






## Effects of face masks and ventilation on the risk of SARS-CoV-2 respiratory transmission in public toilets: a quantitative microbial risk assessment

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### ABSTRACT

Public toilets may increase the risk of COVID-19 infection via airborne transmission; however, related research is limited. We aimed to estimate SARS-CoV-2 infection risk through respiratory transmission using a quantitative microbial risk assessment framework by retrieving SARS-CoV-2 concentrations from the swab tests of 251 Thai patients. Three virus-generating scenarios were investigated: an infector breathing, breathing with a cough, and breathing with a sneeze. The infection risk (95th percentile) was as high as  $10^{-1}$  with breathing and increased to 1 with a cough or a sneeze. No significant gender differences for toilet users (receptors) were noted. The highest risk scenario, namely breathing with a sneeze, was further evaluated for risk mitigation measures. Mitigation to a lower risk under  $10^{-3}$  succeeded only when the infector and the receptor both wore N95 respirators or surgical masks. Ventilation of up to 20 air changes per hour (ACH) did not decrease the risk. However, an extended waiting time of 10 min between an infector and a receptor resulted in approximately  $1.0\text{-log}_{10}$  further risk reduction when both wore masks with the WHO-recommended 12 ACH. The volume of expelled droplets, virus concentrations, and receptor dwell time were identified as the main contributors to transmission risk.

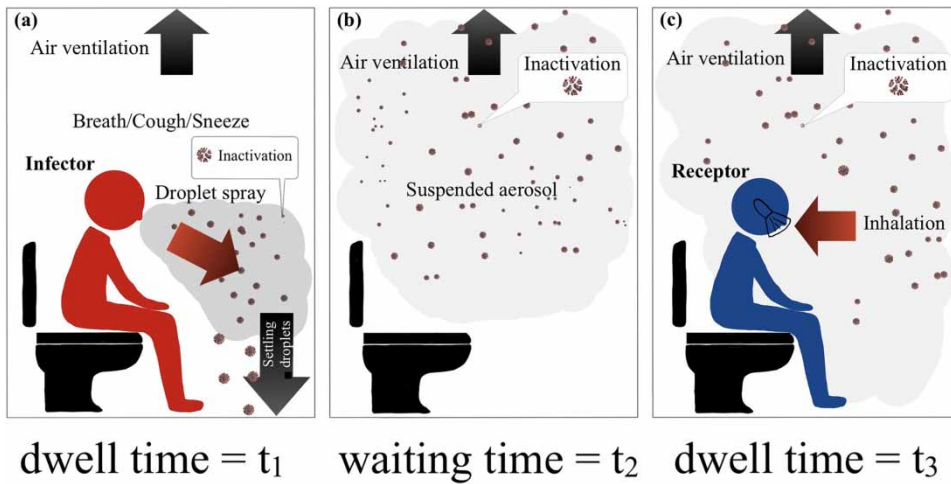
**Key words:** aerosol, COVID-19, mask, restroom, risk management, toilet, ventilation

### HIGHLIGHTS

- The use of public toilets poses a risk of SARS-CoV-2 respiratory transmission.
- No gender differences in risk by counteracting dwell times and inhalation rates.
- Ventilation alone did not reduce risk at 20 ACH with immediate receptor entrance.
- 10-min waiting time further mitigated risks beyond face masks at 12 ACH ventilation.
- N95 and surgical masks offer the most effective risk mitigation to toilet users.

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## GRAPHICAL ABSTRACT



## INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic has affected the global population since its emergence in December 2019. The three main transmission routes of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the etiological agent of COVID-19, have been identified as (1) the inhalation of respiratory fluids carrying infectious viruses, (2) direct splashes or sprays of infectious respiratory droplets and aerosol particles, and (3) the touching of contaminated surfaces (Centers for Disease Control & Prevention 2021). Communal confined spaces such as public toilets in shopping centers, schools, restaurants, airports, theaters, and hospitals may be significant areas for SARS-CoV-2 transmission (Dancer *et al.* 2021). Surface contamination with SARS-CoV-2 in toilets and bathrooms has been reported (Ding *et al.* 2021; Maestre *et al.* 2021); however, transmission risk from fomite exposure could be reduced significantly through the simple yet effective interventions of hand washing, hand sanitizing, and surface disinfection (Dancer *et al.* 2021; Pitol & Julian 2021; World Health Organization [WHO] 2020a). Airborne transmission, on the other hand, is deemed the main route of COVID-19 spread (Centers for Disease Control & Prevention 2021) and could be aggravated by the use of busy, confined public toilet spaces, especially if appropriate steps are not taken to mitigate the risk of virus transmission (Dancer *et al.* 2021).

The positive detection of SARS-CoV-2 RNA has been reported in 23.8% of air samples from hospital toilets, which have demonstrated higher viral loads than clinical areas (Birgand *et al.* 2020). However, risk assessments of respiratory exposure to SARS-CoV-2 in public toilets are limited. Potential sources of infectious respiratory droplets and aerosol particles in toilet settings include exhalation and expelling, such as sneezing, speaking, and coughing, by infected toilet users, and the aerosolization of infected feces and urine after toilet flushing (Dancer *et al.* 2021; Schijven *et al.* 2021). Although infectious SARS-CoV-2 was isolated from the feces of a severely infected patient (Xiao *et al.* 2020), studies confirming that feces and urine in wastewater remain infectious for SARS-CoV-2 are limited, with supporting evidence showing poor virus survival in gastrointestinal tracts due to the low pH of gastric fluids, bile, digestive enzymes, and bacterial byproducts (Jones *et al.* 2020; Zang *et al.* 2020; Albert *et al.* 2021). Consequently, even though flushing activities can produce airborne droplets and aerosols, the associated risks may be low because contamination by infectious virus particles is less likely (Shi *et al.* 2021). In this study, we, therefore, focused on characterizing the risk of SARS-CoV-2 respiratory transmission introduced by normal breathing and expelling (i.e., coughing and sneezing) in a public toilet setting.

Quantitative microbial risk assessment (QMRA) is a valuable tool used to quantitatively estimate human health risks associated with exposure to pathogens in different environmental matrices (Rose & Gerba 1991; Haas *et al.* 2014). The QMRA framework has been applied to estimate SARS-CoV-2 transmission risk among wastewater treatment plant workers (Dada & Gyawali 2021; Zaneti *et al.* 2021), Tokyo 2020 Olympic Games attendees (Murakami *et al.* 2021), and confined vehicle passengers and shared room users (Schijven *et al.* 2021). In the present study, we aimed to estimate the risk of infection associated with public toilet exposure to SARS-CoV-2 through airborne transmission using the QMRA approach. For convenience, we called a healthy person who is exposed to transmission risk a receptor, while a disease-carrying person, either

symptomatic or asymptomatic, was termed an infector. We gathered the input parameters from a variety of sources. These included COVID-19 concentration data obtained by swab testing 251 Thai patients from a public hospital in Bangkok and the exposure factors related to three droplet- and aerosol-generating activities of infectors, namely breathing, breathing with a cough, and breathing with a sneeze, which were all identified from published sources (Duguid 1946; Loudon & Roberts 1967; Fabian *et al.* 2011; Han *et al.* 2013; Schijven *et al.* 2021). The risk of infection was calculated separately for the male and female receptors because of their different respiratory rates and periods of time spent in the toilet, the so-called dwell times. To include uncertainty and variability in the risk characterization, we applied the Monte Carlo simulation (MCs) technique to calculate the risks. The sensitivity of the model parameters was evaluated to determine which input parameters could help reduce the associated uncertainty. Finally, three risk mitigation measures, namely face mask wearing, increased ventilation, and extended waiting times, were assessed to ascertain their efficacy. The calculated risks and associated mitigation measures may be beneficial in the development of public health policies aimed at providing the effective control of SARS-CoV-2 transmission.

## MATERIALS AND METHODS

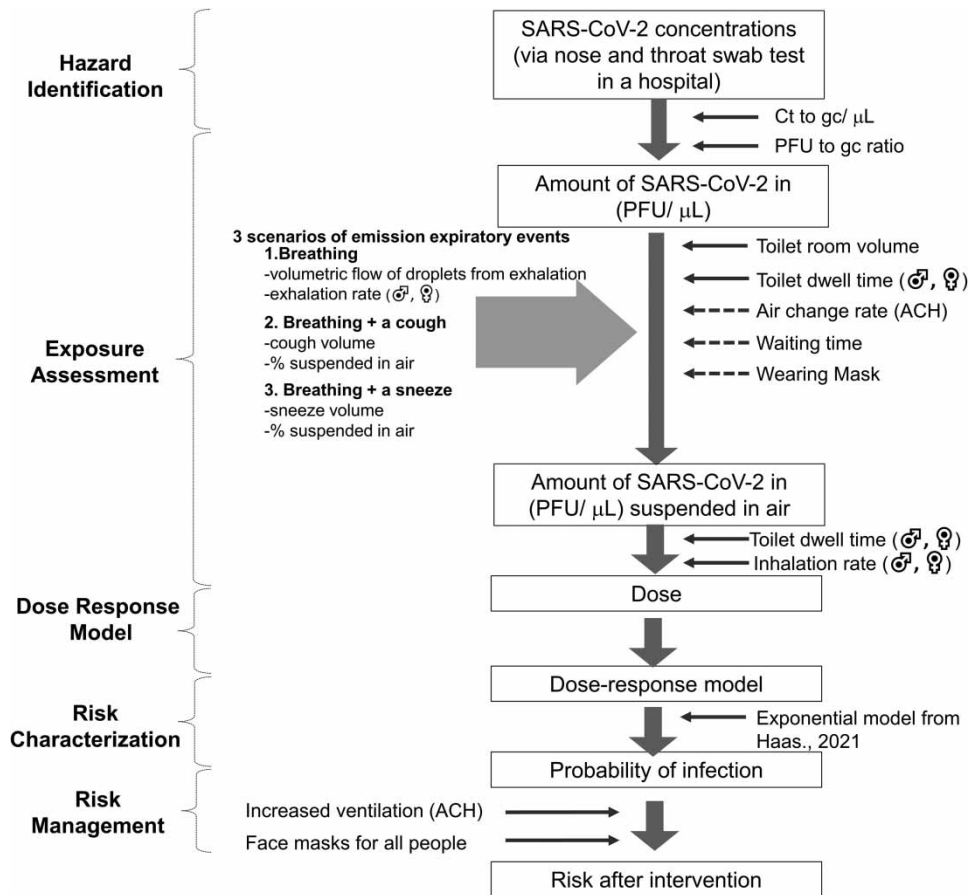
### Risk scenarios

We evaluated the infection risk in various scenarios with and without preventive measures. For the public toilet model, a Thailand standard cubicle size of  $1.5 \times 0.8 \times 2.7$  m ( $3.24 \text{ m}^3$ ) was set for the risk evaluation (Ministry of Public Health 2016). The three scenarios used in this study that can cause an infector to generate infectious droplets and aerosols included breathing (Br), breathing with a cough (Br+Co), and breathing with a sneeze (Br+Sn). The scenario that provided the highest risk was further investigated to determine the efficacy of the identified mitigation measures (i.e., face mask wearing, ventilation, and waiting times). To evaluate the effects of mask wearing, different types of masks (i.e., N95 respirator and surgical and denim fabric masks) were modeled when worn by either an infector or a receptor, or both. For the ventilation evaluation, the air changes per hour (ACH) were varied at 0 (no ventilation), 0.5 (poor ventilation), 10 (DIN 1946 ventilation standard for public toilets), 12 (WHO-recommended standard ventilation 2021), and 20 (extreme ventilation). In addition, the waiting times (i.e., the time the toilet was unoccupied) between an infector and a receptor were set at 0, 3, 5, and 10 min. An outline of the QMRA steps for all the scenarios is presented in Figure 1.

### Virus levels generated by an infector

The SARS-CoV-2 concentrations used in this study were retrieved from the reverse-transcription quantitative polymerase chain reaction quantification cycle ( $C_t$ ) values of 251 positive swab test results of an N2 gene from patients in a public hospital in Bangkok from March to May 2021. Due to the absence of a standard curve for clinical swab testing in Thailand, the viral concentrations were estimated using a published standard curve (Sherchan *et al.* 2020), which ranged from  $4.4 \times 10^{-1}$  to  $6.4 \times 10^8$  gene copies (gc)/ $\mu\text{L}$ . The SARS-CoV-2 concentrations ( $A$ ) were fitted with a triangular distribution as shown in Supplementary Table S1. To convert the virus concentrations from gc to an infectious plaque-forming unit (PFU), the ratio of the PFU/gc ( $R$ ) of SARS-CoV-2 was calculated as 1:10 because the ratio of the median tissue culture infectious dose (TCID<sub>50</sub>) to the viral RNA copies in the saliva of ferrets has been found to be 0.15 (Kim *et al.* 2020), and the ratio of PFU to TCID<sub>50</sub> has been established as 0.7 (Covés-Datson *et al.* 2020). The product of 0.15 and 0.7 was rounded to 0.1 (Murakami *et al.* 2021).

The number of infectious viruses suspended in the ambient air of the toilet cubicle was calculated using the mass balance equations (Equations (1.1) and (1.2)) in which each term in the equation has units of mass per time. Under the completely mixed condition, the accumulation of virus particles as aerosols was obtained from the summation of the breathing of an infector, virus inactivation, and virus removal by mechanical ventilation and inhalation. Under typical conditions of 20–70% relative humidity, a 20 °C temperature, and no direct sunlight, an average SARS-CoV-2 inactivation rate of  $0.008 \text{ (min}^{-1}\text{)}$  was applied (Schuit *et al.* 2020). We assumed that the virus particles were released continuously during the time the infector was in the toilet cubicle (infector's dwell time =  $t_1$  minutes). The dwell times for men and women, which were in line with those indicated in an airport study of 250 men and 237 women, were fitted with a log-normal distribution (Supplementary Table S1; Gwynne *et al.* 2019). The remaining infectious virus concentrations generated by the infector after leaving the toilet ( $C_{t_1}$ ) were calculated according to Equation (2) by integrating Equation (1.2) with no initial virus particles ( $C=0$ ). The inhalation rates ( $q_{in}$ ) following the uniform distribution ranged between 8.40 and 14.93 L/min for men and between 7.71 and 11.32 L/min for women (Stifelman 2007). The additional concentrations of infectious SARS-CoV-2 expelled by coughing ( $C_{co}$ ) and sneezing ( $C_{sn}$ ) were calculated using Equations (3) and (4), respectively. The volumetric



**Figure 1** | Outline of the QMRA steps for the scenarios associated with SARS-CoV-2 respiratory transmission in a public toilet setting.

flow of droplets from an infector’s exhalation ( $q_{br}$ ) ranged from  $5 \times 10^{-9}$  to  $6 \times 10^{-6}$   $\mu\text{L}/\text{min}$  (Schijven *et al.* 2021). In addition, the volume of aerosol droplets expelled per cough ( $V_{co}$ ) and per sneeze ( $V_{sn}$ ) was set in accordance with the results of the study by Schijven *et al.* (2021). However, the size of the droplets can play an important role in their activity. Larger droplets tend to deposit quickly, whereas smaller droplets (aerosols) can remain suspended in the air for a longer period. Thus, the volumetric ratios of aerosols to total droplets expelled ( $F$ ) were considered using a droplet size  $\leq 70 \mu\text{m}$  based on the size distribution of droplets in the literature for breathing (Fabian *et al.* 2011), coughing (Duguid 1946; Loudon & Roberts 1967; Han *et al.* 2013), and sneezing (Duguid 1946; Han *et al.* 2013; Supplementary Table S1).

$$\left\langle \text{Accumulation} \right\rangle_{\text{within system}} = \left\langle \text{Flow in through} \right\rangle_{\text{system boundary}} - \left\langle \text{Flow out through} \right\rangle_{\text{system boundary}} - \left\langle \text{Reaction} \right\rangle_{\text{within system}} \tag{1.1}$$

$$V \frac{dC}{dt} = q_{br}AR - \mu VC - q_{vent}C - q_{in}C \tag{1.2}$$

$$C_{t_1} = \frac{q_{br}AR}{(\mu V + q_{vent} + q_{in})} \left( 1 - e^{-\frac{\mu V + q_{vent} + q_{in}}{V} t_1} \right) \tag{2}$$

$$C_{co} = \frac{A V_{co} R F}{V} \tag{3}$$

$$C_{sn} = \frac{A V_{sn} R F}{V} \tag{4}$$

where the parameters related to virus generation by an infector are:  $q_{br}$  is the volumetric flow rate of droplets from an infector’s exhalation ( $\mu\text{L-droplet}/\text{min}$ );  $A$  is the virus concentrations in the genome copies per volume of droplets ( $\text{gc}/\mu\text{L-droplet}$ );

$C_{co}$  is the additional infectious virus concentrations in the air caused by a cough (PFU/L);  $C_{sn}$  is the additional infectious virus concentrations in the air caused by a sneeze (PFU/L);  $R$  is the PFU/gc ratio;  $F$  is the fraction of aerosol volume per total volume of droplets expelled (dimensionless);  $V_{co}$  is the volume of droplets expelled per cough ( $\mu\text{L}/\text{cough}$ );  $V_{sn}$  is the volume of droplets expelled per sneeze ( $\mu\text{L}/\text{sneeze}$ );  $q_{in}$  is the inhalation rate (L/min);  $q_{vent}$  is the ventilation rate (L/min) that equals  $\text{ACH} \times V/60$ ;  $V$  is the volume of air in a cubicle (3,240 L-air);  $t_1$  is infector's dwell time (min);  $\mu$  is the inactivation rate in the air at 20–70% relative humidity levels ( $\text{min}^{-1}$ ).

### Virus levels during the waiting time

The toilet was unoccupied during the waiting time ( $t_2$ ), and there was no virus input source (i.e., no infector). From Equations (1.1), (5), and (6), the infectious virus concentration decreased based on the ventilation and inactivation mechanism. The initial virus concentration ( $C_0$ ) was applied in line with the three scenarios (i.e., breathing only, breathing with a cough, and breathing with a sneeze [Equations (7)–(9)]). In this study, practical waiting times of 3, 5, and 10 min were considered. If the receptor entered the cubicle immediately after the infector exited the cubicle, the waiting time was equal to 0.

$$V \frac{dC}{dt} = -\mu VC - q_{vent}C \quad (5)$$

$$C_{t_2} = C_0 \left( e^{-\frac{\mu V + q_{vent}}{V} t_2} \right) \quad (6)$$

Given  $C_0$  based on the following specific scenarios:

$$\text{Breathing only } C_0 = C_{t_1} \quad (7)$$

$$\text{Breathing with a cough } C_0 = C_{t_1} + C_{co} \quad (8)$$

$$\text{Breathing with a sneeze } C_0 = C_{t_1} + C_{sn} \quad (9)$$

### Virus doses inhaled by the receptor

After the waiting time ( $t_2$ ), the receptor entering the toilet was exposed to an infectious virus concentration of  $C_{t_2}$ . However, this infectious virus concentration was continuously removed by ventilation, inactivation, and inhalation. During the receptor's dwell time ( $t_3$ ), the exposure to infectious virus concentrations ( $C_{t_3}$ ) was calculated using Equation (10):

$$C_{t_3} = C_{t_2} \left( e^{-\frac{\mu V + q_{vent} + q_{in}}{V} t_3} \right) \quad (10)$$

The virus doses ( $d$ ) that would be inhaled by a receptor were calculated by incorporating the inhalation rate ( $q_{in}$ ) with a definite integral of the infectious virus concentration-time function ( $C_{t_3}$ ). The limits of integration were set from the time of entering the cubicle to the receptor's dwell time ( $t_3$ ) (Equations (11.1) and (11.2)). The initial exposure concentrations ( $C_{t_2}$ ) also followed Equation (6) in line with the desired scenarios.

$$d = q_{in} \int_{t=0}^{t=t_3} C_{t_3} dt \quad (11.1)$$

$$d = \left( \frac{q_{in} V C_{t_2}}{\mu V + q_{vent} + q_{in}} \right) \left( 1 - e^{-\frac{\mu V + q_{vent} + q_{in}}{V} t_3} \right) \quad (11.2)$$

where  $t_3$  is receptor's dwell time (min) and  $d$  is SARS-CoV-2 infectious dose (PFU).

### SARS-CoV-2 dose-response models

The risk assessment was conducted by following the QMRA framework. Given the lack of dose-response information for SARS-CoV-2, the SARS-CoV data sets (Watanabe *et al.* 2010) utilized in various SARS-CoV-2 QMRA studies (Cortellessa

*et al.* 2021; Dada & Gyawali 2021; Murakami *et al.* 2021; Zaneti *et al.* 2021) were applied. The risk of infection followed the exponential model (Equation (12)):

$$P_{\text{event}} = 1 - e^{-\left(\frac{d}{k}\right)} \quad (12)$$

where  $P_{\text{event}}$  is the probability of infection per event (ppe) and  $k$  is the optimal dose-response function value of 18.54 obtained from Coronavirus 229E, which is equivalent to the chance that a single pathogen would initiate an infection response (Watanabe *et al.* 2010; Haas 2021).

### Risk characterization and sensitivity analysis

To estimate the  $P_{\text{event}}$  for a receptor exposed to SARS-CoV-2, the data from the previous steps were integrated into the MCs with 10,000 iterations for each condition using Oracle Crystal Ball software version 11.1.2.4.850. The MCs is a randomization technique that uses repeated random sampling from distributions given to key input variables in a model, including corresponding sensitivity profiles. The risk of infection was displayed in the 5th percentile, mean, and 95th percentile using a forest plot in GraphPad Prism version 7.0. A sensitivity analysis was also conducted to determine the effects of the input variables on the risk calculation.

### Risk management evaluation

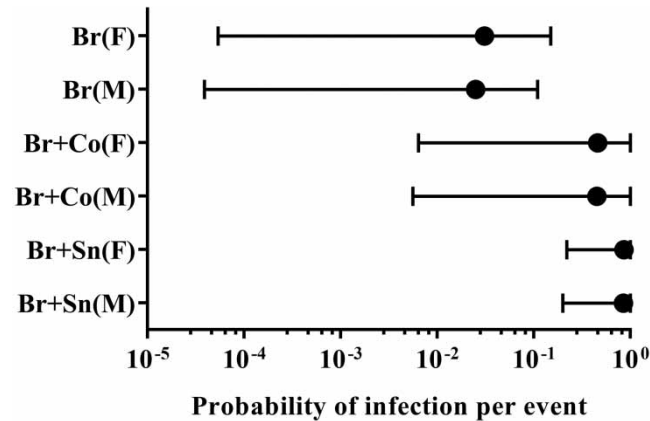
Three risk mitigation interventions were investigated: face mask wearing, ventilation, and waiting times. The universal wearing of face masks has been recommended as a low-cost and efficient means of mitigating virus transmission (WHO 2020b). Among the different types of face masks, predominantly N95 respirators and surgical and fabric masks are used worldwide. Viral filtration efficiency (VFE) characterized using a bacteriophage MS2 following the ASTM F2101-14 standard testing method has revealed 99.8–100% VFE for N95 respirators, 99.3–99.8% VFE for surgical masks, and 54.8–92.1% VFE for denim fabric masks (Whiley *et al.* 2020; Supplementary Table S1). MS2 bacteriophages were selected as the model microbes because they are two to three times smaller in size than SARS-COV-2 (70–90 nm in diameter).

Ventilation is also an important element used to control indoor air quality in public toilets. Depending on the applicable regulatory building standard, either the installation of a mechanical ventilation system or the use of natural ventilation may be necessary. The effect of air change rates on SARS-CoV-2 transmission risk was considered in this study. For the pandemic, the WHO has suggested that ventilation in indoor spaces with aerosol-generating potential be greater than or equal to 12 ACH (WHO 2021). In this study, five air change rates were tested: 0 ACH (no ventilation), 0.5 ACH (poor ventilation), 10 ACH (DIN 1946 ventilation standard for public toilets), 12 ACH (WHO-recommended standard ventilation), and 20 ACH (extreme ventilation) (Supplementary Table S1). Furthermore, the waiting times, during which the infector exited the cubicle and the receptor entered the cubicle, of 3, 5, and 10 min were considered.

## RESULTS AND DISCUSSION

### Infection risk from respiratory transmission in public toilets

The risk of infection from SARS-CoV-2 transmission through three respiratory exposure scenarios, namely breathing, breathing with a cough, and breathing with a sneeze, were characterized in this study. The probability of infection per event was not found to be significantly different between men and women ( $p > 0.05$ ; Mann–Whitney  $U$  test) across all scenarios (Figure 2 and Supplementary Table S2). Although men usually have a higher breathing rate (Stifelman 2007), which results in greater exposure to viruses, they spend on average around 22% less time in toilets than women (Gwynne *et al.* 2019), leading to a reduced risk of virus transmission. When a receptor without a protective mask was in an unventilated public toilet, the mean infection risk was  $2.5 \times 10^{-2}$  ppe for men and  $3.1 \times 10^{-2}$  ppe for women in the infector breathing scenario (Figure 2 and Supplementary Table S2). Interestingly, the risk values increased sharply when additional viral loads were expelled into the air by an infector either sneezing or coughing. Coughing and sneezing can produce saliva droplets of various sizes (Duguid 1946; Loudon & Roberts 1967; Han *et al.* 2013) and thus generate infectious virus-containing aerosols in public toilet facilities. For breathing with a cough, the mean risk was higher at  $4.5 \times 10^{-1}$  ppe for men and  $4.6 \times 10^{-1}$  ppe for women. Similarly, sneezing increased the risk of infection to  $8.5 \times 10^{-1}$  ppe and  $8.6 \times 10^{-1}$  ppe for men and women,



**Figure 2** | The risk of infection per event for a male (M) receptor and a female (F) receptor in three virus-generating scenarios: an infector breathing (Br), breathing with a cough (Br+Co), and breathing with a sneeze (Br+Sn). The forest plots show the mean values in solid circles and 90% confidence intervals (ranging from the 5th [left whiskers] to the 95th [right whiskers] percentiles).

respectively. We, therefore, showed that receptors have a high risk of infection when using an unventilated public toilet without wearing a protective mask.

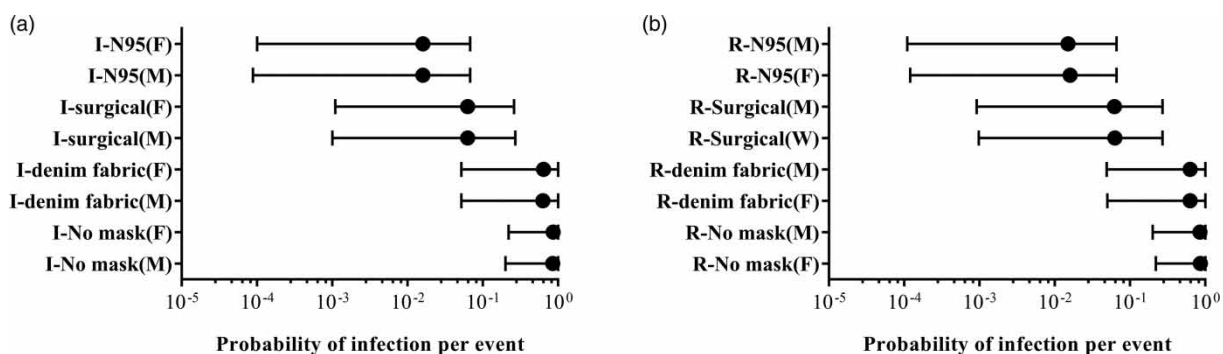
### Risk mitigation

#### Face mask wearing by either an infector or a receptor

Because it delivered the highest risk, the scenario with an infector breathing with a sneeze was selected to further evaluate the effectiveness of face mask wearing to reduce infection risk in a receptor. The probabilities of infection per event for different mask types are shown in Figure 3 and Supplementary Table S3. The highest risk reduction at an approximately 2-log was observed when an N95 respirator was worn by either an infector or a receptor. The results indicated that a high risk of virus transmission in confined spaces like public toilets is, therefore, still possible even if an N95 respirator or surgical mask is worn. This could be because the risk of infection is associated with several factors, including a high concentration of virus aerosols in the ambient air due to insufficient ventilation, long dwell times by toilet users, the low inactivation rate of SARS-CoV-2, and the leakage of air around a mask's edges (Brooks *et al.* 2021; Gwynne *et al.* 2019; Schuit *et al.* 2020; Whiley *et al.* 2020; Stabile *et al.* 2021). We also observed a reduction in risk as low as  $0.14 \cdot \log_{10}$  when an infector or receptor wore a denim fabric mask.

#### Face mask wearing by both an infector and a receptor

In a scenario with an infector breathing with a sneeze, the infection risk could be further reduced if both the infector and receptor wear masks (Supplementary Table S3). The receptor's gender did not affect their risk significantly in any of the



**Figure 3** | The risk of infection per event among male (M) and female (F) receptors in the scenario with an infector (I) breathing with a sneeze (Br+Sn) when (a) only the infector wore different types of masks and (b) only the receptor (R) wore different types of masks. The forest plots show the mean values in solid circles and 90% confidence intervals (ranging from the 5th [left whiskers] to the 95th [right whiskers] percentiles).

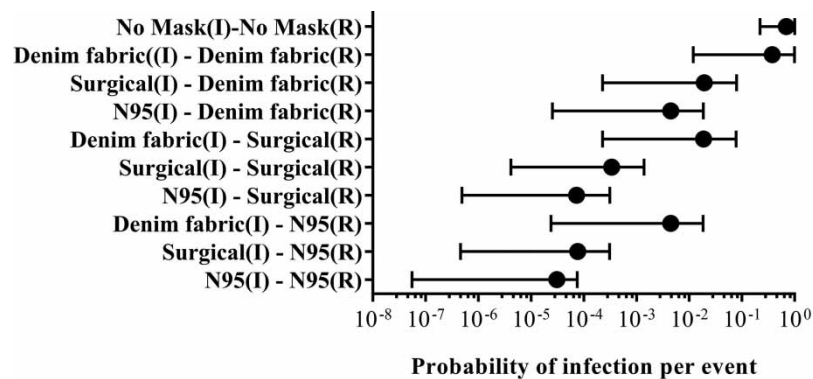
conditions. The risks to a female receptor are illustrated in Figure 4. When an infector and a receptor both wore either a surgical mask or an N95 respirator, the mean infection risk decreased by 3.4–4.7  $\log_{10}$ . With only a 0.4  $\log_{10}$  reduction, denim fabric masks may not provide sufficient protection, even when worn by both an infector and a receptor. Based on our study findings, we recommend that people, as receptors, wear either a surgical mask or an N95 respirator as personal protective equipment to minimize the risk of infection in unventilated public toilets and potentially in other confined communal spaces. In general, we found that wearing a mask was one of the most low-cost and simple yet effective intervention measures that can be used in daily life to minimize transmission risk, which is consistent with reports from other studies (Asadi *et al.* 2020b; Chu *et al.* 2020; WHO 2020b; Cheng *et al.* 2021; Goyal *et al.* 2021). Moreover, in confined communal spaces, other measures, including increasing ventilation, and extended waiting times, should be implemented to reduce the risk of infection.

### Ventilation and extended waiting times

The effects of ventilation (0–20 ACH) were characterized for three virus-generating scenarios: infector breathing, breathing with a cough, and breathing with a sneeze (Supplementary Table S4). The results showed that increasing ACH did not significantly mitigate the risk of COVID-19 infection in a public toilet setting if there was a high turnover of people using a toilet. Although a high ventilation rate has been suggested as a way to reduce the number of virus-containing droplets and aerosols in the air (Morawska *et al.* 2020; Li *et al.* 2021; Stabile *et al.* 2021; WHO 2021), reducing infectious virus concentrations to a low-risk level is time-consuming under completely mixed conditions. Moreover, the continuous expelling of the SARS-CoV-2 virus from breathing and/or sneezing by an infector without a mask appeared to be a significant cause of virus aerosol accumulation in ambient air. To exchange the clean air in a toilet cubicle, a sufficient waiting time should be considered. For example, in a scenario with an infector breathing with a sneeze and at the 12 ACH suggested by the WHO (2021), the mean probabilities of infection per event for the female receptor were as high as  $7.4 \times 10^{-1}$ ,  $6.7 \times 10^{-1}$  and  $4.7 \times 10^{-1}$  ppe for waiting times of 3, 5, and 10 min, respectively (Supplementary Figure S1). This study demonstrated that indoor ventilation alone cannot effectively reduce SARS-CoV-2 transmission risk in a public toilet setting with small cubicles and is less effective in risk reduction than face mask wearing. Another study similarly found that masks could reduce the infection risk caused by the Middle Eastern respiratory syndrome coronavirus in an indoor hospital setting better than ventilation (Adhikari *et al.* 2019). Multiple measures should, therefore, be implemented to mitigate infection risk to an acceptable level.

### Combination of face mask wearing, ventilation, and waiting time

The addition of face mask wearing was investigated for its combined effectiveness in mitigating SARS-CoV-2 transmission risk when used simultaneously with increased ventilation and extended waiting times. In the virus-generating scenario with an infector breathing with a sneeze, mask wearing by both the infector and receptor was assessed using the 12 ACH



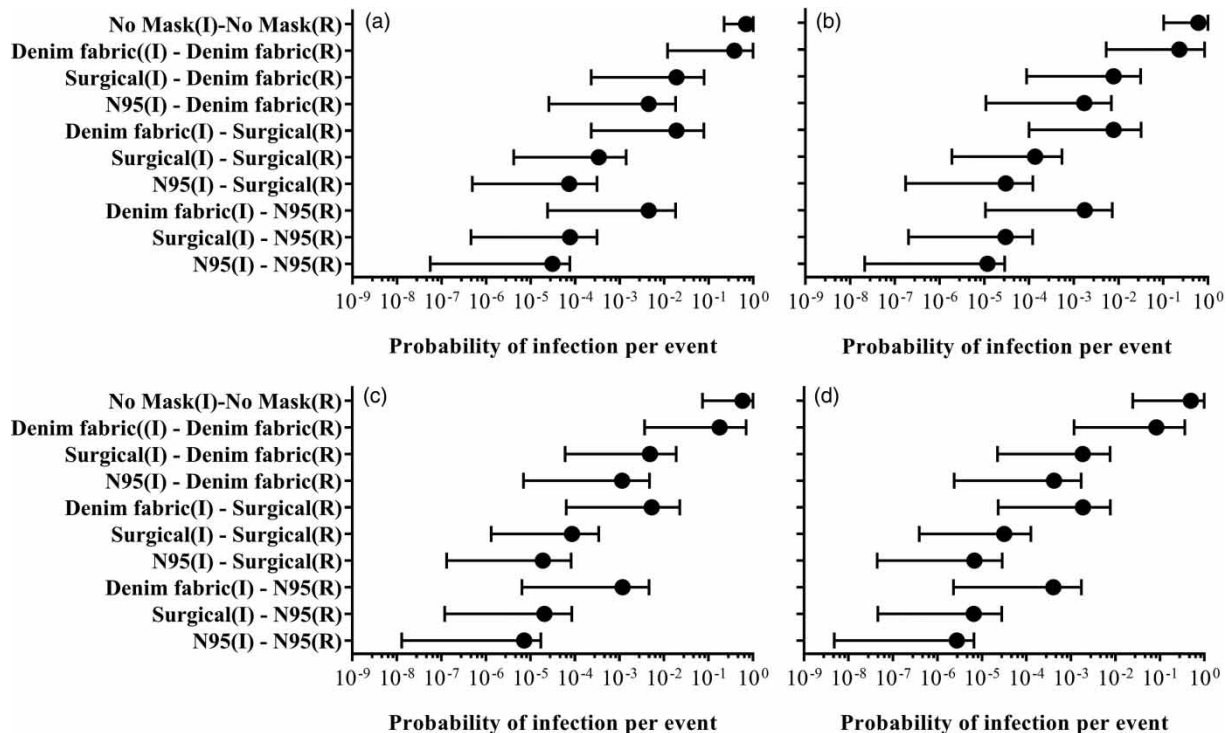
**Figure 4** | The risk of infection per event among male (M) and female (F) receptors in the scenario of no ventilation (0 ACH) with an infector (I) breathing with a sneeze (Br+Sn) and the receptor (R) wearing different types of masks. The risk of infection per event in a scenario with an infector (I) breathing with a sneeze (Br+Sn) when both the infector and receptor (R) wore different types of masks. The risks to a female receptor are represented because no gender effect was evident. The forest plots show the mean values in solid circles and 90% confidence intervals (ranging from the 5th [left whiskers] to the 95th [right whiskers] percentiles).



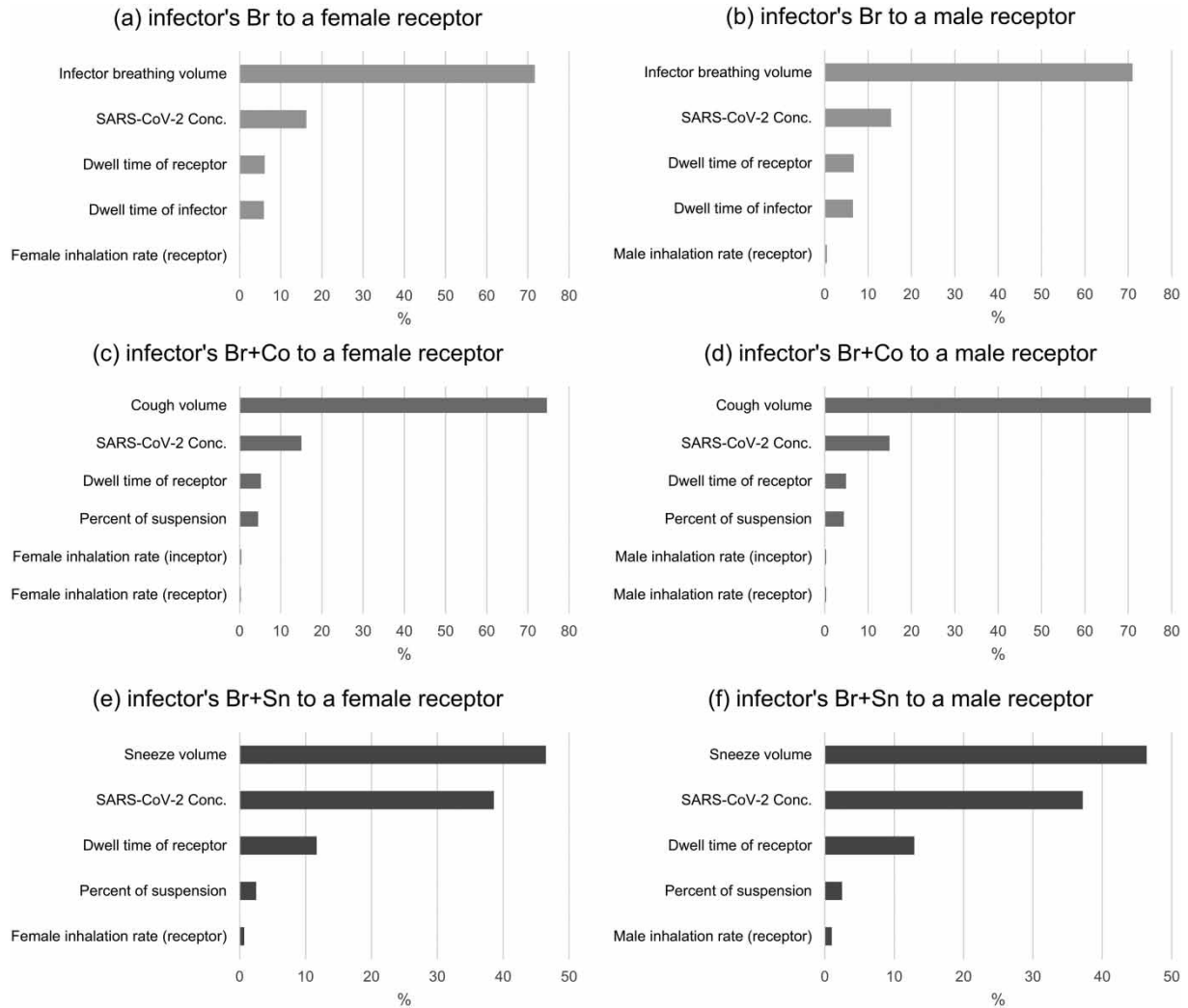
WHO-recommended value and varied waiting times of 3, 5, and 10 min (Figure 5 and Supplementary Table S5). Compared with no waiting time (Figure 5(a)), increasing the waiting time to 10 min at 12 ACH showed a 1.0- $\log_{10}$  reduction in the mean and 95th percentile infection risks in most double-mask-wearing cases. Nevertheless, a waiting time of 10 min for public toilets may not be practical in certain circumstances. Consequently, we reiterate that proper ventilation did not impact the risk mitigation for SARS-CoV-2 transmission in a public toilet setting, especially in confined toilet cubicle conditions. Face mask wearing should, therefore, be promoted as a normal practice when entering public indoor spaces. Moreover, wearing a more tightly fitting mask is also critical for risk mitigation because it can reduce leakage at the mask's edges (Brooks *et al.* 2021).

### Sensitivity analysis

A sensitivity analysis of the QMRA was conducted to identify the input variables that most contributed to the risk estimation. An infector's expelled volumes and concentrations of SARS-CoV-2 in droplets (saliva and mucus) play an important role in infection risk. For all three transmission scenarios, namely an infector breathing (Br), breathing with a cough (Br+Co), and breathing with a sneeze (Br+Sn), the infector's expelled volume ( $\mu\text{L}$ ) of the virus (breathing, coughing, and sneezing volumes) was the most sensitive parameter, accounting for 71.7, 74.6, and 46.5% contribution in the probability of infection in women, respectively (Figure 6 and Supplementary Table S6). The second-most sensitive parameter was concentrations of the SARS-CoV-2 virus in  $\text{gc}/\mu\text{L}$  droplets (saliva and mucus), which accounted 16.2, 15.0, and 38.6% in women, respectively. The other parameters, namely a receptor's dwell time, an infector's dwell time, and the percentage of droplet suspension, were minor parameters in terms of sensitivity (Figure 6 and Supplementary Table S6). Since breathing with a sneeze was the highest virus-generating risk scenario, a sensitivity analysis was performed in which both the infector and receptor wore masks (Supplementary Table S7). Virus concentrations in  $\text{gc}/\mu\text{L}$ , sneeze volume, and the receptor's dwell time were the three parameters that most influenced infection risk. Since controlling for virus concentrations and an infector's expelled volumes are a challenge, particularly among asymptomatic patients, individuals should avoid spending prolonged time in closed indoor settings (Dancer *et al.* 2021; Stabile *et al.* 2021).



**Figure 5** | The risk of infection per event in a scenario with an infector (I) breathing with a sneeze (Br+Sn) when both the infector and receptor (R) wore different types of masks. The risks to a female receptor are represented because no gender effect was evident. The influence of waiting times of (a) 0 min, (b) 3 min, (c) 5 min, and (d) 10 min at 12 ACH are displayed. The forest plots show the mean values in solid circles and 90% confidence intervals (ranging from the 5th [left whiskers] to the 95th [right whiskers] percentiles).



**Figure 6** | The sensitivity analysis representing the contribution of input variables to the risk of infection per event for male and female receptors with no ventilation and no mask wearing in three transmission scenarios, namely with the infector breathing (Br), breathing with a cough (Br+Co), and breathing with a sneeze (Br+Sn): (a) infector's Br to a female receptor, (b) infector's Br to a male receptor, (c) infector's Br+Co to a female receptor, (d) infector's Br+Co to a male receptor, (e) infector's Br+Sn to a female receptor, and (f) infector's Br+Sn to a male receptor.

### Limitations of this study and future perspectives

While this study evaluated the risk of SARS-CoV-2 transmission according to the QMRA framework, its limitations and uncertainties should be carefully acknowledged. SARS-CoV-2 concentrations in  $\text{gc}/\mu\text{L}$ , the most sensitive parameter affecting the calculation of risk, are subject to natural variations in the saliva and mucus of infected patients (Azzi *et al.* 2020; Wölfel *et al.* 2020). In this study, 251 swab test  $C_t$  values were used to represent the virus levels in Thai patients. Due to the lack of a standard curve from Thai hospital laboratories, we used a published standard curve of the N2 gene (Sherchan *et al.* 2020) to estimate the virus concentrations in this study. However, heterogeneity in published standard curves for SARS-CoV-2 has been observed (Bivins *et al.* 2021). Moreover, variations in technical and laboratory analyses (e.g., data analysis methods and control materials) could intensify bias, leading to variability in the calculated virus concentrations (Kongprajug *et al.* 2020; Bivins *et al.* 2021). Adhering to standards and quality control measures is, therefore, highlighted to support data sharing and referencing in future research, especially for emerging infectious diseases. Notwithstanding, even with consideration of the aforementioned uncertainties, the calculated virus concentrations in droplets used in this study, which ranged from  $4.4 \times 10^{-1}$  to  $6.4 \times 10^8$   $\text{gc}/\mu\text{L}$ , were in agreement with those of Schijven *et al.* (2021).

We chose to evaluate three virus-generating scenarios: an infector breathing, breathing with a cough, and breathing with a sneeze. However, infectors may sneeze and/or cough more than once depending on the individuals' symptoms, which could increase the risk of infection. Coughing is the predominant symptom in COVID-19 (Wang *et al.* 2020), rendering coughing potentially more important than sneezing. Nevertheless, some studies have suggested that the airborne transmission of infectious diseases is possible without coughing or sneezing and simply through the exhaled breath of individuals with few symptoms (Asadi *et al.* 2020a). In addition, the lack of dose-response information and infection risk benchmarks for SARS-CoV-2 poses a challenge when evaluating the infection risk of this virus. We assumed that the dose-response of SARS-CoV-2 was similar to that indicated in the SARS-CoV data from Haas (2021) study, which refers to Coronavirus 229E. With the recent emergence of various SARS-CoV-2 variants, much remains unknown regarding the behavior and characteristics of this virus. In this study, we used the available inactivation coefficients of SARS-CoV-2 at 20 °C (Schuit *et al.* 2020), which could have overestimated the calculated risks in Thailand given its average daily temperature of 27 °C (Denpetkul & Phosri 2021).

Even though virus protection efficiencies using different mask types are important for risk protection, we excluded the factor of leakage from a mask's edges due to the different methods of mask wearing (i.e., double masks, knotted and tucked masks). Brooks *et al.* (2021) indicated that mask wearing methods play a role in mask protection efficiencies. In their study, wearing a surgical mask alone blocked 56.1% of the particles from a cough, while a cloth mask alone blocked 51.4% of such particles. Meanwhile, a knotted and tucked surgical mask blocked 77.0% of cough particles, and double masking blocked 85.4%. Leakage based on wearing methods should be included in future risk assessments.

The scope of this study excluded the risk of SARS-CoV-2 respiratory transmission potentially produced by toilet flushing, as well as other transmission risks (e.g., direct splashing and surface transmission) (Verani *et al.* 2014). As evident from the study by McKinney *et al.* (2006) on SARS-CoV-1 in-building transmission in Hong Kong, it must be emphasized that well-designed and standard maintenance procedures for the plumbing and ventilation systems in toilets could play an important role in controlling infectious droplets. By integrating all the known risk sources, comprehensive knowledge regarding risk estimation could be achieved to accurately inform public health policies and help further reduce transmission risk. It is apparent that research related to SARS-CoV-2 is continuing, and additional data will greatly benefit future studies that aim to better understand its characteristics. The QMRA-based risk models developed in this study could facilitate future risk assessments through modifications for particular risk scenarios and the updating of the input parameters based on newly available data. Such improved risk models will be crucial tools in assessing the impact of different risk mitigation strategies during the COVID-19 and future pandemics.

## CONCLUSION

Indoor public toilet facilities could be hubs of virus transmission during the COVID-19 pandemic. This study investigated the risk of the airborne transmission of SARS-CoV-2 in public toilets for three virus-generating scenarios: an infector breathing, breathing with a cough, and breathing with a sneeze. The risk analysis, which followed the QMRA framework, revealed that the highest risk was when an asymptomatic or symptomatic infector sneezed. Both genders were found to be exposed to similar risks. A combination of measures that includes suitable ventilation, extended waiting times, and mask wearing should be applied as an effective intervention for public toilet users.

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## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest in this work. This study was approved by the Ethics Committee of the Faculty of Tropical Medicine, Mahidol University, Thailand (Ethical number MUTM 2021-060-01).

## DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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