, for the combined effect of natural and mechanical ventilation scenario (Fig. 3d), the adult male at 25 min after flushing and adult female at 20 min to 25 min after flushing satisfied the benchmark under an optimistic estimate. The health infection risks of elder male and female at 20–25 min after flushing were almost in the same order of magnitude as the benchmark but still over the benchmark under the conservative estimate. Moreover, at 30 min after flushing, the health infection risk of adult male was endurable under the optimistic estimate. Furthermore, all exposed persons were generally below the benchmark except for those of adult female and elder male under conservative estimates.

The health infection risk for all exposed persons to the mechanical ventilation scenario (Fig. 3b) was lower than that for the natural ventilation scenario (Fig. 3c). Thus, in the mechanical ventilation scenario (Fig. 3b), at 20 min after flushing, the health infection risk for adult male was above the benchmark, whereas that for the rest of all exposed persons under optimistic estimate was below the benchmark. At 25–30 min after flushing, the health infection risks for all exposed persons were almost in the same order of magnitude. Therefore, the health infection risk of adult males was acceptable under the best case scenario. The health infection risks of the remaining exposed persons were generally below the benchmark except for that under conservative estimates. On the other hand, in the natural ventilation scenario (Fig. 3c), at 20–25 min after flushing, the health infection risk of all exposed persons was intolerable except for that of the elder female under the optimistic estimate. At 30 min after flushing, the health infection risk of adult male satisfied the benchmark under the optimistic estimate, but those of the remaining exposed persons were generally below the benchmark, except for the adult female under the conservative estimate.

[Fig. 3 inserts here]

3.4. Diseases burden

Fig. 4 and Supplementary Materials Table 4 and 5 show the DB for the health risk of bioaerosol at 0–35 min after flushing under different ventilation scenarios. The estimations of $P_{(a)inf}$ and DB results were nearly identical in various ventilation scenarios. However, at 20 min to 35 min after flushing in all ventilation scenarios for all exposed persons, the disease health burdens were bearable and below the recommended DB benchmark by the WHO. The exception was for the adult male after flushing 20 min in the poor ventilation scenario (Fig. 4a); the value was over the benchmark under conservative estimate.

The disease health burdens in poor ventilation scenario (Fig. 4a) for all exposed persons at 0–5 min after flushing were unbearable except for adult female, elder male, and elder female, whose burden, at 5 min after flushing, satisfied the benchmark under optimistic estimate. However, at 10 min after flushing, the burden results changed significantly due to the variation in bioaerosol concentration (Table 4). Therefore, given the high breathing rate (Table 3), the burdens of the adult male under optimistic estimate were below the benchmark, whereas the values for the rest of all exposed persons generally satisfied the benchmark except under conservative estimates. Furthermore, the results of burdens revealed no significant differences between elder males and females. Their burdens were in the same order of magnitude in all ventilation scenarios because the breathing rates of elder males and females are almost the same (Table 3). At 15 min after flushing, the burdens of adult male and female were generally over the benchmark under conservative estimates, whereas those for the other exposed persons were below the benchmark in all estimates.

Nevertheless, referring to the combined natural and mechanical ventilation scenario (Fig. 4d), after flushing 0 min, the disease health burden of the adult male was intolerable, whereas that of the remaining exposed persons satisfied the benchmark under the optimistic estimate. At 5 min after flushing, the burden of adult males satisfied the benchmark under optimistic estimate, and those for the remainder of all exposed persons were below the benchmark except for the adult female under conservative estimates. At 10 min after flushing, the burden of the adult male was unbearable under conservative estimates, whereas those for the remaining exposed persons were below the benchmark.

In the mechanical ventilation scenario (Fig. 4b), at 0 min after flushing, the disease health burden of the adult male was one order of magnitude over the benchmark, whereas those for the remaining exposed persons satisfied the benchmark under the optimistic estimate. At 5 min after flushing, the burden of the adult male was bearable under the optimistic estimate, whereas those for the other exposed persons satisfied the benchmark except those for adult female and elder male under conservative estimates. However, after flushing 10 min, the burden of adult males was unbearable under conservative estimates, whereas that for the remaining exposed persons was below the benchmark.

Furthermore, in the natural ventilation scenario (Fig. 4c), after flushing 0 min, the disease health burden of all exposed persons was above the benchmark except for the elder male and female under optimistic estimates. After flushing 5 min, the adult male and female burdens were endurable under optimistic estimates, whereas those for the elder male and female were over the benchmark under conservative estimates. After flushing 10 min, the burden of an adult male was endurable based on the benchmark under optimistic estimate, whereas those for the other exposed persons were below the benchmark except for adult female and elder male under conservative estimates. Furthermore, after flushing 15 min, the burden was bearable only for the adult male under conservative estimates.

However, the discussion about disease health burden in this study only presents the potential impact that a particular health risk exists in the indoor toilet environment rather than setting compulsory guidelines for public health protection or decision making in real life.

[Fig. 4 inserts here]

4. Conclusion

At the initial phase, the highest and lowest bioaerosol concentrations were found in the poor and combined natural and mechanical ventilation scenarios. Furthermore, the bioaerosol concentration in the natural ventilation scenario was 1.1 times higher than that in the mechanical ventilation scenario. However, after flushing, a significant decreasing trend was generally observed in the bioaerosol concentrations in all ventilation scenarios. The peak proportion of bioaerosol particle size distribution was generally observed in the size range of respirable particles, and it increased under all ventilation scenarios after flushing 0 min. The maximum respirable particle size percentage was recorded in the poor ventilation scenario. The percentage of the respirable bioaerosol particle in the natural ventilation scenario was higher than that in the mechanical ventilation scenario.

The health risks (health infection risk and disease health burdens) of adult male were consistently higher in each exposure ventilation scenarios compared with those of the other exposed persons. Furthermore, for all exposed persons in various ventilation scenarios, the maximum health risk was obtained in the poor ventilation scenario, and the minimum was observed in the combined ventilation scenario. The health risks for all exposed persons in the mechanical ventilation scenario were lower than those for the natural ventilation scenario. However, the health infection risk varied with time for all exposed persons.

The present research provided novel data and enhanced the knowledge of the emission characteristics of bioaerosol and its health implication on exposed persons after toilet flushing with the passage of time quantitatively in various ventilation scenarios. The QMRA framework used in this study can be an effective tool to identify the implication of human health risks. For further research, sensitivity analysis is recommended to quantify the contributions of inputted variable parameters to health risk assessment and to determine the most influential parameter for the Monte Carlo simulation-based QMRA framework.

Declaration of competing interest

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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Table 1 Exposure scenarios

Items	Exposure scenarios
Ventilation scenarios	Closed window/turned off air exhaust (poor ventilation scenario), closed window/turned on air exhaust (mechanical ventilation scenario), open window/turned off air exhaust (natural ventilation scenario), open window/turned on air exhaust (combined natural and mechanical ventilation scenario)
Exposure time per day	5 min/day
Exposed position	Sitting posture (sampling height 0.8 m)
Exposure site	Bidet toilet
Exposed persons	Adult male, adult female, elder male, and elder female The adult is between 18 and 60. The elder is above 60.
Exposure frequency per year	365 days (for all exposed person)

Table 2 Mean±SD of temperature and relative humidity during sampling campaign for each ventilation scenario

Meteorological factors		**Closed window/turned off air exhaust**	**Closed window/turned on air exhaust**	**Open window/turned off air exhaust**	**Open window/turned on air exhaust**
Temperature (°C)	Maximum	14.6	13.8	13.4	13.6
	Minimum	12.1	11.3	10.5	10.2
	Median	13.1	12.9	12.4	12.7
	$\bar{x} \pm s$	13.3±1.3	12.7±1.3	12.1±1.5	12.2±1.8
Relative humidity (%)	Maximum	78.1	60.1	65.3	59.1
	Minimum	73.1	52.4	58.1	52.3
	Median	75.5	54.3	62.4	55.3
	$\bar{x} \pm s$	75.6±2.5	55.6±4.0	61.9±3.6	55.6±3.4

Table 3 Parameters for quantitative microbiological risk assessment calculation

Parameters	Unit	Values	Reference
EC: Exposure bioaerosol concentration	CFU/m³	Table 4	-
BR: Breathing rate	m³/day	Elderly, age >60 (male: 13.65, female: 12.65) Adults, age 18~60 (male: 18.65, female: 14.80）	[65]
T: Exposure time in an exposure per day	h/day	Table 1	-
AG: Aerosol ingestion rate	0.5 %	Size distribution of bioaerosol particles for the sixth stage of the Andersen six-stage impactor	Fig. 2
Exposure dose $d=EC\times BR\times T\times AG$	CFU/day	Calculation	[64]
Daily probability of infection $P_i(d)=1-e^{-dk}$		$k=8.05\times10^{-8}$	[39]
Annual probability of infection $P_y=1-(1-P_i(d))^n$	pppy	n means exposure frequency n=365	[64]
Disease burden $DB=P_y\times HB$	DALYs pppy	Health burden (HB)=0.0455	[29]

Table 4 Mean±SD of *Staphylococcus aureus* bioaerosol concentration (CFU/m^3)

Ventilation scenarios	Before attending toilet	The 8 different time sampling periods							
		After flushing							
	-5 min*	0 min**	5 min	10 min	15 min	20 min	25 min	30 min	35 min
Closed window/turned off air exhaust (Poor ventilation scenario)	10.60±0.41	855.15±10.82	685.51±7.96	494.70±5.54	416.96±3.87	339.22±3.74	233.22±1.76	176.68±1.17	84.81±0.63
Closed window/turned on air exhaust (Mechanical ventilation scenario)	7.06±0.40	628.98±5.85	480.57±4.46	332.16±3.25	254.42±2.61	162.54±0.98	134.28±0.75	113.07±0.52	63.60±0.55
Open window/turned off air exhaust (Natural ventilation scenario)	9.18±0.40	699.65±6.25	544.17±4.79	459.36±3.43	367.49±1.75	289.75±1.94	204.95±0.98	127.21±0.63	70.67±0.52
Open window/turned on air exhaust (Combined natural and mechanical ventilation scenario)	5.65±0.32	466.43±3.29	360.42±2.17	282.69±2.25	197.88±1.37	127.21±0.63	120.14±0.98	98.94±1.03	49.47±0.41

* "-5 min" means 5 min before attending the toilet.

** "0 min" means the moment of pressing the flush button.

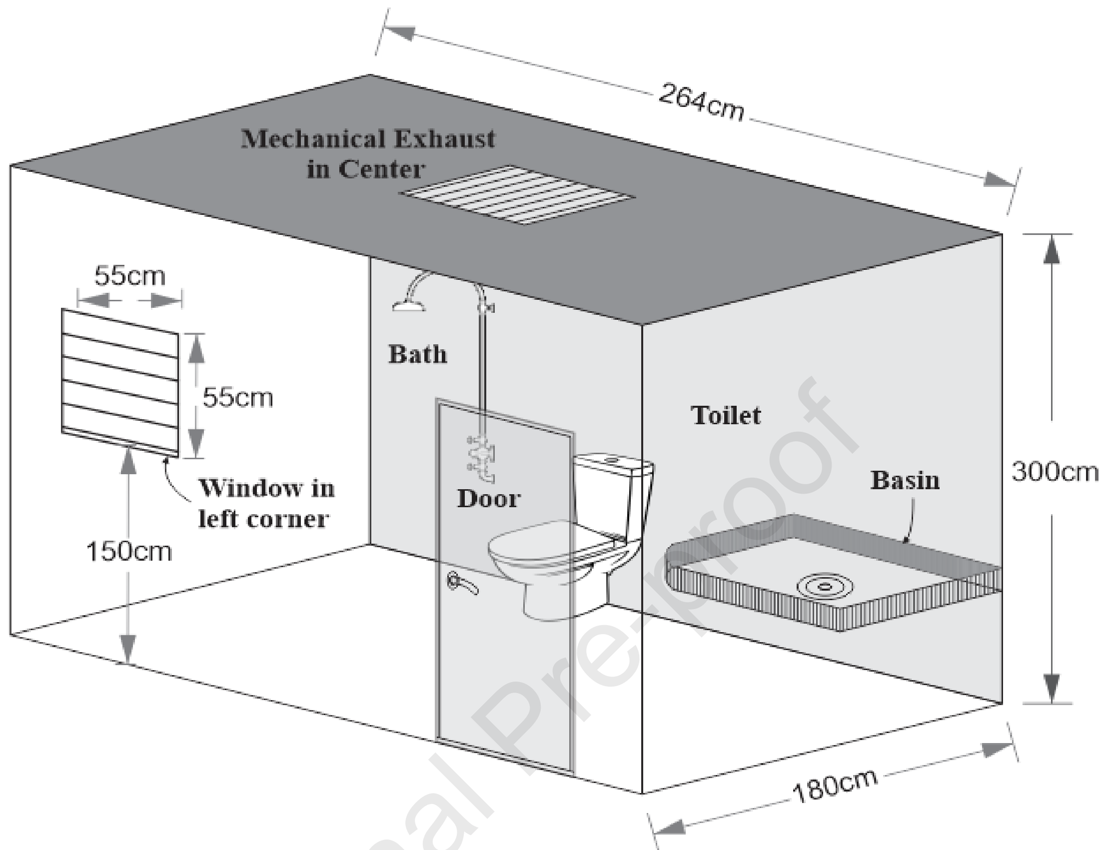

Fig. 1 Indoor bidet toilet description

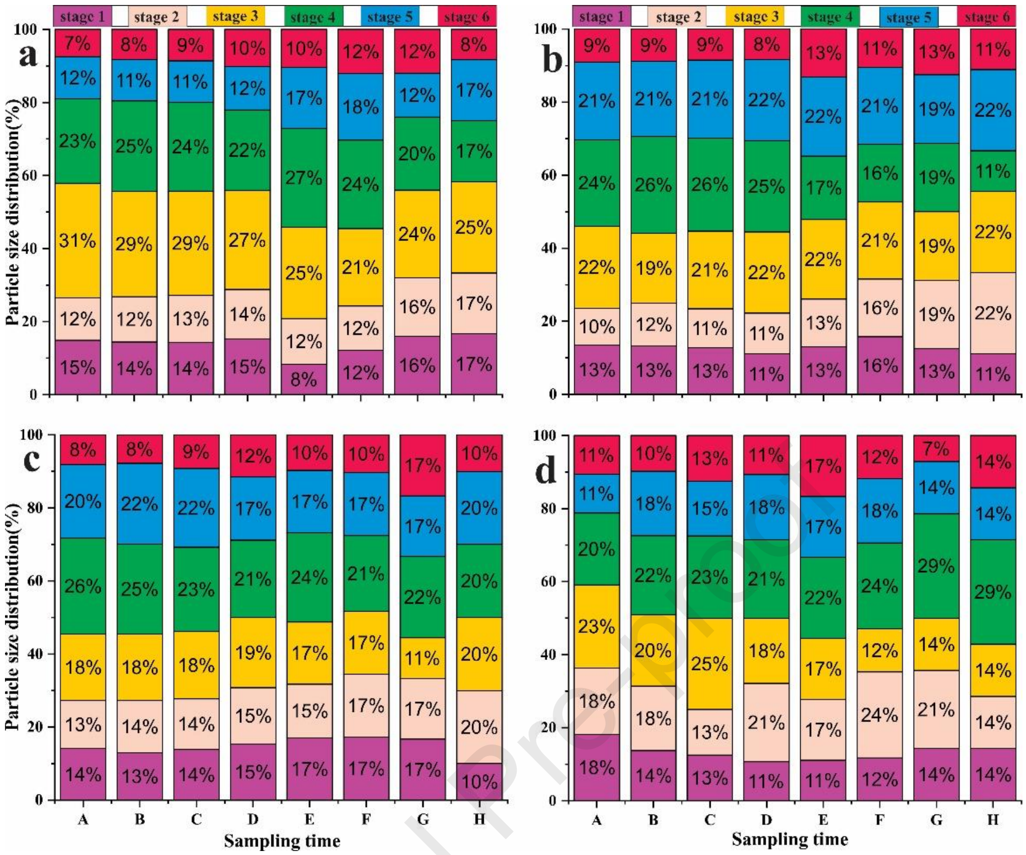

Fig. 2 Proportion of size distribution of *Staphylococcus aureus* bioaerosol particles in various ventilation scenarios: (a) closed window/turned off air exhaust (poor ventilation scenario), (b) closed window/turned on air exhaust (mechanical ventilation scenario), (c) open window/turned off air exhaust (natural ventilation scenario), and (d) open window/turned on air exhaust (combined natural and mechanical ventilation scenario).

The 8 different time sampling periods: A= 0 minute after flushing (the moment of pressing the flush button), B=5 minute after flushing, C=10 minute after flushing, D=15 minute after flushing, E=20 minute after flushing, F=25 minute after flushing, G=30 minute after flushing, H=35 minute after flushing.

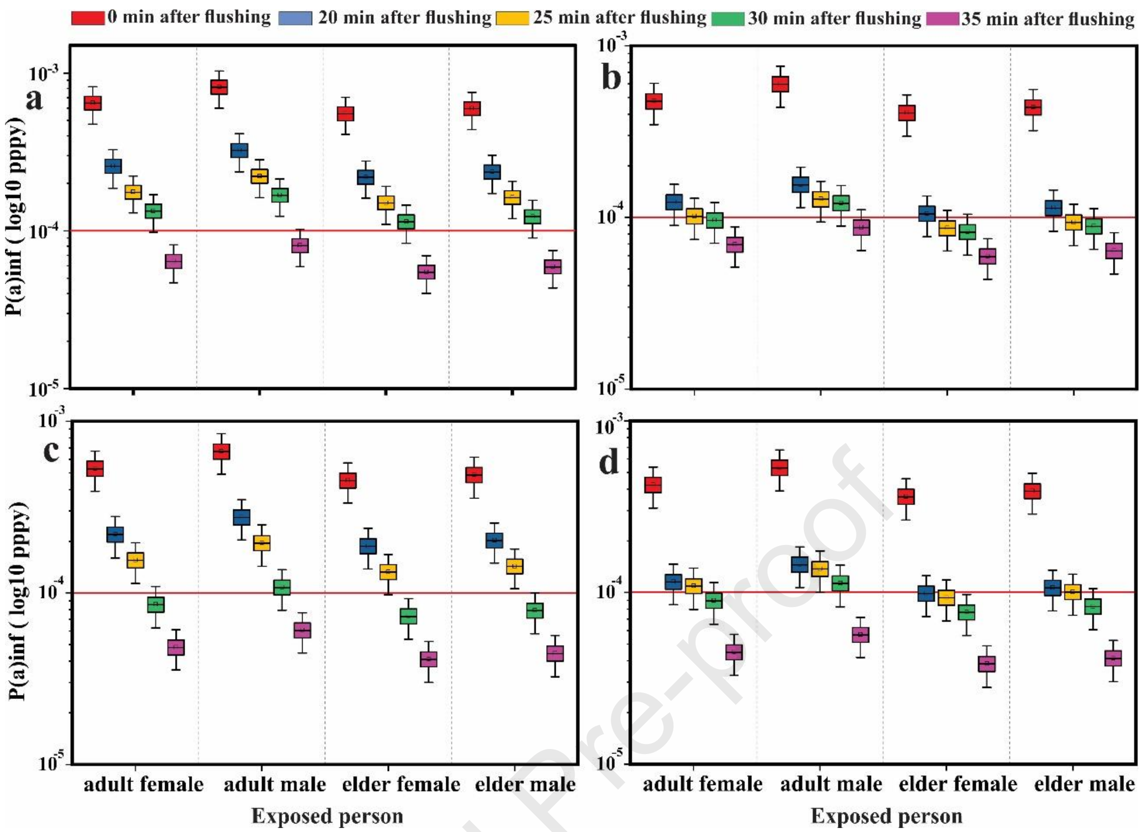

Fig. 3 Box-and-whiskers diagram showing the annual infection risk under various ventilation scnarios: (a) closed window/turned off air exhaust (poor ventilation scenario), (b) closed window/turned on air exhaust (mechanical ventilation scenario), (c) open window/turned off air exhaust (natural ventilation scenario), and (d) open window/turned on air exhaust (combined natural and mechanical ventilation scenario).

The bottom and top of the box represent the first (25th percentile) and third quartiles (75th percentile), respectively. The band inside the box represents the second quartile (median), and the tetragon inside the box denotes the average value. The bottom and top of the whiskers respectively represent the 5th (optimistic estimate in best case scenario) and 95th percentile values (conservative estimate in the worst case scenario).

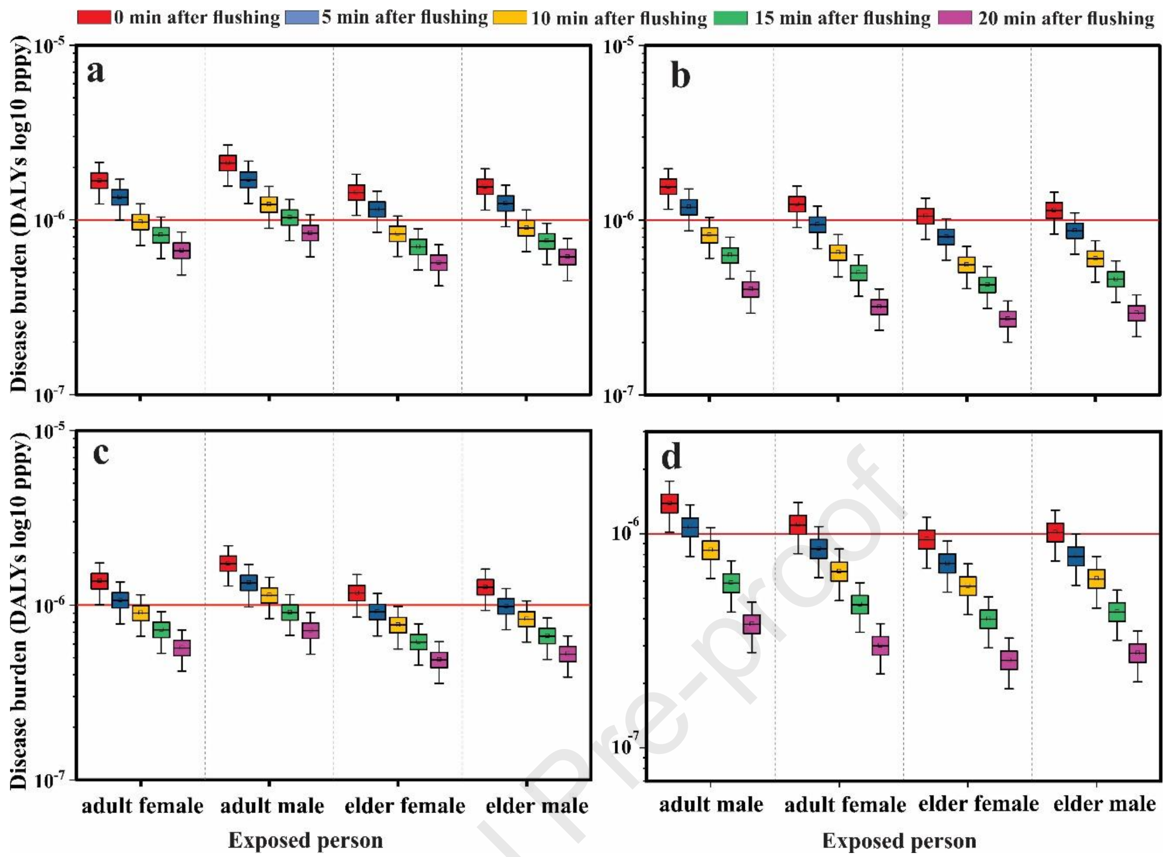

Fig. 4 Box-and-whiskers diagram showing the DB under various ventilation scnarios: (a) closed window/turned off air exhaust (poor ventilation scenario), (b) closed window/turned on air exhaust (mechanical ventilation scenario), (c) open window/turned off air exhaust (natural ventilation scenario), and (d) open window/turned on air exhaust (combined natural and mechanical ventilation scenario).

The bottom and top of the box represent the first (25th percentile) and third quartiles (75th percentile), respectively. The band inside the box represents the second quartile (median), and the tetragon inside the box denotes the average value. The bottom and top of the whiskers respectively represent the 5th (optimistic estimate in best case scenario) and 95th percentile values (conservative estimate in the worst case scenario).

>Poor ventilation scenario has the highest bioaerosol concentration >Health risks of adult male were consistently higher than other exposed persons >Health risks in mechanical were lower than that in natural ventilation scenario >Health infection risk unsatisfied U.S. EPA benchmark after flushing from 0 to 15 min >Disease burdens were below the WHO benchmark after flushing from 20 to 35 min

Declaration of interests

☒ 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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: