

Characteristics, fate, and effects of *Helicobacter pylori* in municipal wastewater treatment plant

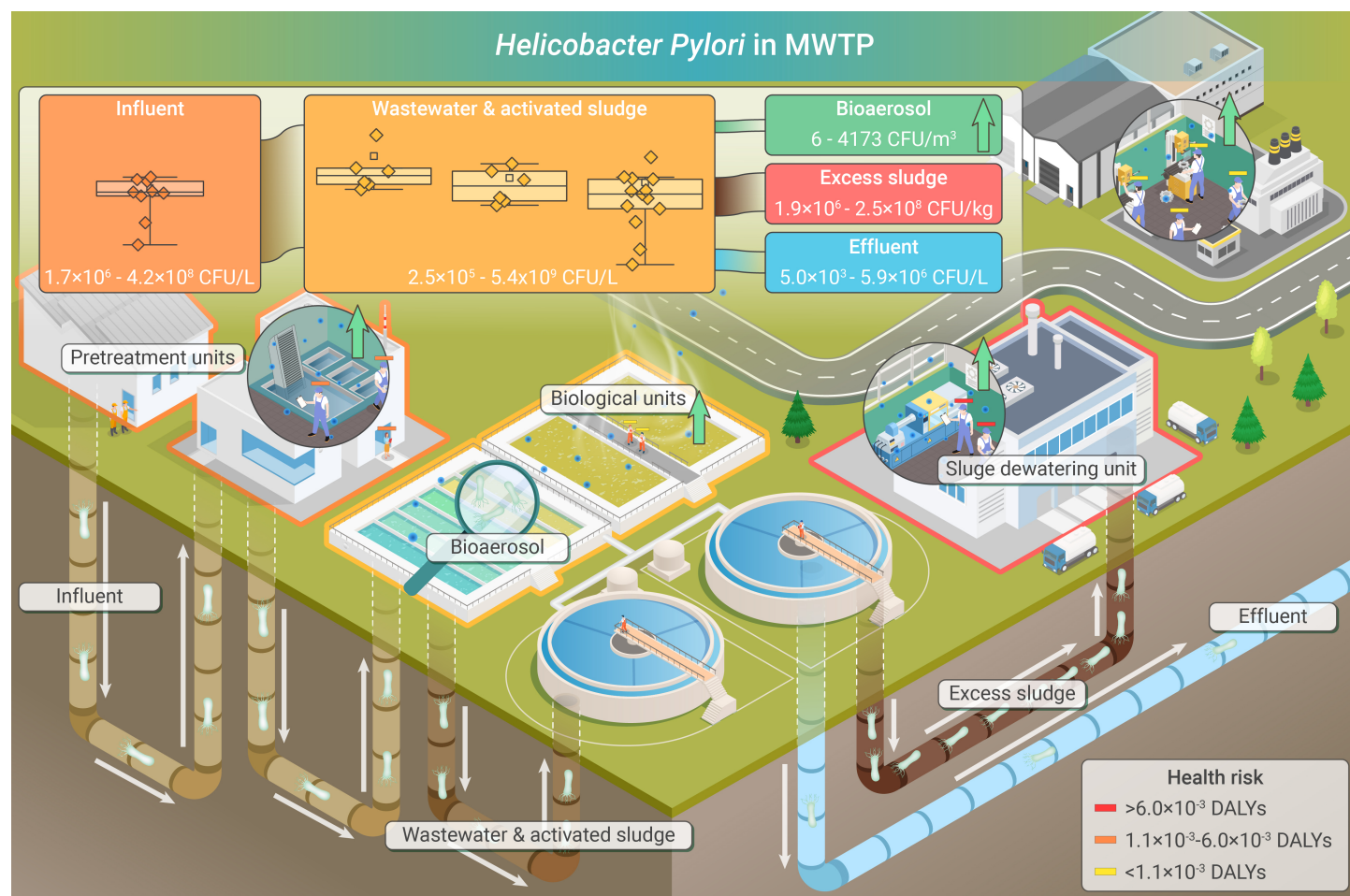
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GRAPHICAL ABSTRACT



PUBLIC SUMMARY

- *H. pylori* was detected throughout the wastewater treatment process.
- Most of the *H. pylori* in wastewater was transferred to the excess sludge.
- *H. pylori* can be released into the air as bioaerosols during wastewater treatment.
- Dispersion of airborne *H. pylori* affected the ambient air quality.
- *H. pylori* infection risk existed in all sections of the wastewater treatment plant.

Characteristics, fate, and effects of *Helicobacter pylori* in municipal wastewater treatment plant

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Helicobacter pylori (*H. Pylori*) is a highly pathogenic bacterium that can be transmitted through various means, including wastewater, which serves as both a source and sink for *H. pylori*. In the wastewater treatment process, the exposure risk of *H. Pylori* escaping through bioaerosols is easily overlooked. In this study, the pollution characteristics and variation patterns of *H. Pylori* in wastewater, sludge, and air medium were systematically investigated in a typical municipal wastewater treatment plant (MWTP) applying anaerobic-anoxic-aerobic process through long-term sampling. The results indicated a significant reduction of *H. Pylori* in wastewater and accumulation in excess sludge following the wastewater treatment process. *H. Pylori* was also detected in bioaerosols due to its escape during wastewater treatment and sludge dewatering. The highest detection concentrations of *H. Pylori* in the air were found in the sludge dewatering house. O₃, solar radiation, ambient temperature, and wind speed all had negative effects on the relative abundance and respirable proportion of airborne *H. pylori*. In addition, *H. Pylori* in bioaerosols had the potential to contaminate the ambient environment and posed a potential health risk to operational workers. This study will help to recognize the pollution of *H. Pylori* in MWTPs and raise the attention to occupational exposure among MWTP workers.

INTRODUCTION

Helicobacter pylori (*H. Pylori*) is a spiral-shaped, Gram-negative bacterium that adheres to the gastric mucosa and intercellular spaces. Owing to its high pathogenicity, *H. pylori* is listed as a Class I carcinogen by the World Health Organization (WHO).¹ *H. pylori* causes chronic and persistent bacterial infections. These infections are associated with a variety of diseases, including gastritis, peptic ulcer, gastric cancer, mucosa-associated lymphoid tissue lymphoma, non-steroidal anti-inflammatory drug-associated gastropathy, functional dyspepsia, and gastroesophageal reflux disease.² With a high infection rate, approximately half of the world's population has *H. pylori* infection, particularly in Asia and developing countries.³ In approximately 15% of people with *H. pylori* infection, the gastrointestinal disease progresses further and eventually leads to gastric cancer in 1–3% of infected people.⁴ Based on statistics, *H. pylori* infection is currently one of the clearest major risk factors for gastric cancer, one of the most common malignancies worldwide.^{5,6} The various transmission pathways of *H. pylori* included oral-oral, fecal-oral, and gastro-oral routes.⁷ Close contacts with infected individuals or their body fluids can lead to *H. pylori* infection, posing a risk of *H. pylori* transmission and infection in families and occupational places such as hospitals. Occupational exposures were found to increase the probability of *H. pylori* infection. Health professionals, especially gastroenterology staff, had a statistically significantly higher prevalence of *H. pylori* infection compared to controls.⁸

In addition to the healthcare industry, the discharge and treatment of fecal contaminated wastewater may also be potential sources of *H. pylori* transmission and infection. In water environment, *H. pylori* can survive for 20–30 days under appropriate conditions.^{9,10} Previous studies have reported the detection of *H. pylori* in the influent of municipal wastewater treatment plants (MWTPs). *H. pylori* was also detected in the final effluent after secondary treatment and tertiary disinfection.^{11,12} This indicates that *H. pylori* is more tolerant to disinfection than traditional fecal indicators. Therefore, *H. pylori* in the effluent will enter and pollute the natural water. Some developing countries with low economic levels will be at risk of drinking water safety due to their poor drinking water management.² In addition, an increasing amount of

MWTP effluent is reused for landscaping, urban miscellaneous, industrial, and agricultural uses. This allows *H. pylori* in reclaimed water to enter the soil and even the atmosphere, contaminating the environment and increasing the risk of public exposure. In MWTPs, direct or indirect contact with wastewater may pose a potential risk of *H. pylori* infection to workers. More importantly, a variety of potentially pathogens spread with bioaerosols during wastewater treatment and sludge dewatering. The common detected opportunistic pathogens include *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Aspergillus fumigatus*, which can cause infectious, respiratory, and other diseases when exposed.^{13,14} The potential risks can also arise if *H. pylori* escapes with bioaerosol. A multi-dimensional *H. pylori* infection pathway of wastewater, sludge, and air will be formed in MWTPs, which will increase the probability of *H. pylori* exposure for workers.

Quantitative Microbial Risk Assessment (QMRA) is a statistical modelling framework that can help provide quantitative estimates of the health risks posed by pathogens to humans. This methodology includes four typical steps: (1) hazard identification; (2) exposure assessment; (3) dose-response models; (4) exposure assessment. The health risk is calculated based on the exposure concentration of a certain pathogen in the environment.¹⁵ Two benchmarks are used to quantify the infection risks, including the acceptable annual probability of infection ($\leq 10^{-4}$ per person per year (pppy)) recommended by the U.S. EPA, and the acceptable disease burden (DB; $\leq 10^{-6}$ disability-adjusted life years (DALYs) pppy) recommended by WHO. QMRA has been applied to assess exposure to various microorganisms. In recent years, the QMRA assessment method for pathogen risk assessment in bioaerosols of MWTPs has received widespread attention.^{16,17} In a Monte Carlo simulation-based QMRA case study, wastewater treatment plant (WWTP) workers without personal protective equipment (PPE) exposed to *S. aureus* or *E. coli* bioaerosols had an annual probability of infection that far exceeded 10^{-4} pppy.¹⁸ The DB value from *S. aureus* exposure also exceeded 10^{-6} DALYs pppy. Reverse QMRA was used to estimate the threshold limit value (TLV) of acceptable exposure concentration. It was found that the TLV benchmark for workers wearing masks (0.54–6315.96 colony forming unit (CFU/m³)) was 1–2 orders of magnitude higher than that of workers not wearing masks (0.02–93.59 CFU/m³).¹⁹ Therefore, the use of PPE help to reduce related occupational health risks of workers in WWTPs. In QMRA, sensitive analysis was commonly used to determine the main factors that affect the calculation results. When estimating the daily probability of infection risk for sewage workers exposed to viable infectious viral airborne particles, exposure time was found to contribute significantly to the daily infection risk in addition to the concentration of SARS-CoV-2 in human stool.²⁰ This suggests that increasing worker rotations may effectively reduce the inhalation of pathogens. Therefore, the application of QMRA to assess the health risks of exposure to bioaerosols from WWTPs, especially airborne pathogens, can provide a basis for the development of relevant standards, help optimize WWTP management measures, and improve the protection of workers.

As one of the most infectious pathogen, *H. pylori* infected individuals have a much higher risk of developing gastric cancer than the normal population. The pollution of *H. pylori* in various media of MWTPs increases the pathways and probability of *H. pylori* infection among the workers. However, the current status of *H. pylori* pollution in MWTPs and potential hazards are still unclear due to insufficient attention to *H. pylori* pollution in MWTPs and the lack of relevant standards. Based on the above, this study systematically determined the variations in *H. pylori* abundance from influent to effluent during

the entire wastewater treatment process of a typical MWTP. The transmission mechanisms of *H. pylori* from wastewater to sludge and air medium were resolved, and the potential impacts of airborne *H. pylori* on the environment and workers were assessed. This study aimed to enhance the protective awareness of workers and provide a scientific basis for a comprehensive understanding of the future control of *H. pylori* contamination in MWTPs.

MATERIALS AND METHODS

Sampling site descriptions

This study was conducted in a MWTP with an approximate service population of 3.64×10^5 people located in Beijing (39.83°N, 116.27°E). An anaerobic-anoxic-aerobic (A²/O) process was applied, and 10^5 m³/d of domestic wastewater was treated at the MWTP. Bioaerosols were collected from six typical sites including the grille, aerobic tank (AT), mechanical maintenance house (MMH), sludge dewatering house (SDH), office area (OA), and an inside site downwind of the MWTP (DW), as shown in Figure S1. Sampling was carried out from September to December 2021 and February to August 2023 during the working hours of the workers (9:00–17:00).

Sample collection

Wastewater samples were collected during the entire wastewater treatment process, including the influent, grille, biological units, effluent from secondary sedimentation tank (SST), and final effluent. The sludge samples included the excess sludge (ES) and dewatered sludge (DS). Bioaerosols were captured using a Six-stage Viable Andersen Cascade Impactor via a Quick Take 30 Sample Pump (228-9530 K, SKC Gulf Coast Inc., USA). Each air sample was pumped into an impactor at a flow rate of 28.3 L/min for 3 min. After entering the impactor, the airborne particles were separated into stages I to VI with particle sizes of >7.0, 4.7–7.0, 3.3–4.7, 2.1–3.3, 1.1–2.1, and 0.65–1.1 μm. The *H. pylori* selective culture Petri dishes with a diameter of 90 mm were placed directly below each stage to receive and capture *H. pylori*. Three samples were collected in parallel from each sampling site. The sampling height was the average respiratory height at a distance of 1.5 m from the horizontal ground. After collection, the sampled plates were cultured at 37°C for 72–96 h under microaerobic conditions, as described in previous studies.^{21,22} The selective *H. pylori* medium was prepared as follows: every 5.2 g of *H. pylori* solid medium (HopeBio, China) was added to 93 ml of distilled water and then sterilized at 121°C under high pressure for 15 min. When cooled to 50–55°C, 7 ml of defibrinated sheep blood and one inhibitor were added to the sterilized liquid medium. The optimized inhibitor consists of vancomycin (10 mg/L), cefuroxime (5 mg/L), trimethoprim (5 mg/L), and amphotericin B (5 mg/L). It inhibits the growth of other microorganisms such as quality control strains *Staphylococcus aureus* and yeast.²³ After mixing, approximately 20 ml of liquid medium was poured into each sterile petri dish, solidified, and then refrigerated at 4–8°C for use. All the surgeries were performed under sterile conditions.

The total bacterial population in the bioaerosols was mainly determined by the total suspended particles sampled. A medium-volume sampler (TH-150C, TianHong, Wuhan, China) was used to collect the particles on sterilized quartz filters (diameter, 90 mm) at a flow rate of 100 L/min. Each sample was collected for 8 h to obtain sufficient biomass and maintain the microbial activity. The subsequent pre-treatment method and process of the sampled filters were referenced from a previous study.²⁴

During sample collection, the temperature and relative humidity were recorded with a Humidity&Temperature Meter (GM1361, Shenzheng Benetech, China). Wind speed (WS) and solar radiation were monitored using an Anemo Meter (HD2303.0, Delta OHM, Italy) and an Irradiance Meter (HD2302.0, Delta OHM, Italy), respectively. Other meteorological data, including PM_{2.5}, PM₁₀, NO_x, SO₂, O₃, and CO, were obtained from the real-time monitoring data released by the Beijing Municipal Ecological and Environmental Monitoring Center (<http://www.bjmemc.com.cn/>).

H. pylori quantification

The number of *H. pylori* contained in the bioaerosol samples (expressed as CFU/m³) was calculated based on the counted colonies formed by the growth and multiplication of individual cells on the petri dishes after cultivation. Despite the selectivity of the medium used, attention was paid to the

morphology of the colonies during counting. *H. pylori* grown on the medium should be smooth, raised, neatly edged, grey-white translucent round colonies. The positive-hole method was utilized for correction of counting results as described in a previous study.²⁵ *H. pylori* in wastewater, sludge, and soil was quantified using the dilution plate coating method. The samples to be tested were first made into a homogeneous series of dilutions. Then 100 μl of the dilutions were obtained and inoculated into the *H. pylori* medium and evenly distributed in the medium on the plate. After incubation, colonies were formed by the growth and multiplication of individual cells. The number of colonies was counted to calculate the number of bacteria present in the sample. The appropriate number of colonies for counting was in the range of 30 to 300. However, the presence of viable but nonculturable cells may lead to an underestimation of the number of *H. pylori*.

Microbial identification

The metagenomic methods were used to assist in identifying *H. pylori* and calculating its abundance in total bacteria. DNA was extracted from wastewater, sludge, and quartz membrane samples using the E.Z.N.A.® soil DNA kit (Omega Bio-tek, Norcross, GA, USA). The DNA extracts were examined on a 1% agarose gel. A NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, USA) was used to determine DNA concentration and purity. The hypervariable region V3-V4 of the bacterial 16S rRNA gene was amplified using an ABI GeneAmp® 9700 PCR Thermocycler (ABI, CA, USA) based on primers 338F and 806R. Polymerase chain reaction (PCR) was performed in triplicates. PCR products after extraction from 2% agarose gel were purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and quantified using Quantus™ Fluorometer (Promega, USA). Purified amplicons were sequenced on an Illumina MiSeq PE300 platform/NovaSeq PE250 platform (Illumina, San Diego, USA) for paired-end sequencing. Operational taxonomic units (OTUs) with 97% similarity were clustered using UPARSE version 7.1 (Edgar, 2013). The classification of each OTU representative sequence was analyzed against the 16S rRNA database using the RDP classifier version 2.2 with a confidence threshold of 0.7.

Net growth rate of *H. pylori* in wastewater

An ecogenomics-based mass balance approach was used to investigate the migration of *H. pylori* during the wastewater treatment process.²⁶ Under normal and stable operating conditions of the MWTP, the microbial population in the wastewater system remains in mass balance. The abundance of *H. pylori* does not change over time in one reactor. The sum of *H. pylori* cells in the influent and effluent and their growth is zero (equation (1)). This allows for the calculation of the net growth rate (μ) (equation (2)). The entire biological treatment unit was considered as a whole, with the influent and pre-treatment units upstream and the SST effluent and residual sludge discharge downstream.

$$N_{in} = N_{eff} - Growth \quad (1)$$

$$\mu_x = \frac{Q_{eff} \cdot C_{eff} - Q_{in} \cdot C_{in}}{V_{re} \cdot C_{re}} \quad (2)$$

where N_{in} is the number of *H. pylori* cells in the influent, and N_{eff} is the number of *H. pylori* cells in the effluent. *Growth* represents the variation in *H. pylori* distribution in each reactor. μ_x is the net growth rate constant. A positive μ_x (d⁻¹) represents the net growth and active function, while a negative μ_x represents the net decay and minimal ecological function. Q_{in} and Q_{eff} are the flow rates of the influent and effluent, respectively. C_{in} and C_{eff} are the concentrations of *H. pylori* in the influent and effluent (CFU/m³), respectively. V_{re} is the volume of wastewater in one specific reactor (m³). C_{re} is the concentration of *H. pylori* in the reactor (CFU/m³).

Aerosolization factor (AF)

From the perspective that *H. pylori* can be cultivated in bioaerosols of the MWTP, the contribution of wastewater and sludge was evaluated by means of a ratio. The ratio was obtained by dividing the difference between the concentrations of airborne *H. pylori* at the sampling points and the blank (OA) by the concentration of *H. pylori* in wastewater or sludge. In terms of microbial populations in bioaerosols from MWTPs, the AF was used to evaluate the

contribution of wastewater and sludge to *H. pylori* emissions into the air using the following equation:²⁷

$$AF = \frac{RB_i - RB_{0A}}{RB} \quad (3)$$

where RB_i is the relative abundance of *H. pylori* among the total bioaerosol bacteria at a specific sampling site (%). RB_{0A} is the relative abundance of airborne *H. pylori* detected in the blank (%). RB is the proportion of *H. pylori* among the total bacteria in the wastewater or sludge (%). If the calculated AF is greater than 1, it indicates that the microorganisms are highly susceptible to aerosolization from wastewater or sludge and can enter the air, and vice versa.

Health risk assessment

Hazard identification and exposure assessment. QMRA was used to calculate the inhalation risk of airborne pathogens in MWTP workers. The pathogen of concern in this study was *H. pylori* in bioaerosols originating from wastewater treatment. The exposed individuals included both adult males and adult females. The exposure scenarios were for the operational units, including the grille, MMH, SDH, and AT, and non-operational points, including the OA and DW. All the exposure assessment parameters referring to the QMRA calculation frameworks are listed in Table S2. The exposure dose of the pathogen per person per day was calculated using equation (4).

$$d = C \times BR \times T \times AG \quad (4)$$

where d represents the daily exposure dose (CFU). C is the concentration of airborne *H. pylori* (CFU/m³). BR is the exposed person respiration rate (m³/d). T is the exposure time per day (h). AG is the bioaerosol ingestion rate, which is the ratio of bioaerosol concentration from stages 4–6 (<3.3 μm) to stages 1–6 of the Six-stage Viable Andersen Cascade Impactor.

Dose-response models. The exponential distribution was found to be a better fit for the *H. pylori* dose-response model,²⁸ which was used for QMRA in this study. The daily health infection risk was calculated using equation (5).

$$P_i = 1 - \exp(-k \times d) \quad (5)$$

where P_i is the daily health infection risk, and k is the model parameter.

Exposure assessment. The annual probability of infection (pppy) was calculated using equation (6).

$$P_y = 1 - (1 - P_i(d))^n \quad (6)$$

where P_y is the annual probability of health infection risk (pppy). n is the annual exposure frequency per year for each exposure scenario.

The annual probability of illness risk (pppy) was calculated using equation (7).

$$P_{(a)ill} = P_y \times P_{illinf} \quad (7)$$

where $P_{(a)ill}$ is the annual probability of illness risk. P_{illinf} is the risk of disease due to an infection.

The disease health burden related to the illnesses was evaluated using equation (8).

$$DB = HB \times P_{(a)ill} \quad (8)$$

where DB is the disease burden (DALYS pppy). HB is the health burden (DALYS/case). As there are few studies on *H. pylori*, the HB of *E. Coli* was used instead.

Monte Carlo simulation and sensitivity analysis. Monte Carlo simulations and sensitivity analyses were performed to calculate the probability distribution of microbial risks in different scenarios using the Oracle Crystal Ball software and Microsoft Excel 2016. The output parameters were computed over 10,000 iterations such that the distributions could reach a steady state. The dose-response model parameters and all input variables were chosen randomly from their probability distributions. A small variation in terms of the average between replicates of the distribution was used to check the reproducibility of the model output. The input variables were the detected airborne concentration of *H. pylori*, exposure time, and bioaerosol ingestion ratio. The distributions of these parameters are listed in Table S3.

Diffusion simulation

The diffusion of airborne *H. pylori* in the downwind of MWTP was simulated using the NOAA online Hybrid Single-Particle Lagrangian Integrated Trajectory (HYSPPLIT) model with the Global Data Assimilation System (GDAS) database (https://www.ready.noaa.gov/HYSPPLIT_traj.php). The HYSPPLIT model was commonly used to simulate the transportation, diffusion, and deposition of atmospheric pollutants (including pollutants, harmful substances, and bioaerosols).^{29,30}

RESULTS

H. pylori in wastewater

Once wastewater enters an MWTP through a wastewater pipeline, the number and community structure of microorganisms undergo dynamic changes throughout the wastewater treatment process. The variations in the abundance of *H. pylori* in the MWTP wastewater are presented in Figure 1. The concentration of *H. pylori* in the inlet ranged from 1.65×10^6 CFU/L to 4.20×10^8 CFU/L. The majority of the inlet samples maintained concentrations in the order of 8 and a median of 1.24×10^8 CFU/L. After the interception of large floating and suspended substances by the screen, the concentration of *H. pylori* in the wastewater remained at approximately 10^8 CFU/L. Upon entering the biological treatment units, an order of magnitude decrease in the concentration of *H. pylori* was observed. The average concentration decreased from 1.08×10^9 CFU/L in the anaerobic tank (ANA) to 2.03×10^8 CFU/L in the anoxic tank, and then to 5.78×10^7 CFU/L in AT. After secondary sedimentation, the concentration of *H. pylori* in the effluent eventually dropped to 5×10^3 – 5.90×10^6 CFU/L, achieving a substantial reduction. As the SST effluent at the MWTP was taken directly to the water reclamation plant for further treatment without disinfection, the biological units and their subsequent sedimentation appeared to help reduce *H. pylori* in wastewater. This is mainly attributed to the formation of bacterial colloids in the activated sludge by flocculant bacteria with mucus or pods held together, which can adsorb free microorganisms in wastewater.³¹ Due to the excellent settling performance of the activated sludge, most of the adsorbed *H. pylori* could be separated from the wastewater by ES discharge after SST sedimentation, thus ensuring its removal.

The relative abundance of *H. pylori* represents its dominance in the total bacterial population. The proportion of *H. pylori* among the total bacteria in the influent was determined to be between 0.0001 and 0.0236%. Its proportion decreased in the biological units, with a median abundance of 0.0004%, as the wastewater contained a large number of microorganisms when mixed with activated sludge. After secondary sedimentation, the median relative abundance of *H. pylori* reached 0.0009% (Figure 1), indicating its slightly increased predominance. This could be explained by the fact that although the wastewater treatment process reduced the total number of bacteria, the weak reduction in *H. pylori* abundance still resulted in a higher proportion of *H. pylori* in the effluent.

Water quality affects the community composition of microorganisms in wastewater.³² To discern the roles of individual water qualities on their effect on *H. pylori* abundance, a correlation heatmap was constructed based on the Spearman correlation (Figure 2A). The results showed moderately positive relationships between *H. pylori* concentrations and water quality indicators, including chemical oxygen demand (COD, $r=0.50$), total nitrogen (TN, $r=0.44$), ammonia nitrogen (NH_4^+-N , $r=0.44$), and total phosphorus (TP, $r=0.37$). Similar relationships have been observed in previous studies, where environmental pathogens such as *Acinetobacter* spp. and *Aeromonas* spp. were positively correlated with NH_4^+-N , 5-day biochemical oxygen demand, and TP in urban sewer infrastructure.³³ *Aeromonas* spp. and *A. johnsonii* were also found to be positively correlated with NH_4^+-N , the dissolved organic carbon (DOC) or soluble TP.³⁴ *A. lwoffii* was positively correlated with DOC and the suspended solid. These positive relationships suggest that some of the nutrients in wastewater may favor the growth of these pathogens, as there are various types of organics, nitrogenous, and phosphorus-containing substances in wastewater. In addition, due to the positive correlation between TP, COD, and TN (Figure 2B), it was also possible that the high concentrations of *H. pylori* were detected in wastewater at precisely the time of high concentrations of the nutrients. However, negative relationships were found

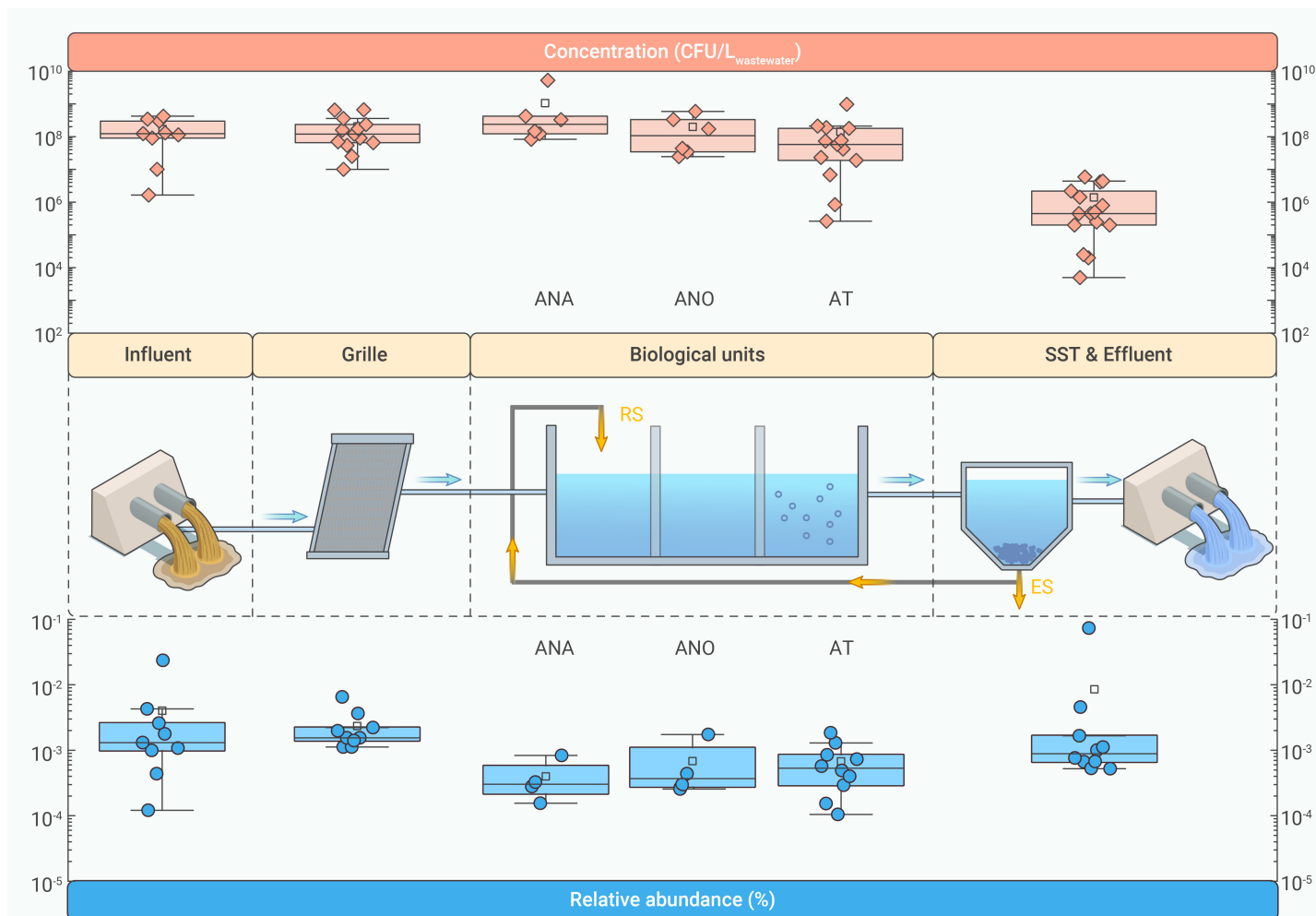


Figure 1. Variations in *H. pylori* abundance in wastewater during treatment processes ANO, anoxic tank. RS, returned sludge.

between TP, COD, and TN and the relative abundance of *H. pylori*. This could be explained by the fact that under higher nutrient conditions, other microorganisms grow and reproduce at faster rates. However, *H. pylori* accounts for only a small proportion of the wastewater and has a weak competitive advantage. In addition, there may also be antagonistic microorganisms of *H. pylori* in wastewater, which inhibit the growth of *H. pylori*.³⁵ The chloride ion (Cl^-) displayed a negative relationship with the concentration of *H. pylori*. Chlorine has been used as a disinfectant for *H. pylori* in water. It has been found to have an inhibiting effect on enzymatic processes and cause physical damage to the membrane cells of *H. pylori*. A disinfection concentration and exposure time of 20 mg min/L resulted in a reduction of 5-log cells/mL of *H. pylori*.³⁶ In this study, the detected concentrations of Cl^- in the influent were 114.19 ± 22.61 mg/L (Table S1). Nitrite (NO_2^-) showed a strong positive correlation ($r=0.90$) with the relative abundance of *H. pylori*, indicating that NO_2^- may also promote the growth of *H. pylori*. However, due to the complex impacts of environmental conditions on microorganisms in wastewater, further research is needed to explore the role of water qualities in the survival and reproduction of *H. pylori*.

H. pylori in sludge

After wastewater was treated by the biochemical units, most *H. pylori* cells were wrapped in activated sludge and discharged through the ES. As a result, high concentrations of *H. pylori* were detected in the ES with an average concentration of 4.22×10^8 CFU/kg (Figure 3A), which was three orders of magnitude higher than that in the effluent. After dehydration, the concentration of *H. pylori* in DS increased by two orders of magnitude compared to the ES, ranging from 4.00×10^7 to 3.28×10^{11} CFU/kg. The relative abundances of *H. pylori* in the ES and DS were approximately minus four orders of magni-

tude equivalent.

Notably, the average concentration of *H. pylori* in the environmental soil upwind (S-OA) of the MWTP was 1.22×10^7 CFU/kg higher than that in the downwind (S-DW) (Figure 3B). Moreover, according to the macro-genomic analysis, the relative abundance of *H. pylori* was detected in almost all samples of S-DW, while it was not detected in all S-OA samples. Two factors might have contributed to this observation. First, the DW was once used to store DS, and stacking of the sludge increased the concentration of *H. pylori* in the soil. In addition, the proximity of the DW to SDH allowed some *H. pylori* to escape into the air as bioaerosols during sludge dewatering. The escaping *H. pylori* were then deposited in the soil through atmospheric deposition and rainfall.

H. pylori in bioaerosols

Bioaerosols are formed when wastewater or sludge is mechanically disturbed during wastewater treatment processes, such as agitation, aeration, and mechanical dewatering. In this study, the detected concentrations of *H. pylori* in bioaerosols at the sampling points ranged from 6 to 4173 (CFU/m³) (Figure 4A). According to the macro-genomic results, *H. pylori* was identified in the air at all sampling points, accounting for 0.00002% to 0.00199% of the total bacteria detected (Figure 4B). *H. pylori* is one of the multiple pathogens identified in the bioaerosols of MWTPs. Due to its high infectivity, *H. pylori* may pose a health risk to the exposed population. The low relative abundance of *H. pylori* in total bacteria indicated that *H. pylori* was not dominant in the airborne bacterial community. However, the quantitative results of plate collection and cultivation showed high concentrations of *H. pylori* in the air of the MWTP. This indicated the presence of active *H. pylori* in the air. For airborne pathogens, their activity has a significant impact on

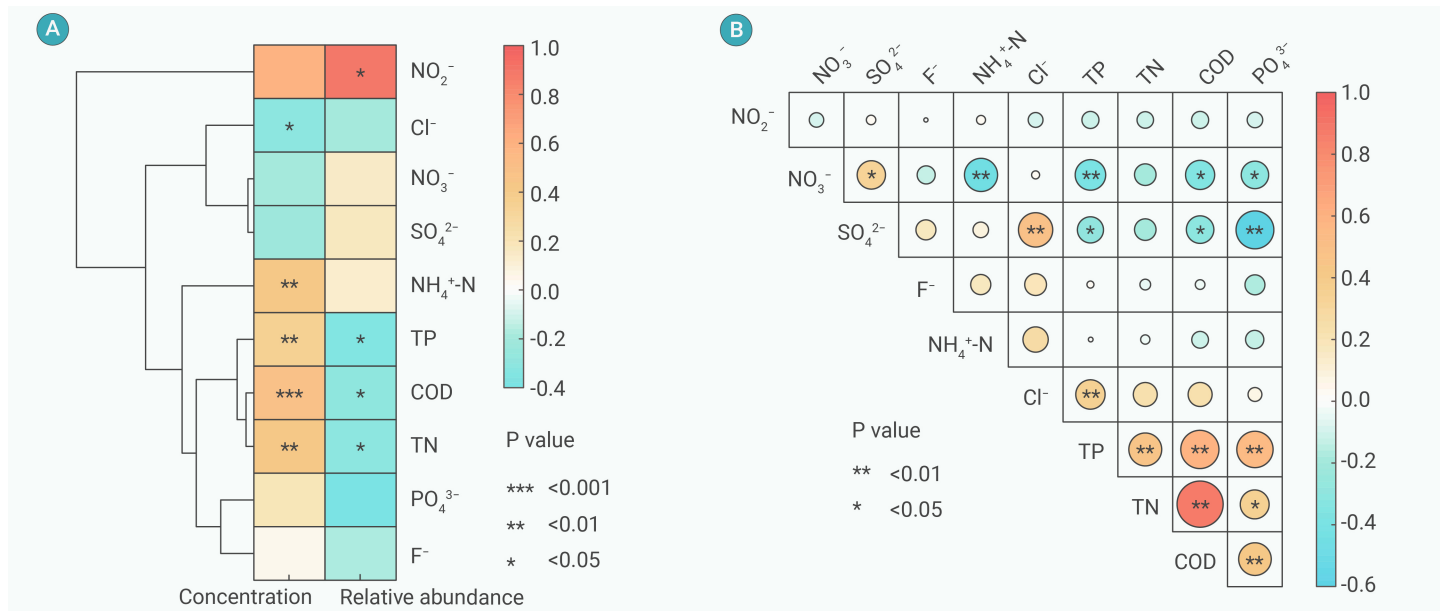


Figure 2. The response of wastewater quality characteristics on the abundance of *H. pylori* in wastewater (A) Correlation of water quality with *H. pylori* abundance. (B) Correlation wastewater qualities. F⁻, fluoride ion. NO₃⁻, nitrate. PO₄³⁻, phosphate radical. SO₄²⁻, sulphate.

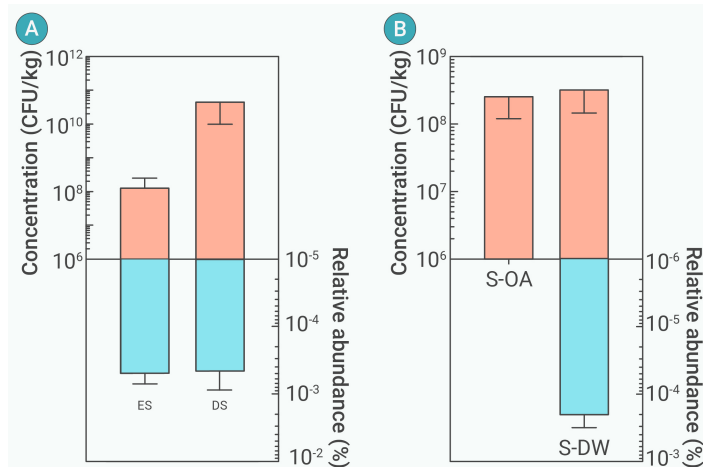


Figure 3. *H. pylori* in sludge and environmental soil (A) Abundance of *H. pylori* in sludge. (B) Abundance of *H. pylori* in environmental soil.

pathogenicity. Therefore, the health threat of *H. pylori* in the air of MWTPs to MWTP workers could not be ignored.

There were differences in the detected concentrations of *H. pylori* at each sampling point. Among the indoor units, the SDH had the highest concentrations (549±516 CFU/m³). The SDH is functional for the dewatering and reduction of ES. Various microorganisms in ES are released into the environment during the continuous squeezing of sludge-by-sludge dewatering equipment.²⁴ In addition, high relative humidity is suitable for the survival of microorganisms in the SDH. The lack of wind diffusion and dilution under confined conditions also makes it easy to accumulate and form a high concentration of microorganisms. The grille was also a major source of *H. pylori* escape, with the detected concentrations of *H. pylori* ranging from 6 to 411 CFU/m³. Pre-treatment units are usually those with high microbial air contamination. Processes, such as mechanical treatment or forced aeration, tend to aerosolize microorganisms.³⁷ The grille was used as a pre-treatment unit to trap larger floating and suspended substances in the wastewater influent. The metal grating bars move on a chain of teeth and continuously disturb the water surface during operation. This brings microorganisms in the wastewater out of the water surface via particles to form bioaerosols.³⁸ In MMH, *H. pylori* was also detected at concentrations of 6–147 CFU/m³ air, indicating the risk of *H. pylori* inhalation by workers when servicing machinery.

Unlike in enclosed indoor spaces, bioaerosols generated by aeration are

easily diffused and diluted in open spaces. The concentration of *H. pylori* in the air above the AT was lower than that in the SDH, with a median concentration of 41 CFU/m³. Moreover, AT had higher airborne *H. pylori* concentrations than the grille, indicating that aeration was more susceptible to the emission of bioaerosols in wastewater. Compared with OA upwind, the average concentration of *H. pylori* detected in DW was 28.57 times higher. The highest *H. pylori* concentration (4173 CFU/m³) was detected in the air in the DW. There are two main reasons for this finding. First, the DW sampling point was close to the parking area of sludge transportation vehicles. The escape of microorganisms during sludge loading increased the abundance of *H. pylori* in the air. Second, the effect of wind caused the microorganisms in the air of other sampling points to migrate and transport to the downwind factory boundary.

The particle size of bioaerosols determines where they are deposited in the human respiratory tract and how they interact with human tissues, which is critical for human health. Larger particles are deposited in the upper respiratory tract, nasal cavity, and bronchial tubes. Particles smaller than 3.3 µm are inhalable fragments that can penetrate deep into the human alveoli and diffuse deep into the lung tissues, posing a risk of infection.^{39,40} The particle size distribution of *H. pylori* in the bioaerosols in this study showed clear indoor and outdoor differences (Figure 4C). *H. pylori* cells in indoor units were mainly distributed in the fine particle size range, i.e., <3.3 µm, while those in outdoor units were distributed in the larger particle size (>3.3 µm). At the SDH and grille, the average proportion of respirable fragments of *H. pylori* in the air exceeded 66% and 48%, respectively. This indicates that these *H. pylori*-carrying particles may penetrate deep into the respiratory system, posing an inhalation risk to operating workers in the corresponding operating rooms. *H. pylori* in the air at the outdoor sampling points were mainly distributed in the larger particle size range. This was because that dust on the outdoor ground was easily carried into the air by wind and human activities. The wrapping of particles increased the diameter of the bioaerosols.

The survival of airborne microorganisms is influenced by various environmental factors. To identify the determinants affecting the survival of airborne *H. pylori*, correlation analysis was used to determine the relationship between environmental factors and the concentration of *H. pylori* detected (Figure 4D). The vast majority of the filtered factors were negatively correlated with the relative abundance and respirable proportion of airborne *H. pylori* based on a significant p-value of less than 0.05. Among them, O₃ in the near surface atmosphere is produced by a series of photochemical reactions of nitrogen oxides and volatile organic compounds emitted by human activities into the atmosphere under solar radiation.⁴¹ O₃ is a spectroscopic disinfectant with a strong killing effect on a wide range of microorganisms and can therefore act

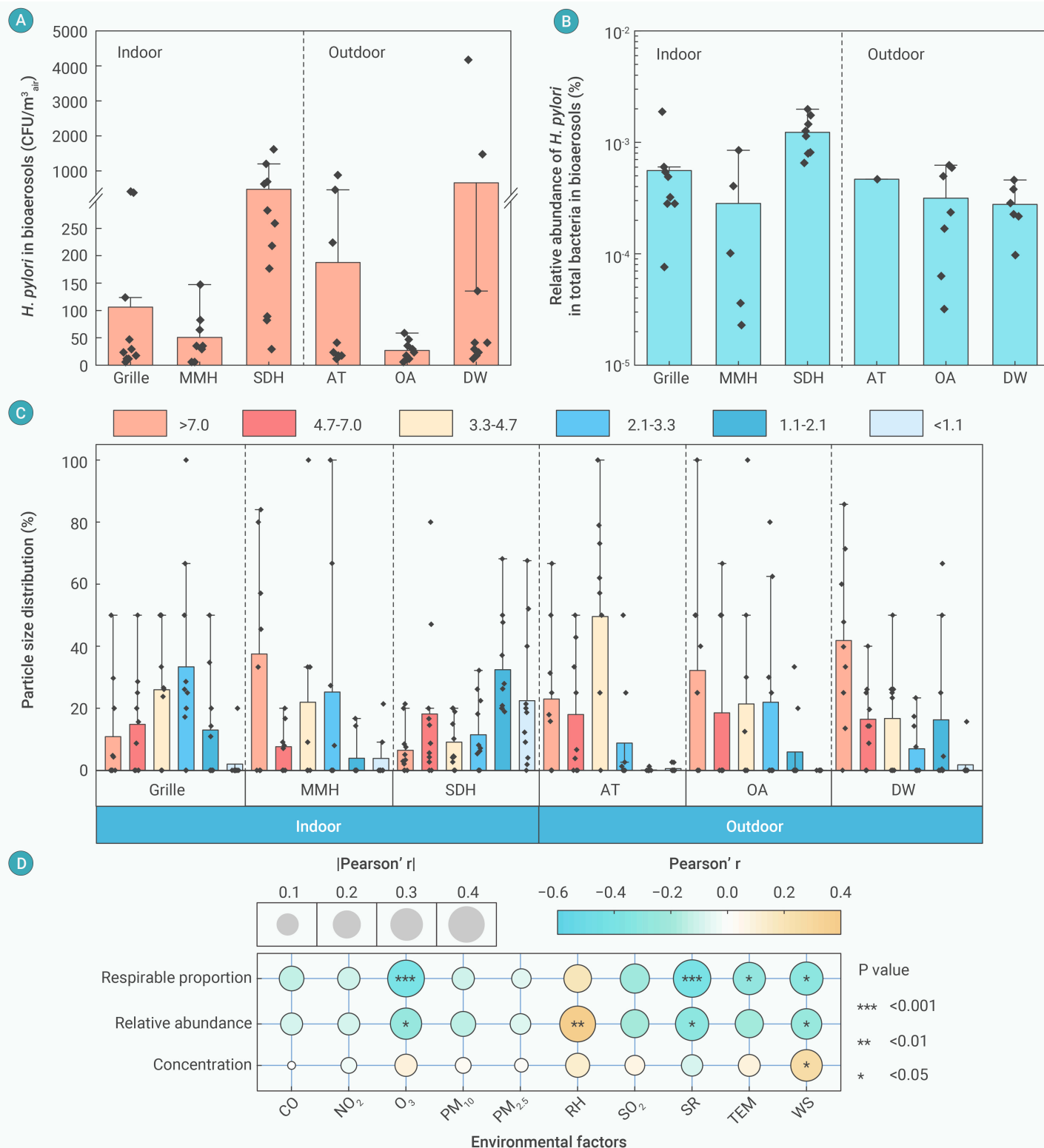


Figure 4. *H. pylori* in bioaerosols (A) Concentration. (B) Relative abundance. (C) Particle size distribution. (D) Environmental factors. SR, solar radiation. TEM, temperature.

as an *H. pylori* inhibitor.⁴² Adequate outdoor solar radiation includes strong ultraviolet (UV) radiation, which can cause the inactivation or death of microorganisms and is detrimental to the survival of airborne *H. pylori*.⁴³ *H. pylori* is a humidity-loving bacterium, and higher relative humidity is more conducive to its survival and reproduction.⁴⁴ A negative correlation was also found between the ambient temperature and the respirable proportion of *H. pylori* bacteria. This is because the dilution and diffusion of inhalable particles depend on air movement. Higher temperatures increase the vertical

movement of the atmosphere, which is conducive to the diffusion and dilution of small particles. Meanwhile, higher ambient temperatures are not favorable for the survival and reproduction of microorganisms, resulting in lower bioaerosol concentrations. However, in contrast to other studies, WS was inversely proportional to the concentration of airborne *H. pylori*. As WSs were only used for microbiological analyses at the outdoor sampling sites, microorganisms from other areas may have been transported to the sampling sites at high WSs.

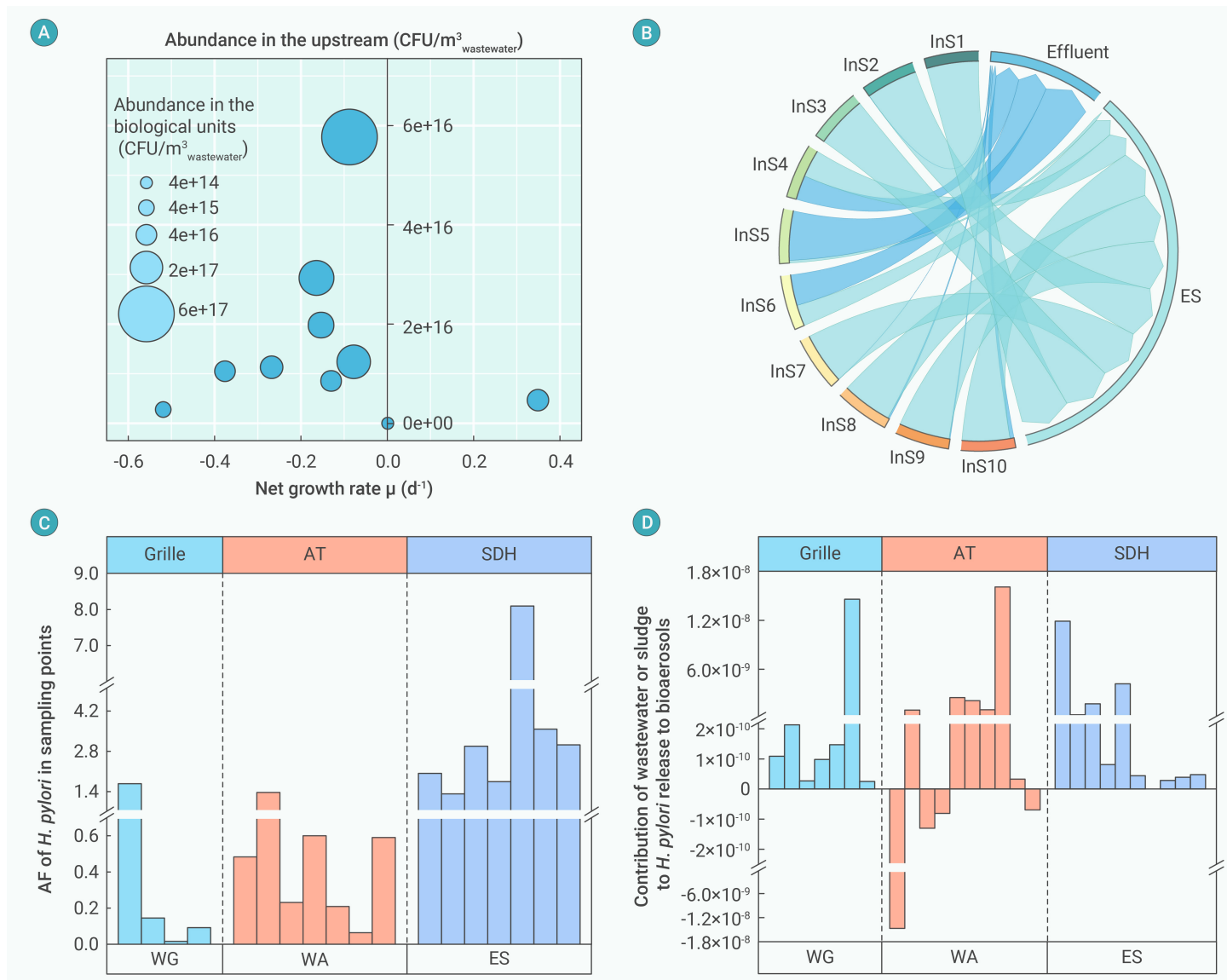


Figure 5. Transmission of *H. pylori* in the MWTP (A) Net growth of *H. pylori* in the biological units. (B) Proportion of *H. pylori* transferred to ES and effluent. (C) Aerosolization factor (AF) of *H. pylori* in different sampling points. (D) Contribution of wastewater or sludge to *H. pylori* release into bioaerosols. InS, influent sample. WG, wastewater sample from Grille. WA, wastewater sample from AT.

DISCUSSION

Fate and transmission of *H. pylori* in the MWTP

The fate of *H. pylori* in the wastewater revealed that the majority of the *H. pylori* bacteria had negative net growth rates (Figure 5A). The average net growth rate was calculated to be -0.143 d^{-1} . This suggests that *H. pylori* bacteria in the upstream effluent exhibit low activity despite their higher abundance, and the biological treatment units can reduce *H. pylori* bacteria. After positive or negative proliferation by the biological units, some of the *H. pylori* cells in the wastewater are precipitated and discharged in the form of ES through the SST. The other proportion flows out with the effluent. The surviving *H. pylori* bacteria in the ES were stored in DS after dehydration treatment. According to the calculation results, most of the *H. pylori* bacteria were transferred to the ES, with an average transfer proportion of 78.49% (Figure 5B). Therefore, the potential risks of the escape and spread of *H. pylori* still exist in the subsequent treatment and disposal of DS, which should be addressed in future studies.

The emission possibility of *H. pylori* from wastewater and sludge into the air was investigated based on the aerosolization potential of *H. pylori* in the main treatment units of the MWTP. The results showed that all the AFs of *H. pylori* from ES in the SDH were greater than one (Figure 5C). Therefore, *H. pylori* was highly susceptible to aerosolization during sludge dewatering. The AT was also determined to have a high aerosolization potential, with average

and median AFs of 0.51 and 0.48, respectively. Moreover, the AF values of *H. pylori* in AT were higher than those in the grille, suggesting that aeration is more likely to aerosolize microorganisms in wastewater. During aeration, fine bubbles carrying activated sludge flocs containing microorganisms reach the water-vapor interface. Then the bubbles burst and cause particles in the wastewater mixture to splash into the gas phase. According to the contributions of wastewater and sludge to *H. pylori* release in the bioaerosols, close contributions of approximately minus nine orders of magnitude were observed at several sampling points (Figure 5D). Although only a small proportion of *H. pylori* escapes into the air, its aerosolization potential and the potential risks it poses are still of concern because of its high infectivity and pathogenicity.

Potential effects of *H. pylori*

The potential risks of *H. pylori* in MWTP effluents and DS are unclear because they need to be reprocessed or disposed of. However, *H. pylori* can enter the ambient air directly or be inhaled into the human body. Therefore, the potential risks posed by *H. pylori* in bioaerosols were evaluated in this study. *H. pylori* in outdoor air is carried by aerosol particles and dispersed by wind beyond plant boundaries. According to the HYSPLIT results, the particles containing *H. pylori* spread in an oval shape (Figure 6). The diffusion directions and areas of bioaerosols were distinct over the sampling months

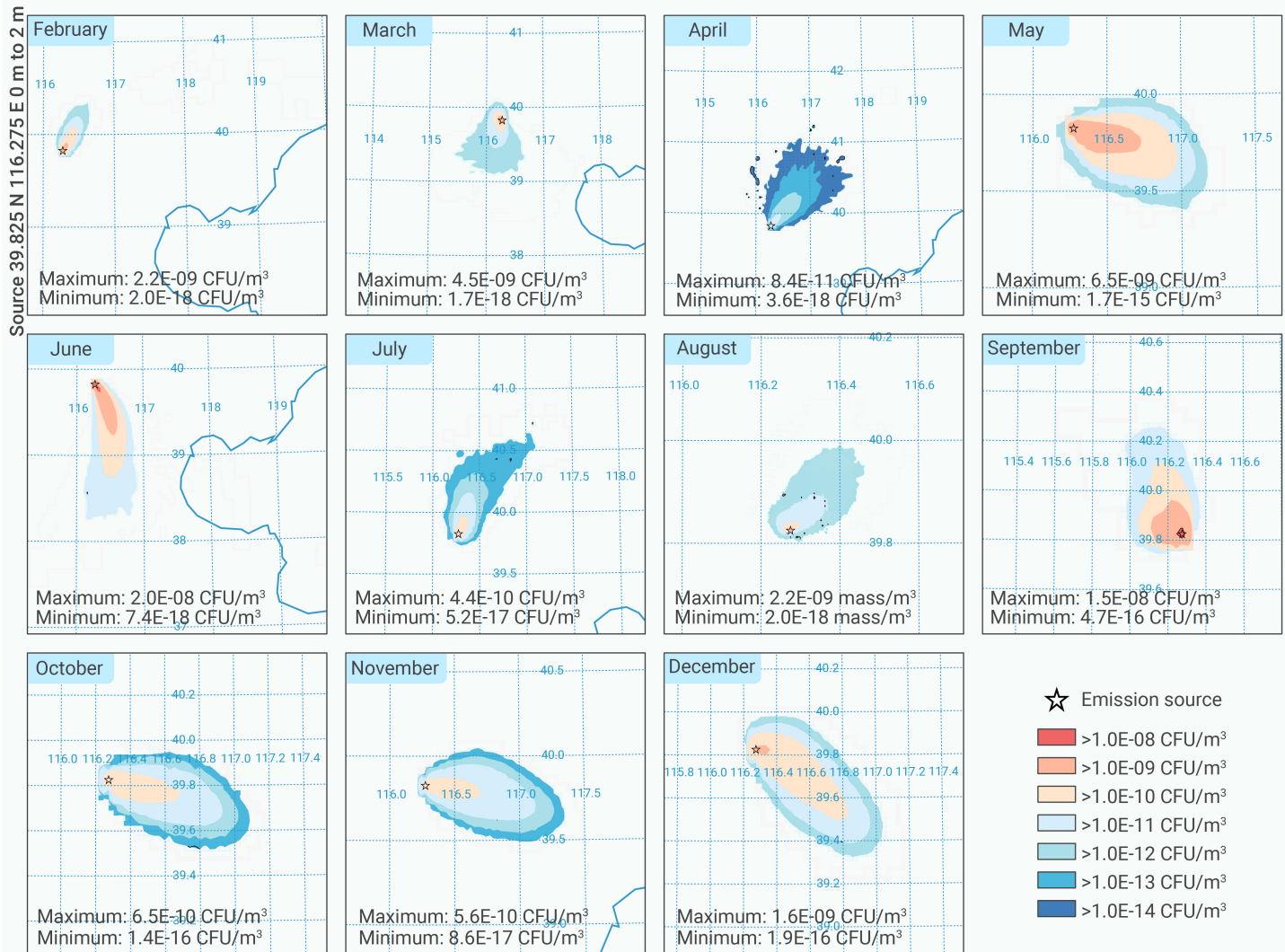


Figure 6. Diffusion of airborne *H. pylori* downwind.

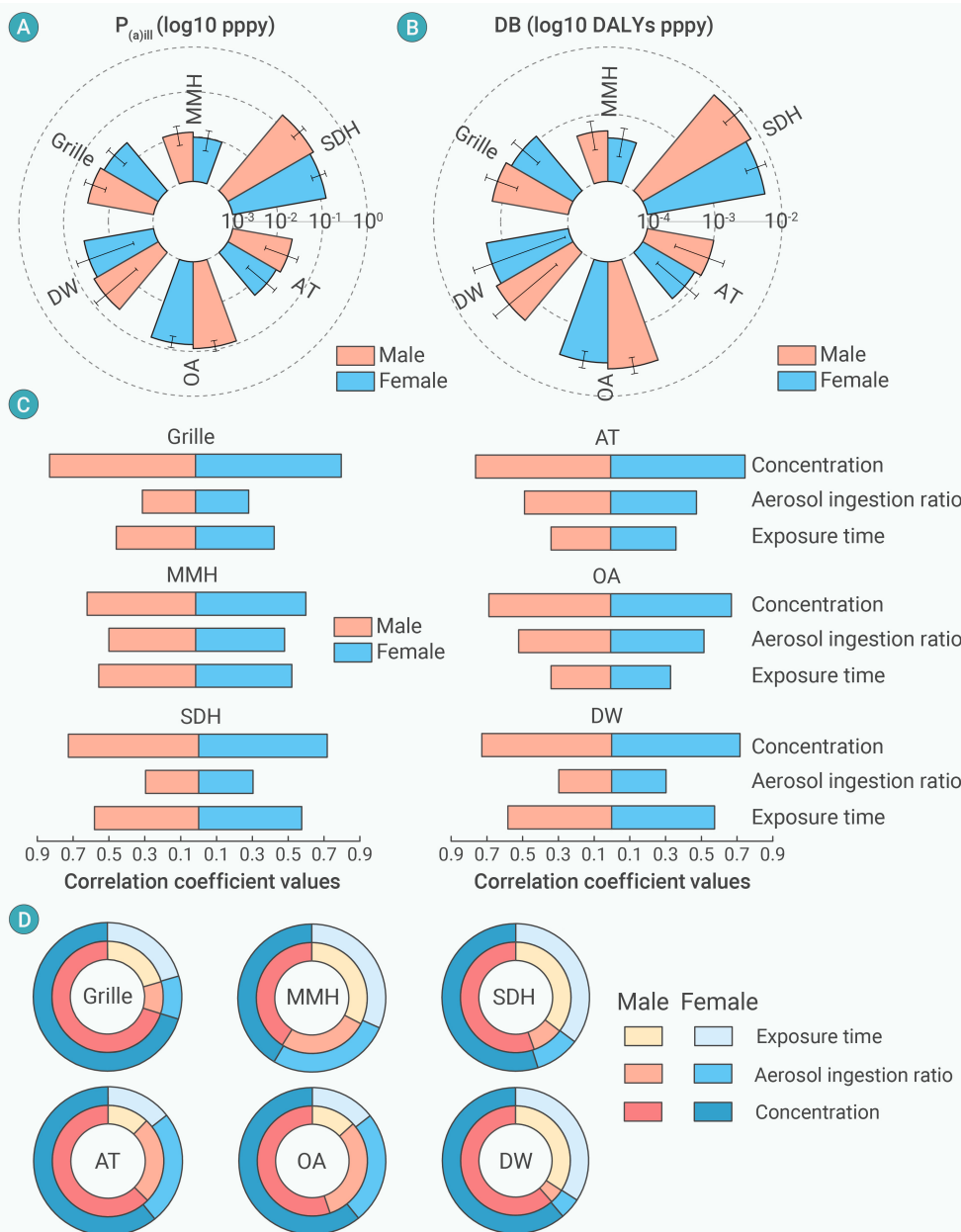
owing to variations in wind direction and meteorological conditions. Due to the continuous operation of MWTPs, bioaerosols are constantly discharged and have a larger impact area after diffusion. Therefore, residents close to MWTPs should also take good health protection measures in their daily lives.

Airborne *H. pylori* may enter the body through the respiratory tract when MWTP workers are exposed for a prolonged time in the working environment. The QMRA results showed that the prevalence probabilities of *H. pylori* infection due to occupational exposure for male and female workers ranged from 9.69×10^{-3} to 1.62×10^{-1} pppy (Figure 7A). These values exceeded the U.S. EPA's annual infection benchmark (10^{-4} pppy). In the operational units, $P_{(a)ill}$ is the highest in the SDH (0.13 – 0.16 pppy), followed by the grille (9.69×10^{-3} – 1.25×10^{-2} pppy). The DB values were also much higher than the benchmark of 10^{-6} DALYs pppy proposed by the WHO (Figure 7B). Similar to the results of the annual illness probabilities, the DB values of the workers in the SDH was also much higher than that of the workers at other sampling points. The DB values of male and female workers were 7.33×10^{-3} DALYs pppy and 6.01×10^{-3} DALYs pppy, respectively. Notably, the exposure risks of airborne *H. pylori* at non-operational points, including the OA and DW, had high values of $P_{(a)ill}$ and DB. Moreover, the exposure risks of OA are higher than DW. This is due to the fact that the daily office hours of office workers are more than 8 h. However, the DW had a higher risk of microbial inhalation owing to its proximity to the SDH and the former DS stockpile area, which was influenced by the SDH. In addition, male workers had a higher risk of exposure than female workers, which stems from their different respiration rates.

Figures 7C & D show the ranking and relative contribution of each input

parameter to the variability of infection risk and DB. The three parameters included the exposure concentration of *H. pylori* bioaerosol, the aerosol ingestion ratio, and exposure time. Among the model inputs, the exposure concentration of airborne *H. pylori* was the most sensitive to exposure risk. The correlation coefficient values ranged from 0.61 to 0.81 (Figure 7C), and the contributions ranged from 41.07% to 70.12% (Figure 7D). Therefore, the development of appropriate reduction technologies to control the fugitive concentrations of bioaerosols during wastewater treatment is essential to reduce the risk of exposure to workers. In addition, ventilation in indoor units, including the grille, MMH, and SDH, could be strengthened to reduce the bioaerosol concentrations.⁴⁵ The contribution of exposure time to exposure risk ranked second in the main operating units including the grille, MMH, and SDH. The average correlation coefficient values were 0.44, 0.54, and 0.58, and the average contributions were 20.55%, 32.16%, and 35.31%, respectively. Previous studies have reported findings that the probability of infection risk increases with increasing working hours,^{20,46} so worker rotation may be able to reduce the exposure of pathogens. Based on the calculation results, there is a certain risk of *H. pylori* infection in all major operational sections of the plant and even in the OA. Moreover, *E. Coli*'s HB values were used in this study instead of *H. pylori*, which should be less than the actual HB values for *H. pylori*. Large prospective studies are needed to identify *H. pylori* strains or subtypes associated with human diseases to determine the DB regarding *H. pylori*.

The evaluation used in this study may be inadequate. However, it reflects the risk of *H. pylori* exposure to mouth and nose through bioaerosols in MWTPs, in addition to the potential risk associated with its transmission



through water, soil, and feces. Although different from traditional respiratory diseases, the presence of airborne pathogens poses a greater health threat to some risk populations such as MWTP workers.⁴⁷ Integrating this risk into management allows for early detection and treatment, as well as prevention and control of its spread in risk workplaces. Prior to this, workers are required to take daily protective measures, such as wearing masks, washing hands carefully, and disinfecting frequently.⁴⁸ Moreover, further research is needed to investigate whether inhalation and exposure to aerosols can contribute to the increase in *H. pylori* infection among MWTP workers.

CONCLUSION

In this study, the pollution characteristics, variation patterns, and potential effects of *H. Pylori* in a typical MWTP were systematically investigated using field sampling and data analyses. *H. pylori* in the influent was at a median concentration of 1.24×10^8 CFU/L. After passing through the biological treatment units, most of the *H. pylori* was transferred to the ES with an average concentration of 4.22×10^8 CFU/kg. During wastewater treatment and sludge dewatering, a large amount of *H. pylori* was released into the air. SDH was the main unit of escape and accumulation. According to the QMRA results, the annual probability of illness risk and DB owing to occupational exposure far exceeded the related benchmark. Therefore, there is a need to strengthen

Figure 7. Exposure risk of airborne *H. pylori* (A) The annual probability of illness risk. (B) Disease burdens. (C) Tornado charts showing the ranking of input variables that affect the QMRA results. (D) Pie charts showing the contribution to variance of input parameters that affect the QMRA results.

disinfection in MWTPs, and staff should take protective measures to reduce exposure. Still, further research is necessary regarding the reduction and control of *H. pylori* in different media of MWTPs in order to reduce the potential risks to the environment and human health.

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AUTHOR CONTRIBUTIONS

Y.W. was involved in methodology, data processing and analyses and writing. Y.H. undertook conceptualization, methodology, funding acquisition, supervision and writing. W.W. performed research and made the visualizations. L.L. supervised and reviewed the manuscript. All authors contributed to and approved the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

DATA AND CODE AVAILABILITY

All data are available in the main text and/or the supplementary materials.

SUPPLEMENTAL INFORMATION

It can be found online at <https://doi.org/10.59717/j.xinn-geo.2024.100080>

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