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Opportunistic bacterial pathogens in bioaerosols emitted at municipal wastewater treatment plants, South Africa

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Aeration tanks at wastewater treatment plants (WWTPs) emit significant amounts of bioaerosols containing potentially hazardous infectious material. Occupational exposure to airborne pathogens can pose health risks to WWTP workers. Bioaerosol samples collected at aeration tanks of two typical municipal WWTPs that use different aeration modes were analysed to investigate the composition and diversity of airborne bacteria in wastewater environments, using the Illumina MiSeq platform. Thirty-six potential airborne bacterial pathogens were identified in the air samples, and these were dominated by *Bacillus*, *Enterococcus*, *Clostridium*, *Streptococcus*, *Acinetobacter*, *Enterobacter*, *Pseudomonas*, *Bacteroides fragilis*, *Acinetobacter baumannii*, and *Escherichia/Shigella*. Bioaerosols from mechanical aeration tanks (72%, 26/36) had a relatively higher richness and diversity of airborne bacterial pathogens than diffused aeration tanks (17%, 6/36). Furthermore, most of the identified airborne bacterial pathogens (78%, 28/36) were classified as Risk Group 2 according to the revised South African Regulation for Hazardous Biological Agents, 2022, and up to 70% of these were gram-negative bacteria. The presence of potentially pathogenic bacteria in the ambient air at WWTPs suggests an elevated risk of bioaerosol exposure for workers. Therefore, further research and site-specific risk assessments are recommended to guide the implementation of effective bioaerosol strategies to protect workers' health, with special attention paid to WWTPs that use mechanical aerators.

Keywords Airborne bacteria, Opportunistic pathogens, Occupational exposure, Municipal wastewater

Wastewater treatment plants (WWTPs) are a typical source of hazardous airborne biological particles, also known as bioaerosols[1]. Bioaerosols are primarily emitted from aeration tanks due to external forces such as aeration, mechanical agitation, and sludge dewatering. Other important sources of bioaerosols include bar screens, grit chambers, primary settling tanks, pump stations as well as sludge storage sites[1–3]. However, the conventional layout of WWTPs is not designed to prevent aerial dispersion of wastewater contaminants, thus posing an unavoidable risk to human health[2,4].

Bioaerosols emitted from wastewater contain different types of biologically harmful components, including bacteria, fungi, viruses, spores, allergenic pollen, and toxins among others[5]. Human exposure to bioaerosols has been associated with respiratory symptoms and impaired lung function[5]. Additionally, other studies have reported adverse health effects such as infections[6,7], allergies[8], acute toxic effects[9], and cancer[5]. Due to their microscale size (aerodynamic diameter smaller than 5 µm), bioaerosol particles can be disseminated by wind over distances of up to 10 km, and easily deposited into the respiratory tract[2]. Consequently, bioaerosol emissions at WWTPs could pose serious health risks to WWTP workers as well as residents of nearby communities[2].

A plethora of opportunistic bacterial pathogens have been identified in bioaerosols originating from WWTPs, including those belonging to the genera *Staphylococcus*, *Acinetobacter*, *Pseudomonas*, *Klebsiella*, *Mycobacterium,* and *Enterococcus* among others[4,10–13]. Various factors such as wastewater source, aeration

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technology used, inactivation rate of the bacteria, the amount of aerosolised material, meteorological conditions (e.g., temperature, sunlight, wind), and humidity may influence the composition, load, dispersion, and survival of aerosolised bacteria[14,15]. However, comprehensive analysis of bacterial communities in WWTP generated bioaerosols using high throughput sequencing technologies are scarce in many developing countries[10,11,16,17]. Since the 1970s, WWTP workers have commonly reported symptoms, also termed 'sewage worker's syndrome', fatigue, unexplained tiredness, headache, respiratory diseases, and fever among others ([18,19]. Most importantly, research has found a correlation between sewage worker's syndrome and bioaerosol emission from WWTPs[20]. Therefore, a comprehensive identification of pathogenic bacterial species in bioaerosols from WWTPs is crucial to provide scientific evidence on the causal agents associated with health outcomes among workers, and also provide a basis for the control and prevention of potential health risks posed by bioaerosols for protection of WWTP workers and nearby communities. Hence, the aim of the present study was to investigate bacterial communities in bioaerosols emitted from two municipal WWTPs in South Africa using high throughput sequencing to better understand potential occupational exposure to airborne bacteria. This approach provides a broad profile of bacterial diversity, including pathogenic bacteria.

Materials and methods

Study site description

Bioaerosol samples were collected at two municipal WWTPs in the City of Tshwane Metropolitan Municipality, South Africa. The two WWTPs primarily receive domestic wastewater and, to a lesser extent, wastewater from industrial and healthcare facilities. Poopedi et al.[21], previously outlined a detailed overview of the studied WWTPs. Briefly, the WWTPs comprise the following treatment stages: coarse screening, primary settling, biological treatment, secondary settling, and chlorine disinfection. The two WWTPs use different aeration systems for the biological degradation of organic contaminants. At WWTP1, mechanical aerators (MAER) are used for aeration in activated sludge tanks, where rotating blades introduce oxygen into the water by creating negative pressure on the rear side, causing the activated sludge to splash out into the air. This process releases droplets containing microorganisms, that may dehydrate and atomise to form bioaerosols. In contrast, at WWTP5, diffused aerators (DAER) are used, which provide oxygen through air bubbles released from the bottom of the aeration tank. As these bubbles rise through the water, they carry activated sludge flocs and rupture at the water–air interface, thus forming bioaerosols[11,22].

Bioaerosol sampling

Sampling was carried out on 18th October and 1st November 2022 at WWTP1, while WWTP5 was sampled on the 9th and 15th of November 2022. Airborne biological particles were collected into a sterile 20 mL phosphate buffered saline (PBS) (Sigma-Aldrich, MO, USA) using a stationary SKC Bio-Sampler (SKC Ltd, Blandford Forum Dorset, UK) at a flow rate of 12.5 L/min for 150 min per sample. The Bio-Sampler, mounted securely on a tripod stand 1.5 m above the ground to simulate the breathing zone of WWTP workers, was placed within 1 m to the aeration tank. The SKC Bio-Sampler collects bioaerosol particles with aerodynamic diameters ranging from 0.3 to 8 µm[23]. Air samples upwind of each plant were collected as background controls (BC). For quality assurance purposes, the pump flow rate was calibrated using an SKC rotameter (SKC Ltd, Blandford Forum Dorset, UK) before and after each sampling visit. A field control consisting of 20 mL PBS was processed alongside the samples to ensure the integrity of the sampling procedure. In addition, all sample collecting tools were decontaminated with 75% ethanol and rinsed with sterile water before every sampling event. Samples were transported to the National Institute of Occupational Health Waterborne Pathogen Unit laboratory on ice and stored at − 20 °C until further analysis. Meteorological conditions (e.g., temperature, relative humidity, and wind speed) at the sampling sites were obtained from the South African weather Service (Supplementary Table S1). These data were averaged over the region including the study site.

DNA extraction

Aliquots (2 mL) of the collected PBS samples were centrifuged (Mikro 22R, Hettich, Germany) at 14,000 × *g* for 10 min at 4 °C to concentrate bacterial cells. The supernatant was carefully removed, and genomic DNA was isolated from the pellet using DNeasy Powersoil Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Eluted DNA was stored at − 20 °C until further analysis.

16S rRNA sequencing and data analysis

The V3 and V4 hypervariable regions of the bacterial 16S rRNA gene were amplified using the universal primers (16S Forward Primer = TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG, and 16S Reverse Primer = 5′ GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC)[24]. The libraries were sequenced on the Illumina MiSeq instrument (Illumina, USA) using MiSeq reagent kit v3 (Illumina, USA) and 2 × 300 bp paired-end sequencing reads were generated. Raw reads were processed using DADA2 workflow[25]. The non-chimeric sequences were analysed for taxonomic classification with the silva_nr99_v138.1_wSpecies_train_set.fa.gz database (https://zenodo.org/record/4587955/files/silva_nr99_v138.1_wSpecies_train_set.fa.gz?download=1). The number of amplicon sequence variants (ASV) and alpha diversity indices (Shannon, Simpson and InvSimpson) were calculated using PhyloSeq R package.

Results

Alpha diversity of the air samples

A total of 657,203 quality reads were obtained for further downstream analysis. The number of Amplicon Sequence Variants (ASVs) generated per sample ranged from three (WWTP1-BC1) to 29 (WWTP1-MAER2).

Sample ID	Quality reads	ASV	Shannon	Simpson	InvSimpson
WWTP1-MAER1[a]	76,734	19	2.10	0.83	5.98
WWTP1-BC1[a]	113,638	3	0.10	0.04	1.04
WWTP1-MAER2[b]	81,995	29	2.29	0.87	7.42
WWTP1-BC2[b]	86,826	8	1.60	0.76	4.21
WWTP5-DAER1[c]	28,872	6	1.22	0.61	2.53
WWTP5-BC1[c]	90,791	7	0.93	0.56	2.26
WWTP5-DAER2[d]	102,117	4	0.12	0.04	1.04
WWTP5-BC2[d]	76,230	5	0.69	0.45	1.81

Table 1. Bacterial community diversity of air samples. Note: MAER: mechanical aerators, DAER: diffused aerators, BC: background control, ASV: amplicon sequence variants. [a]Sample collection date, 18th October 2022. [b]Sample collection date, 1st November 2022. [c]Sample collection date, 9th November 2022. [d]Sample collection date, 15th November 2022.

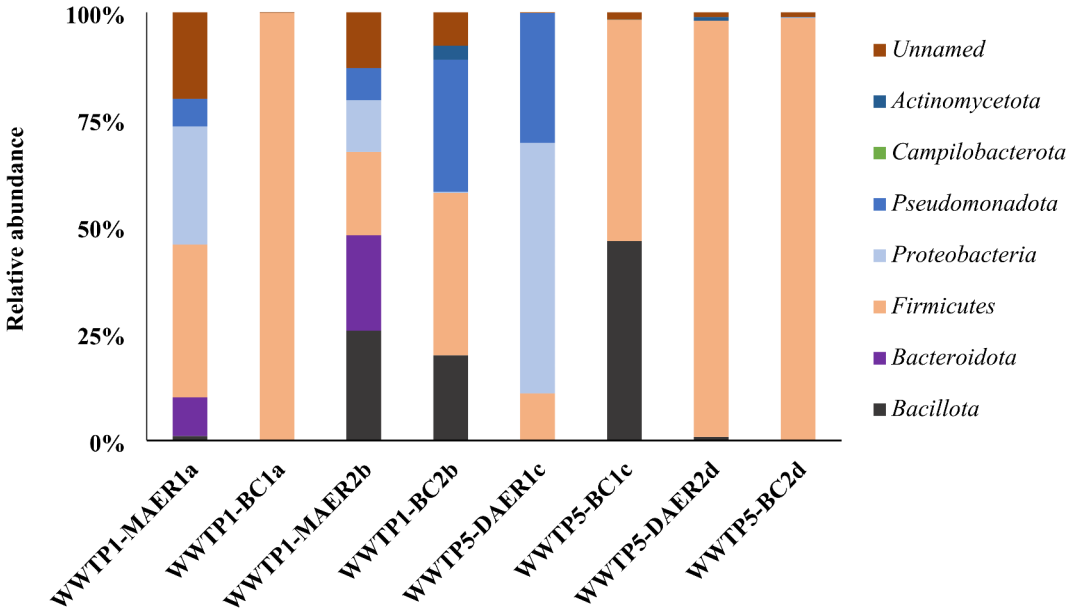


Fig. 1. Relative abundance (%) of phyla identified in bioaerosols from two municipal WWTPs. MAER: mechanical surface aerators; DAER: submerged aerators; BC: background control. [a]Sample collection date, 18th October 2022. [b]Sample collection date, 1st November 2022. [c]Sample collection date, 9th November 2022. [d]Sample collection date, 15th November 2022.

Analysis based on Shannon diversity revealed that the aeration tank (MAER) at WWTP1 had higher species diversity than aeration tank (DAER) at WWTP5 (Table 1). The Simpson and InvSimpson values for species richness and evenness ranged from 0.039–0.865 to 1.041–7.420, respectively. Both the Simpson and InvSimpson indexes revealed higher bacterial richness and evenness in the WWTP1 aeration tank (MAER) compared to DAER at WWTP5, and higher values were found for background control at WWTP1 than at WWTP5.

Airborne bacterial community composition

Seven phyla were identified across the eight bioaerosol samples as shown in Fig. 1. The three predominant phyla were *Firmicutes* identified in all the samples, followed by *Proteobacteria* and *Bacillota*, identified in six and five samples, respectively.

Figure 2 depicts nineteen families identified in the bioaerosol samples studied. *Bacillaceae* (8/8), *Moraxellaceae* (5/8), and *Clostridiaceae* (4/8) were the most frequently identified families detected in at least four samples. The remaining sixteen bacterial families were only identified in three or fewer air samples.

The results for bacterial communities at the genus level are illustrated in Fig. 3, highlighting the most abundant genera (more than 1% relative abundance in at least one sample). *Bacillus* was the predominant genus identified in all samples, followed by *Acinetobacter* (5/8), *Clostridium,* and *Enterococcus*, which were identified in four of the samples. Genera with relative abundance of at least 5% within samples were *Streptococcus* (29.2%), *Enterobacter* (14.8%), *Bacteroides* (9.0%), *Klebsiella* (6.4%), *Escherichia/Shigella* (6.2%), and *Acinetobacter* (6.0%) in WWTP1-MAER1; *Bacillus* (99.9%) was the only identified genera in WWTP1-BC1; *Bacteroides* (22.1%),

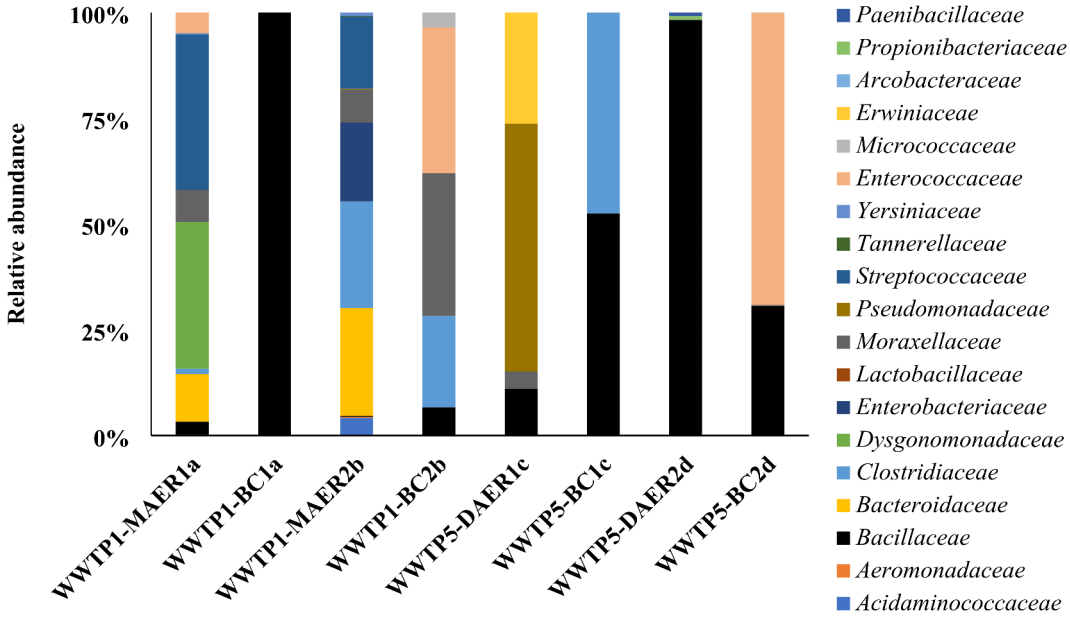


Fig. 2. Relative abundance (%) of the families identified in bioaerosols from two municipal WWTPs. MAER: mechanical surface aerators; DAER: submerged aerators; BC: background control. [a]Sample collection date, 18th October 2022. [b]Sample collection date, 1st November 2022. [c]Sample collection date, 9th November 2022. [d]Sample collection date, 15th November 2022.

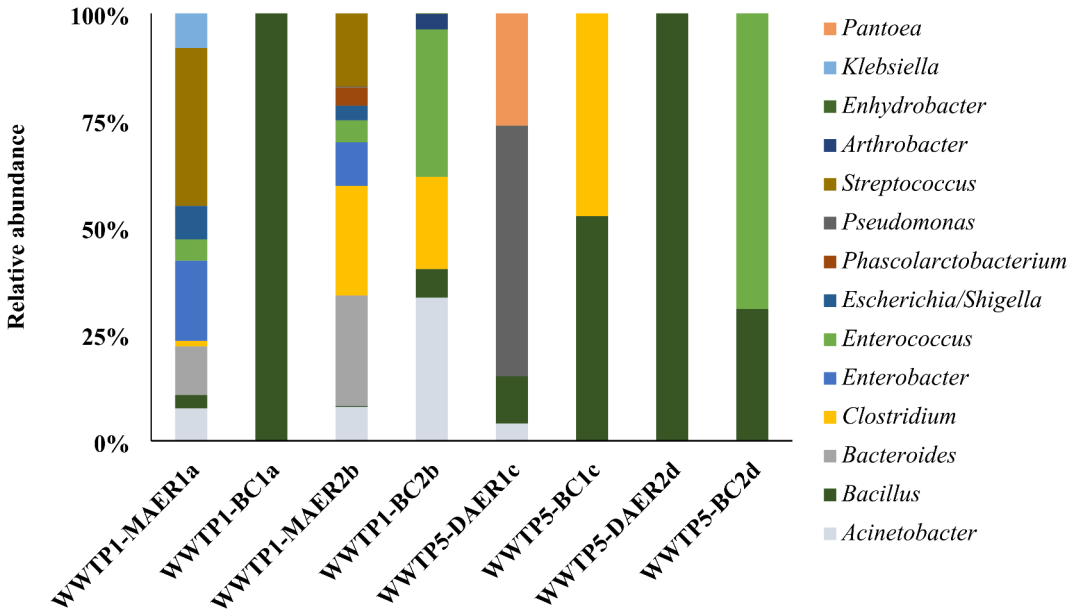


Fig. 3. Most abundant genera (> 1% relative abundance) in bioaerosols from two municipal WWTPs. MAER: mechanical surface aerators; DAER: submerged aerators; BC: background control. [a]Sample collection date, 18th October 2022. [b]Sample collection date, 1st November 2022. [c]Sample collection date, 9th November 2022. [d]Sample collection date, 15th November 2022.

Clostridium (21.9%), *Streptococcus* (14.7%), *Enterobacter* (8.7%), and *Acinetobacter* (6.8%) were dominant in WTP1-MAER2; *Enterococcus* (31.8%), *Acinetobacter* (30.9%), *Clostridium* (19.9%), and *Bacillus* (6.2%) were dominant in WWTP1-BC2. The genera *Acinetobacter, Aeromonas, Bacillus, Bacteroides, Clostridium, Enterobacter, Enterococcus, Escherichia/Shigella, Lactococcus, Serratia,* and *Streptococcus* were the most dominant bacteria in the bioaerosol samples at WWTP1 identified during both sampling events. In addition, *Acinetobacter, Bacillus, Clostridium* and *Enterococcus* were also identified in the background control at WWTP1, while *Arthrobacter* and *Enhydrobacter* were only identified in the background control. Regarding WWTP5 (DAER),

the dominant genera in WWTP5-DAER1 were *Pseudomonas* (58.5%), *Pantoea* (26.3%), and *Bacillus* (11.0%). *Bacillus* and *Clostridium* were the most abundant genera in WWTP5-BC1, accounting for 51.6 and 46.6% of the total relative abundance, respectively. *Bacillus* (97.1%) dominated in WWTP5-DAER2, *Enterococcus* (68.3%), and *Bacillus* (30.3%) were dominant in WWTP5-BC2. In general, bacterial communities in the bioaerosol samples at WWTP1 which uses mechanical aerators exhibited the most diverse genera, with 15 distinct genera compared to diffused aerators.

Shared and distinct genera in bioaerosols

A Venn diagram was used to explore shared and distinct bacterial genera in bioaerosols, and the results are presented in Fig. 4 and Supplementary Table S2 (genera list). A total of 26 genera across all samples were identified in the present study. Of these genera, *Acinetobacter* and *Bacillus* appeared across all sampling sites, *Clostridium* and *Enterococcus* were shared by WWTP1 employing mechanical aerators and the background control samples from both WWTPs. The genus *Pseudomonas* was shared among three samples namely, bioaerosol samples from both bioreactors utilising mechanical aerators (WWTP1) and diffused aerators (WWTP5), as well as background control samples from WWWT5. On the other hand, *Pantoea* was only common in samples from WWTP5. All sampling points had unique genera, albeit the MAER bioaerosol samples had the most, with 15 unique genera detected at this sampling point. Both background control samples had one unique genus with *Enhydrobacter* and *Arcobacter* identified at WWTP1 and WWTP5, respectively.

Potential opportunistic airborne bacterial pathogens present in the air at municipal WWTPs

Several potential opportunistic pathogenic genera were identified in the bioaerosol samples at the two WWTPs and in the background control. This study identified 36 potential airborne bacterial pathogens, which included 22 different genera and 18 species (Table 2). Twenty-eight potential opportunistic pathogens were detected in mechanical aerators bioaerosol samples at WWTP1, while six potential pathogens were identified at WWTP5 (diffused aerators). At WWTP1 and WWTP5, seven and ten potential pathogens, respectively, were identified in the background air samples. The most abundant (>1% relative abundance) potential airborne pathogens in this study were *Bacillus* spp. (44.6), *Enterococcus* spp. (13.2%), *Clostridium* spp. (8.8%), *Streptococcus* spp. (5.2%), *Acinetobacter* spp. (4.3%), *Enterobacter* spp. (2.8%), *Pseudomonas* spp. (2.4%), *Bacteroides fragilis* (2.1%), *Acinetobacter baumannii* (1.2%), and *Escherichia/Shigella* (1.1%) (Table 2).

As shown in Table 2, different potential opportunistic airborne pathogens dominated in individual bioaerosol samples. Opportunistic airborne pathogens *Streptococcus, Enterobacter, Bacteroides fragilis, Acinetobacter baumannii* and *Escherichia/Shigella* were abundant in WWTP1 aeration tank (MAER) samples. However, *Bacillus* and *Pseudomonas* were the main pathogens in WWTP5 aeration tank (DAER) samples. Background controls from the two WWTPs were dominated by pathogens *Bacillus* and *Clostridium*. Three pathogenic genera, *Acinetobacter, Bacillus* and *Pseudomonas*, were identified in the bioaerosol samples from the aeration tank area at both WWTPs. *Acinetobacter radioresistens*, *Arcobacter butzleri*, *Bacillus cereus*, *Clostridium perfringens* and *Arthrobacter* spp. were detected in the background control only and not in any of the samples from the aeration tanks. Furthermore, similar genera (*Acinetobacter*, *Bacillus*, *Clostridium*, and *Enterococcus*) were detected in the background control from both WWTPs.

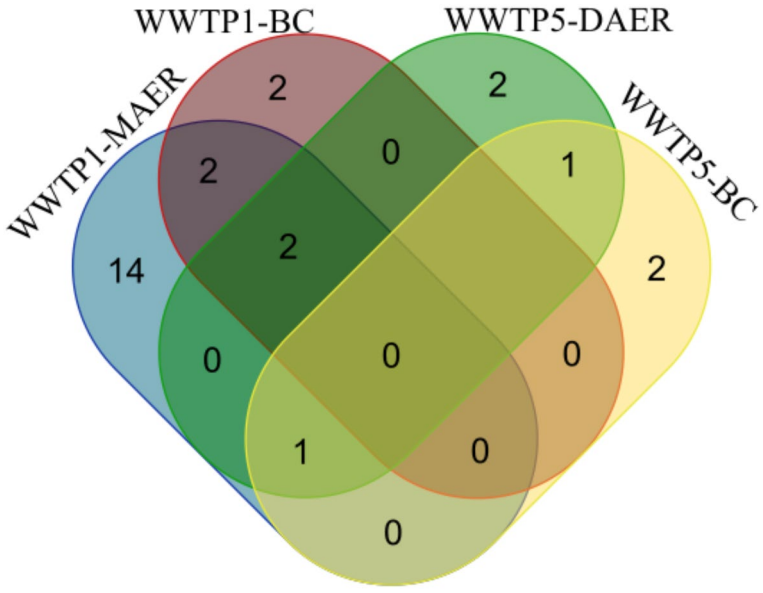


Fig. 4. Venn diagram showing airborne bacterial genera from aeration tanks with mechanical aerators and diffused aerators at WWTP1 and WWTP5, respectively. MAER: mechanical aerators; DAER: diffused aerators; BC: background control. Overlap indicates genera identified in the different sample locations.

Genera	Species	Total relative abundance (%)	Gram stain	HBA Risk group	WWTP5-DAER	WWTP5-BC	WWTP1-MAER	WWTP1-BC
1. *Acinetobacter*	*radioresistens*	0.2	-	2				
2. *Acinetobacter*	*baumannii*	1.2	-	2				
3. *Acinetobacter*	*soli*	0.04	-	2				
4. *Acinetobacter*	*junii*	0.03	-	2				
5. *Acinetobacter*	spp.	4.3	-	2				
6. *Aeromonas*	spp.	0.1	-	2				
7. *Arcobacter*	*butzleri*	0.02	-	2				
8. *Arthrobacter*	spp.	0.4	+	2				
9. *Bacillus*	spp.	44.6	+	2				
10. *Bacillus*	*cereus*	0.3	+	2				
11. *Bacteroides*	*uniformis*	0.02	-	2				
12. *Bacteroides*	*fragilis*	2.1	-	2				
13. *Bacteroides*	*ovatus*	0.03	-	2				
14. *Bacteroides*	*thetaiotaomicron*	0.2	-	2				
15. *Bacteroides*	spp.	0.3	-	2				
16. *Buttiauxella*	spp.	0.3	-	unclassified				
17. *Clostridium*	spp.	8.8	+	2				
18. *Clostridium*	*perfringens*	0.7	+	2				
19. *Enterobacter*	spp.	2.8	-	2				
20. *Enterococcus*	*faecium*	0.02	+	2				
21. *Enterococcus*	spp.	13.2	+	2				
22. *Escherichia/Shigella*	spp.	1.1	-	2 or 3				
23. *Klebsiella*	*pneumoniae*	0.3	-	2				
Klebsiella	spp.	0.5	-	2				
Lactobacillus	spp.	0.003	+	unclassified				
Lactococcus	spp.	0.03	+	unclassified				
Pantoea	*ananatis*	0.6	-	unclassified				
Pantoea	spp.	0.5	-	unclassified				
Propionibacterium	*acnes*	0.1	+	2				
Pseudomonas	*aeruginosa*	0.01	-	2				
Pseudomonas	spp.	2.4	-	2				
Raoultella	*ornithinolytica*	0.01	-	unclassified				
Raoultella	spp.	0.02	-	unclassified				
Serratia	spp.	0.1	-	2				
Streptococcus	spp.	5.2	+	2				
Vagococcus	*fluvialis*	0.01	+	unclassified				

Table 2. Relative abundance of potential opportunistic airborne bacterial pathogens and their risk group evaluation.
Note: Red shade = detected with > 1% relative abundance; orange shade = detected with < 1% relative abundance; no shade or color = not detected.

Risk group classification of the identified opportunistic bacterial pathogens

Twenty-eight bacterial pathogens (89% of the total relative abundance) identified were classified as hazardous biological agents (HBAs) according to the revised South African Regulation for Hazardous Biological Agents, 2022 (Table 2)[26]. Out of the identified HBAs, 27 organisms belong to Risk group 2 agents (organisms that may cause disease but are unlikely to spread to the community and have effective treatment in place). *Escherichia/Shigella* belong to Risk group 2 or 3 depending on the species types. Risk Group 3 HBAs may cause severe disease, presenting a risk of spreading to the community; nevertheless, effective therapy is available. The identified airborne pathogens were further differentiated based on their cell wall properties. Up to 67% (24/36) of the identified bacteria in the bioaerosol samples from aeration tanks and background control of the studied WWTPs were gram-negative bacteria.

Discussion

Utilising high-throughput sequencing, this study assessed the bacterial communities of bioaerosols emitted from two municipal WWTPs that use different aeration modes. The findings shed light on a broad profile of

bacteria, including potential opportunistic pathogenic bacteria, present in the air at WWTPs. The results of this study demonstrated that bacterial composition of bioaerosols generated by MAER was diverse when compared to bioaerosols generated by DAER. These findings are further supported in a recent study where significantly higher bacterial diversity was observed in bioaerosols produced by MAER than the background air[27]. The study further indicated that rotor speed influences species composition, size distribution, and concentration of bioaerosols, and that the levels of bacteria in the air increased with accelerated rotational speed[27]. The secondary treatment step is a key component of the wastewater treatment process, aimed at biologically removing organic components in wastewater[28]. Mechanical (e.g. rotors, fountains, agitators and others) and diffused (e.g. fine and coarse bubble diffusers) aerator systems are commonly utilised to aerate wastewater in aeration tanks, resulting in bioaerosol emission due to intense mixing and turbulence. The type of aeration system used determines the size of bubbles formed, which in turn influences the size of the bioaerosol and its microbial load[16,22]. Additionally, distinct genera were identified in the background controls compared to aeration tanks samples. Differences in bacterial composition across different treatment stages in WWTPs are primarily influenced by factors including mechanical processes (e.g., aeration and agitation), meteorological conditions (e.g., temperature, humidity, solar radiation, and wind), and sources of bacteria (e.g., wastewater, sludge, and background atmospheric sources)[13,15]. Therefore, it is plausible that bacteria from other sources unrelated to wastewater may have been dominant in the background control samples, contributing to the observed differences in bacterial species.

Although the bioaerosol data in this study are presented as relative abundances rather than quantitative measurements, the inclusion of environmental metadata provides a valuable context for interpreting the results. Environmental factors such as relative humidity, temperature, wind and UV index are known to influence bioaerosol dynamics and could partially explain the observed shifts in relative abundance[13,15]. For example, a study investigating bioaerosols from six municipal WWTPs in China demonstrated that meteorological factors, particularly temperature, solar radiation, and relative humidity influence the survival and dispersion of airborne microorganisms[29]. Temperature and relative humidity directly affect bioaerosol dispersal and viability of airborne microorganisms, thus affecting the concentration of bioaerosols. Wind dilute and reduce bioaerosol concentrations, whereas poor ventilation may lead to significant accumulation of microbial aerosols in indoor treatment units[13,15]. Since the environmental metadata is averaged over a larger area, it may not perfectly represent microclimatic conditions at the study site. However, the data provides a reasonable approximation of the prevailing environmental conditions at the studied areas during the sampling period.

In this study, majority of potentially opportunistic pathogenic bacterial genera and species were identified in MAER bioaerosols. These findings are comparable with previous studies that explored bacterial composition in bioaerosols from WWTPs using different aeration modes[16,22,27]. Intestinal bacteria in bioaerosols from six municipal WWTPs in China were substantially higher in mechanical aerators than biochemical reaction tanks utilising diffused aerators[11]. Similarly, Han and co-workers (2020) indicated that mechanical aerators (horizontal rotors) contributed the most total microorganisms and specific pathogens in bioaerosols when compared to fine bubble diffusers in the same WWTP[22]. In a recent study, Lu et al.[4] collected air samples from the grit chamber house and near the aeration tank at a municipal WWTP in Denmark, identifying 91 and 94 bacterial species, respectively, with five Risk Group 2 species (*Enterobacter cloacae*, *Klebsiella oxytoca*, *K. pneumoniae, Escherichia coli*, and *Morganella morganii*) present at both sites.

Potentially pathogenic genera in this study were dominated by *Bacillus* spp., *Enterococcus* spp., *Clostridium* spp., *Streptococcus* spp., *Acinetobacter* spp., *Enterobacter* spp., *Pseudomonas* spp., *Bacteroides fragilis*, *Acinetobacter baumannii,* and *Escherichia/Shigella*. These bacterial species can cause skin, community acquired pneumonia, gastrointestinal, and urinary tract infections (UTI) in humans. Previous studies have found similar pathogenic bacteria in bioaerosols from WWTPs[4,11–13,16,17]. *Bacillus* species are common in soil and wastewater, and the species of medical importance are *Bacillus anthracis* and *Bacillus cereus*, which are responsible for anthrax and food poisoning, respectively[30]. *Enterococcus* species can cause UTI, meningitis, infective endocarditis (IE), and wound infections[31]. Clostridial infections, on the other hand, are typically associated with food poisoning, gas gangrene, botulism, and soft tissue infections[32]. *Acinetobacter* species can cause infections of the respiratory tract, blood and urinary tract, particularly in immuno-compromised individuals. Of note, *A. baumannii* is one of the ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* spp.) pathogens primarily associated with hospital-acquired infections as well as antimicrobial resistance[33]. *Streptococcus* species can cause invasive diseases such as sepsis, meningitis, IE, and pneumonia[34], whereas the *Enterobacter* genus is a well-known nosocomial pathogen that causes UTI, osteomyelitis, IE, respiratory, and soft tissue infections[35]. *Pseudomonas aeruginosa* causes the majority of *Pseudomonas* infections, and can infect virtually any tissue, including the blood, central nervous system, and lungs, among others[36]. *Bacteroides fragilis* can cause peritonitis, lung and brain abscesses, bacteremia, soft tissue infections, and toxin-associated diarrhoea[37]. Common infections of *Escherichia* species include UTI, pneumonia, bacteremia, and acute enteritis[38], whereas *Shigella* is a well-known cause of acute gastrointestinal infections[39].

Twenty-seven human bacterial pathogens identified in this study are classified as Risk Group 2, whereas *Escherichia/Shigella* belong to Risk group 2 or 3[26]. Overall, this study suggests that WWTP workers are potentially exposed to airborne bacterial pathogens that can cause a range of diseases such as respiratory and gastrointestinal infections, UTI, and skin and soft tissue infections. Five species (*E. faecium*, *A. baumannii, K. pneumoniae, P. aeruginosa, and Enterobacter* spp.) that are highly virulent and associated with multidrug resistance were also identified. Furthermore, a majority of the classified bacterial pathogens were gram-negative bacteria, suggesting that workers at WWTPs may be exposed to higher levels of inhalable endotoxins present in their ambient air, potentially leading to chronic diseases such as bronchitis, asthma, wheeze, and organic toxic dust syndrome after long-term exposure[40]. This highlights the importance of effective bioaerosol control strategies to safeguard the health of WWTP workers. This could include engineering controls such as covering aeration tanks, particularly at WWTPs using mechanical aerators, to minimise bioaerosol generation and reduce

the concentration of bioaerosols in the air. In addition, implementing worker exposure controls such as strict adherence to PPE and administrative measures, such as work schedule adjustments and regular training, can further mitigate the risk of bioaerosol exposure among WWTP workers.

Conclusion

The diversity of bacterial composition in bioaerosols varied depending on the mode of aeration at the WWTPs. Furthermore, mechanical aeration tanks exhibited relatively higher richness and diversity of airborne bacterial communities and specific pathogens compared to diffused aeration tanks. Twenty-seven human bacterial pathogens near aeration tanks and background control, classified as Risk Group 2, with *Escherichia/Shigella* potentially belonging to Risk Group 3 were identified. Among these, highly virulent species associated with multidrug resistance were identified, underscoring the urgent need for effective bioaerosol control strategies and worker exposure protocols to safeguard the health of WWTP workers. The predominance of gram-negative bacteria suggests a higher risk of inhalable endotoxin exposure, potentially leading to chronic respiratory conditions. Overall, our findings underscore the critical importance of addressing bioaerosol exposure in WWTP environments to mitigate health risks and ensure worker safety. Future studies should conduct comprehensive investigations to identify additional potentially harmful components in bioaerosols such as viruses, fungi, allergens, and antibiotic-resistant genes, as these may pose serious health risks to humans. Additionally, long-term studies are important for understanding the spatial and temporal variation of bioaerosols. Finally, conducting site-specific risk assessments with a few selected dominant bacterial pathogens determined by culture methods will enable a complementary and more accurate evaluation of the health risks posed by bioaerosols to WWTP workers.

Data availability

The datasets generated and analysed during the current study are available in the NCBI BioProject repository (BioProject ID: PRJNA1182964), https://www.ncbi.nlm.nih.gov/PRJNA1182964.

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Author contributions

E.P: Conception and design of study, Methodology, Acquisition of data, Analysis of data, Writing—original draft, and Writing—review and editing. R.P: Analysis of data, and Writing—review and editing. T.S: Conception and design of study, Resources, Supervision, and Writing—review and editing. A.G: Conception and design of study, Funding acquisition, Methodology, Resources, Supervision, and Writing -review and editing. All authors reviewed and approved the manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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