What are the disease burden and its sensitivity analysis of workers exposing to *Staphylococcus aureus* bioaerosol during warm and cold periods in a wastewater treatment plant?

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**Abstract**

Biological treatment in wastewater treatment plants releases high amounts of pathogenic bioaerosols. Quantitative microbial risk assessment is a framework commonly used for quantitative risk estimation for occupational exposure scenarios. However, the quantitative contributions of health-risk-estimate inputted parameters remain ambiguous. Therefore, this research aimed to study the disease burden of workers exposed to *Staphylococcus aureus* bioaerosol during warm and cold periods and strictly quantify the contributions of the inputted parameters by sensitivity analysis on the basis of Monte Carlo simulation. Results showed that the disease health risk burden of workers in the warm period was 1.15–6.11 times higher than that of workers in the cold period. The disease health risk burden of workers without personal protective equipment was 23.83–36.55 times higher than that of workers with personal protective equipment. Sensitivity analysis showed that exposure concentration and aerosol ingestion rate were the first and second predominant factors, respectively; the sensitivity partitioning coefficient of the former was 1.17–1.35 times the value of the latter. In addition, no remarkable differences were revealed in the sensitivity percentage ratio between warm and cold periods. The findings could contribute to the mitigation measures for the management of public health risks.

**Keywords** Quantitative microbial risk assessment · Monte Carlo simulation · Disease health risk burden · Sensitivity ranking

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**Introduction**

Biological treatment is the most predominant treatment process in wastewater treatment plants (WWTPs), and it is one of the main sources of bioaerosols (Kowalski et al. 2017; Thakur et al. 2018; Han et al. 2019; Han et al. 2020a). Large numbers of microorganisms exist in wastewater and sludge (Uhrbrand et al. 2017; Kozajda et al. 2019). The indispensible oxygen supply (i.e., microporous and mechanical aeration) during the biological treatment process results in the production and release of high amounts of bioaerosols carrying various pathogens (Han et al. 2019; Han et al. 2020b; Tian et al. 2020). Workers in WWTPs encounter potential health risk from these pathogenic bioaerosols, mainly through inhalation (Upadhyay et al. 2013; Masclaux et al. 2014; Han et al. 2020a). Therefore, bioaerosol risk characteristics and measures to reduce risk in WWTPs have attracted increasing attention.

However, at present, no dedicated risk control standards or strategies are available for bioaerosols in WWTPs...
(WHO 2009; Gollakota et al. 2021), because unquantifiable variable parameters are involved in the health risk assessment process. According to prior studies, the health risks of workers in WWTPs could be remarkably reduced by equipping them with personal protective equipment (PPE) (Haas et al. 2017; Zaneti et al. 2021; Nag et al. 2021). In addition, bioaerosol concentrations relating to health risks in WWTPs could be influenced by meteorological parameters, such as temperature, relative humidity, and solar illumination, in different seasons (Zhai et al. 2018; Singh et al. 2020; Nag et al. 2021) and aeration modes, such as mechanical and microporous aeration modes (Sánchez-Monedero et al. 2008; Han et al. 2019). Therefore, sensitivity analysis should be used to quantify the contributions of inputted aforementioned variable parameters to health risk assessment and then determine the most influential parameter (Saltelli et al. 2019; Dean and Mitchell 2020). Xu et al. (2020) reported sensitivity analysis for the health risks of WWTP staff exposed to Legionella pneumophila. Dean and Mitchell (2020) performed sensitivity analysis of subjects exposed to Pseudomonas aeruginosa when showering. Nag et al. (2021) conducted sensitivity analysis of health risk assessment of Escherichia coli in bioaerosols generated from land application of farmyard slurry. Meanwhile, Monte Carlo simulation could be used to perform multiple random samplings and iterative computations for sensitivity analysis (de Matos et al. 2020; Xu et al. 2020). For instance, Shi et al. (2018) used Monte Carlo simulation to build a probabilistic-based risk model. Dean and Mitchell (2020) used 10,000 Monte Carlo iterations in the calculation for QMRA. Nag et al. (2021) estimated human exposure by using a Monte Carlo simulation approach.

Quantitative microbial risk assessment (QMRA) is a framework intended for the quantitative estimation of health risks in occupational exposure scenarios (Haas 2015; Haas et al. 2017; Seis et al. 2020). It is carried out as follows: (i) hazard identification, (ii) exposure assessment, (iii) dose–response assessment, and (iv) risk characterization (Shi et al., 2018). Dose–response assessment is typically carried out through the exponential dose–response model, while risk characterization is performed to assess the health risk by disease burden (Haas 2015; Esfahanian et al. 2019). Disease burden is evaluated by the acceptable disability-adjusted life years (DALYs), as proposed by World Health Organization, and it is the most authoritative and widely-used health risk benchmark (≤ 10−6 DALYs per person per year [pppy]) (WHO 2008; Shi et al. 2018).

Moreover, quantitative analysis of disease burden and health risk for works among different season periods has been studied inadequately, and the literature above failed to explore bioaerosol QMRA sensitivity analysis among different aeration modes. Therefore, the present study aimed to determine the disease burden of workers exposed to Staphylococcus aureus (S. aureus) bioaerosol by using the exponential dose–response model in a WWTP under mechanical aeration and microporous aeration modes during warm and cold periods. Then, the contributions of the inputted parameters of disease burden were strictly quantified to determine the most influential variable parameter by Monte Carlo simulation. This research could further deepen the understanding of the dynamic uncertainty analysis under two extremely different meteorological conditions (warm and cold periods). The findings could also contribute effort to the establishment of mitigation measures and control strategies for the management of public health risks, including exposure to bioaerosols in local utilities.

### Materials and methods

#### WWTP description

This study was performed at a WWTP built in 2014 and located in China. The collected domestic wastewater is distributed into the WWTP by a series of variable-frequency pump stations. The WWTP maintains two independently operated parallel phases, and it is equipped with a rotating-disc aeration tank (mechanical aeration mode) for phase I and a microporous aeration tank (microporous aeration mode) for phase II (Fig. 1). The treatment capacity of phases I and II is 50,000 tons per day.

#### Sampling and analysis

### Sampling procedure

An Anderson six-stage impactor was used to collect S. aureus bioaerosol samples with different size ranges (Byeon et al. 2008; Kowalski et al. 2017). The sampling campaign was conducted on September 20th, September 27th, December 7th, and December 16th of 2020. Samples were taken in the rotating-disc aeration tank and microporous aeration tank at the same time, and each sampling was repeated thrice. The sampling site was located in the middle of the corridor of each aeration tank (Fig. 1). At each sampling site, the Anderson six-stage impactor was mounted at 1.5 m above the ground level of the corridor, and the impactor was operated at a flow rate of 28.3 L/min (Hung et al. 2010; Kowalski et al. 2017). The impactor was sterilized with 75% alcohol pads before and after each sampling to prevent contamination. In addition, sampling conditions, such as temperature, relative humidity, and solar illumination, were recorded (Supplementary Material Table 1). The temperature for warm and cold period was divided as 25–35°C and 5–15°C, respectively (Supplementary Material Table 1). All samples were transported to the lab immediately in a cold box and analyzed within 48 h (Pascual et al. 2003).
Bioaerosol analysis

The media used in the Anderson six-stage impactor for sampling airborne *S. aureus* was egg-yolk mannitol salt agar base (Qingdao Hope Bio-Technology Co., Ltd). The preparation and cultivating methods followed the standard procedures and previous publications (Pascual et al. 2003; Grzyb and Lenart-Boroń 2019). The samples were enumerated as colony-forming unit (CFU) by using an automatic colony enumeration instrument (HICC-B, Wanshen Inc., Hangzhou, China). Only plates containing colonies less than 300 were counted to prevent colony masking due

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Table 1 Calculation parameters of quantitative microbial risk assessment

| Items      | Description                                      | Units       | Values                                      | References                        |
|------------|--------------------------------------------------|-------------|---------------------------------------------|-----------------------------------|
| *C*        | Exposure concentration                          | CFU·m⁻³     | Table 2                                    |                                   |
| *BR*       | Breathing rate                                  | m³·d⁻¹      | Uniform distribution (Min = 7.2; Max = 15.7) | Wang et al. 2018                  |
| *AG*       | Aerosol ingestion rate                          | %           | Uniform distribution (Min = 10; Max = 50)   | Jahne et al. 2015                  |
| *T*        | Exposure time                                   | h·d⁻¹       | Uniform distribution (Min = 0.50; Max = 1)  | Field investigation               |
| *P_(d)*   | Exponential dose–response model                 | Unitless    | *k* = 8.05E-08                             | Esfahanian et al. 2019            |
| *FPPE*     | Removal fraction by employing personal protective equipment | Unitless | Uniform distribution (Min = 0.95; Max = 0.99) | Rosenstock 1995; Cyprowski et al. 2008; Mark et al. 2020 |
| *n*        | Exposure frequency                              | d·year⁻¹    | 250                                         | Field investigation               |
| *P_(inf)* | Prevalence                                      | Unitless    | 1                                           | Busgang et al. 2018               |
| *HB*       | Health burden                                   | DALYs·case⁻¹| *HB* = 0.0026                               | Havelaar et al. 2012              |
to overloading (Hung et al. 2010). The actual number of colonies in each sample was corrected by the positive-hole method (Andersen 1958; Carducci et al. 2018; Han et al. 2020b). All the results of bioaerosol concentrations were expressed as the number of CFU per cubic meter of air (CFU m⁻³) (Yang et al. 2019).

QMRA framework

QMRA was carried out following the classical four-step framework (Haas 2015; Haas et al. 2017). All details and explanations are shown in Table 1 and the Supplementary Material. S. aureus was popularly confirmed as the most frequently detected, widely distributed, and extensively studied potential hazard in most bioaerosol samples (Kozajda et al. 2019). Therefore, for hazard identification, the indicator pathogen of concern in the present study was S. aureus bioaerosol emitted from the two aeration tanks.

The workers in this study were equipped with a wide range of models of PPE, including N-95 and N-99, and other types whose efficacy was presumed to be between that of N-95 and N-99. The dose of the inhaled bioaerosol was analyzed in accordance with previous literature (Rosenstock 1995; Cyprowski et al. 2008; Mark et al. 2020). The exponential dose–response model was utilized for the S. aureus bioaerosol (Esfahanian et al. 2019). The daily infection risk, annual infection risk, and disease burden were estimated.

Monte Carlo simulation and sensitivity analysis

All inputted variable parameters (exposure concentration, aerosol ingestion rate, removal fraction by employing PPE [FPPE], exposure time, and breathing rate) were randomly selected from their corresponding probability distributions (Tables 1 and 2). Then, the disease burden was calculated with 10,000 iterations, as shown in Supplementary Material Eq. (5). Sensitivity analysis was used to quantify the contributions of all QMRA inputted variable parameters to the uncertainty in health risk output (the disease burden) (Saltelli 2002; Dias et al. 2019; Saltelli et al. 2019). The quantification contribution (the sensitivity partitioning coefficient) was calculated by using the Spearman rank correlation coefficient in Oracle Crystal Ball (Dean and Mitchell 2020).

Results and discussion

The mean concentration and standard deviation of exposure bioaerosol under mechanical and microporous aeration mode during warm and cold periods are shown in Table 2.

Characterization of disease health risk burden

Figure 2 demonstrates the disease burden with the first and third quartiles (25th and 75th percentiles), the mean value (general condition), the 2.5th percentile (optimistic estimate at the best situation), and the 97.5th percentile (conservative estimate at the worst situation). They were all compared with the World Health Organization (WHO) benchmark (≤ 10⁻⁶ DALYs pppy).

The disease health risk burden of the workers (with or without PPE) in the warm period was constantly one order of magnitude higher than that in cold period under all estimate situations, and the disease health risk burden in the warm period was 1.15–6.11 times higher than that in cold period (on general condition). This result could be attributed to the higher bioaerosol concentrations in the warm period (Table 2). Niazi et al. (2015) and Singh et al. (2020) stated a similar result, that is, the average concentration of bacterial bioaerosols in summer (warm period) was considerably higher than that in winter (cold period) for the aeration tank in a WWTP. In general, the high temperature and relative humidity in warm period during the sampling campaign of the present research (Supplementary Material Table 1) supported the survival of bioaerosols (Dehghani et al. 2018; Han et al. 2018, 2019; Singh et al. 2020).

All disease health risk burden in the rotating-disc aeration mode was regularly consistently higher by one order of magnitude higher than that in the microporous aeration mode. Literature revealed that the mechanical aeration mode

| Table 2 | Sampling log (CFU·m⁻³) |
|---------|------------------------|
| Periods | Sampling site          | Sampling time (minute) | Exposure concentration (CFU·m⁻³) |
| Warm period | Rotating-disc aeration tank | 5 | Lognormal distribution (mean = 4840.49; Std. Dev. = 3015.88) |
|          | Microporous aeration tank | 5 | Lognormal distribution (mean = 270.52; Std. Dev. = 168.34) |
| Cold period | Rotating-disc aeration tank | 5 | Lognormal distribution (mean = 723.04; Std. Dev. = 437.66) |
|          | Microporous aeration tank | 5 | Lognormal distribution (mean = 118.53; Std. Dev. = 66.52) |
(i.e., surface aeration) differs from the microporous aeration mode (i.e., subsurface aeration), exerts strong shear forces, and constantly generates a high concentration of bioaerosols (Sánchez-Monedero et al. 2008; Li et al. 2016; Han et al. 2019; Bidaki et al. 2019; Burdsall et al. 2020). A study by Ochoxiak and his group showed that the increase in aeration intensity increased the number of bubbles generated per unit time and resulted in the decreased number of large droplets and the increased number of small droplets (Ochowiak and Matuzsak 2017; Wang et al. 2019). Han et al. (2020c) found the same phenomenon for total microorganisms in a WWTP, that is, mechanical aeration mode emitted more bioaerosols than microporous aeration mode and had higher ratio of bacteria. In the rotating-disc aeration mode, the higher bioaerosol concentration contributed to the higher exposure dose and bioaerosol-relative health risk calculated in the QMRA (Dean and Mitchell 2020; Han et al. 2020b). Therefore, reducing bioaerosol emission and switching from mechanical aeration mode to microporous aeration mode are promising strategies (Sánchez-Monedero et al. 2008; Fathi et al. 2017; Han et al. 2019; Burdsall et al. 2020). Literature confirmed this finding. Wang et al. (2019) found that the accelerated aeration rate may create more bioaerosols and thus require a series of protective measures. Carducci et al. (2018) suggested changing the aeration system type applied in the biological treatment to reduce bioaerosol emissions in WWTPs.

For workers without PPE in rotating-disc aeration tank, their disease health risk burden in warm period was unacceptable in accordance with all the conditions in the WHO benchmark. Meanwhile, under the optimistic estimate, their disease health risk burden in cold period satisfied the benchmark. The disease health risk burdens at the worst situation were 23.10 and 14.64 times higher than those at the best situation in warm and cold periods, respectively. For workers without PPE in microporous aeration tank, the disease health risk burdens in warm period under general condition and in cold period under the conservative estimate were on the same order of magnitude as the benchmark. On the contrary, the disease health risk burdens under the conservative estimate were 19.71 and 16.85 times higher than those under the optimistic estimate in warm and cold periods, respectively.

For workers with PPE, their disease health risk burden satisfied the WHO benchmark in the two aeration tanks, except for rotating-disc aeration tank in warm period at the worst situation, wherein the disease health risk burden for workers was 26.54–33.23 times higher than that at the best situation. In addition, the disease health risk burden was consistently lower by one or two orders of magnitude than that in workers without PPE in both periods. The disease health risk burden for workers without PPE was 23.83–36.55 times higher than that workers with PPE (on general condition). Several literatures agreed with these results and recommended equipping PPE to reduce health risks (Teixeira et al. 2013; Carducci et al. 2018; Han et al. 2020c). Zaneti et al. (2021) suggested that workers in WWTP, who execute manual cleaning of screening, should use face protective masks and shields during COVID-19 outbreaks. Nag et al. (2021) mentioned PPE could reduce the exposure
occupational risk to workers who operate slurry tanks. Therefore, disease health risk burden could be remarkably mitigated by equipping workers with PPE.

**Sensitivity analysis of the results of disease health risk burden**

Figure 3 displays the sensitivity partitioning coefficient of parameters in the two aeration tanks in warm and cold periods. Figure 4 demonstrates the sensitivity percentage ratio of each sensitivity parameter in each exposure scenario.

The sensitivity analysis indicated that the exposure concentration and aerosol ingestion rate were the first and second predominant factors for the estimated risk in all exposure scenarios, respectively (Fig. 3). The sensitivity partitioning coefficient of exposure concentration was 1.17–1.35 times the value of the aerosol ingestion rate (Fig. 3), because the degree of dispersion (standard deviation) of bioaerosol concentration was more than 50% of its mean value in all exposure scenarios. This phenomenon led to the highest sensitivity ranking of exposure concentration. Therefore, decreasing the exposure concentration is one of the most important methods to lessen the disease health risk burden in theory. However, the high degree of dispersion of bioaerosol concentration may lead to an overestimation or underestimation of the bioaerosol-linked disease health risk burden and its sensitivity ranking in this study, because the parameter bioaerosol concentration straightly affects the health risk assessment (Xu et al. 2020). Besides, controlling the aerosol ingestion rate, which is highly affected by breathing pattern, is an effective means to mitigate the disease health risk burden (Stuart 1984; Warren et al. 1988; Lim et al. 2015). Shi et al. (2018) reported that nasal breathers had higher infection risks than habitual oral breathers by approximately two orders of magnitude. Therefore, health risk mitigation may be achieved by effective oral breathing.

However, the sensitivity of FPPE ranked third in the contribution to disease health risk burden. The sensitivity partitioning coefficient of FPPE was 0.03 lower (by absolute value) than the aerosol ingestion rate in all exposure scenarios, and they showed almost the same sensitivity partitioning coefficients (by absolute value) and sensitivity percentage ratios (Figs. 3 and 4b). This result further demonstrated that wearing PPE could largely reduce the risk (Haas et al. 2017; Carducci et al. 2018) and proved that it is another effective strategy to mitigate the disease health risk burden.

In addition, breathing rate and exposure time showed an alternating regularity in the two periods. In the rotating-disc aeration tank, the breathing rate ranked ahead of the exposure time in warm period, while the exposure time ranked ahead of the breathing rate in cold period. The opposite was true for the microporous aeration tank. However, in the study of Xu et al. (2020), the single exposure duration and annual exposure frequency were calculated to be the most two critical parameters for the infection risk and illness risk in sensitivity analysis. This finding was different from the results of the present research, which was related to the different exposure scenarios. Wang et al. (2019) found that the intake (i.e., breathing rate) of adult males was commonly higher than that of adult females, and so did the exposure hazard risk. Zaneti et al. (2021) suggested to reduce the work frequency and duration of workers in WWTPs and such occupational exposure areas. The authors of literature mentioned above all proposed that certain control measures should be taken to reduce risks of exposure and protect workers in WWTPs, consistent with the claim of the current research.

Referring to the sensitivity percentage ratio in one particular aeration mode, no remarkable differences were revealed between warm and cold periods (Fig. 4). This finding may be due to the same inputted values and distribution patterns of these sensitivity parameters, except the exposure concentration (Table 2). Therefore, the disease health risk burdens in warm and cold periods are equally important and thus deserve corresponding attention from stakeholders. Gao et al. (2021) reported similar results and emphasized the non-negligible role of seasonal research. Seasonal studies are crucial to capture the longstanding implication of bioaerosol exposure to comprehensively evaluate the airborne aerosol risk exposure.

**Conclusion**

The disease health risk burden of workers exposed to bioaerosol in warm period was constantly 1.15–6.11 times higher than that in cold period (on general condition). The disease health risk burden in the rotating-disc aeration mode was regularly consistently higher by one order of magnitude than that in the microporous aeration mode. For workers without PPE in the rotating-disc aeration tank, their disease health risk burden in warm period was

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**Fig. 3** Tornado graphs of the ranking of sensitivity partitioning coefficient of input sensitivity parameters that affect the output value for workers exposed to *Staphylococcus aureus* bioaerosol in the wastewater treatment plant, referring to a workers without PPE in rotating-disc aeration tank in warm period, b workers with PPE in rotating-disc aeration tank in warm period, c workers without PPE in microporous aeration tank in warm period, d workers with PPE in microporous aeration tank in warm period, e workers without PPE in rotating-disc aeration tank in cold period, and f workers with PPE in microporous aeration tank in cold period. g workers without PPE in microporous aeration tank in cold period, and h workers with PPE in microporous aeration tank in cold period. RD, rotating-disc aeration tank; M, microporous aeration tank; C, exposure concentrations; T, exposure time; AG, aerosol ingestion rate; BR, breathing rate; FPPE, removal fraction by employing PPE; PPE, personal protective equipment.
unacceptable in accordance with all the conditions from the WHO benchmark. For workers without PPE in the microporous aeration tank, the disease health risk burden in warm period under general condition and in cold period under the conservative estimate were on the same order of magnitude as the benchmark. For workers with PPE, their disease health risk burden all satisfied the WHO benchmark in the two aeration tanks, except for the rotating-disc aeration tank in warm period at the worst situation. The disease health risk burden was consistently lower by one or two orders of magnitude than that for workers without PPE in both periods. The disease health risk burden for workers without PPE was 23.83–36.55 times higher than that for workers with PPE (on general condition). The sensitivity analysis indicated that exposure concentration and aerosol ingestion rate were the first and second predominant factors for the estimated risk in all exposure scenarios, respectively. The sensitivity partitioning coefficient of exposure concentration was 1.17–1.35 times higher than the value of the aerosol ingestion rate. However, the sensitivity of FPPE ranked third in the contribution to disease health risk burden. Therefore, decreasing exposure concentration is the most preferred in theory to lessen the health risk. In addition, no notable differences were discovered in the sensitivity percentage ratio between warm and cold periods, revealing that seasonal variation equally contributed to the health risk. This research systematically further delivered novel data on the sensitivity analysis of quantitative health risk assessment framework in one WWTP for comparing exposure to bioaerosols in warm and cold periods. This sensitivity analysis study could provide a theoretical basis for follow-up research on the mitigation measures and then assist local utilities in understanding control strategies for bioaerosol exposure.

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Declarations

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