MICROBIAL AGENTS OF CONCERN DETECTED IN WATER AND AIR AT THE HUDSON RIVER ESTUARY WATERFRONT

A Final Report of the Tibor T. Polgar Fellowship Program

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ABSTRACT

Despite building evidence of the transfer of bacteria from contaminated water surfaces to coastal aerosols, the presence of antibiotic resistant bacteria and sewage indicating bacteria in the air in urban waterfronts has not been sufficiently studied. This represents an understudied route of pathogen exposure to human populations in proximity to sewage-contaminated surface waters. To bridge this gap, this study deployed new methodologies to detect and taxonomically identify these microbial agents of concern in the lower Hudson River Estuary (HRE). Aerosol and water surface samples were collected from the Newtown Creek Nature Walk, which is adjacent to a highly-urbanized tributary of the lower HRE, to assess whether microbial agents of concern were present and detectable. Antibiotic resistant bacteria and sewage indicators were detected in both water and air on each sampling day at this site. This occurred despite the fact that viable bacterial fallout was lower at this site than other previously studied waterfronts on the HRE. The percentage of antibiotic resistance in bacterial aerosols (~20%) was significantly higher than in surface water bacteria (~8%). Molecular analysis of the 16S rRNA gene of viable bacteria sampled revealed a diverse bacterial community with a wide range of possible sources, including water, land, and sewage. Many of the resistant aerosols and water bacteria were members of genera known to contain human pathogens, including *Massilia, Pseudomonas*, and *Roseomonas*. These findings greatly expand the potential public health implications for sewage contamination in the urban coastal environment, adding aerosol exposure as a potential route for human contact with sewage-associated microbial agents of concern.

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INTRODUCTION

Over 39% of the US population now lives near a coastline, and wastewater inputs to rivers, estuaries and marine waters have been increasing (Niemi et al. 2004; NOAA 2013). Raw sewage releases contain many pathogenic bacteria (Tourlousse et al. 2008; Korzeniewska et al. 2009) and antibiotic resistant bacteria (Kim et al. 2010; Young et al. 2013), creating potential public health risks for human contact with these waters. Direct contact with contaminated waters (ingestion, skin contact) is known to lead to illnesses including gastroenteritis, conjunctivitis, and wound infections (US EPA Office of Water 2012); however, contact through inhalation of aerosols created from contaminated waters is an additional and understudied human exposure pathway. Aerosols can be created from water surfaces by surface disruption such as wind-wave interactions, wave-shore interactions, and the recreational use of water (Monahan et al. 1983; Blanchard 1989; de Leeuw et al. 2000). This results in the movement of water surface materials to the air, which may include viable bacteria, as established in previous studies of coastal environments including the Hudson River Estuary (HRE) (Aller et al. 2005; Dueker et al. 2012a; Dueker et al. In Review).

This water-air movement of materials creates the opportunity for airborne human exposure to microbial agents of concern, including sewage pathogens and antibiotic-resistant bacteria, when raw sewage is present in urban surface waters. Research conducted at Louis Valentino Pier (LVP) in Red Hook, Brooklyn showed that the number of culturable bacteria in the air correlated with the culturable bacteria residing in surface waters (Dueker et al. In Review). Untreated sewage released to estuarine and coastal environments gets restricted to the surface in a density-stratified layer.

Therefore, any surface disruption of contaminated waters may cause the release of raw sewage materials into the atmosphere as indicated by previous research conducted at Newtown Creek (Brooklyn, NY) (Dueker et al. 2012a).

Enterococci are routinely used as an indicator of human and animal fecal waste in HRE surface waters and are associated with microbes that are of serious public health concern (US EPA Office of Water 2012). Although *Enterococcus* presence in HRE surface waters has been closely monitored, there is no current literature outlining their presence in HRE waterfront air. Several studies have investigated the negative public health prospects of aerosol exposure to aerosolized sewage microbes in relation to sewage treatment plants (Woodcock 1955; Muscillo et al. 1997; Brandi et al. 2000; Carducci et al. 2000; Radke 2005; Baertsch et al. 2007; Heinonen-Tanski et al. 2009; Korzeniewska et al. 2009); however, the presence of these bacteria and their dominant source in urban aerosols is a novel field of research.

Over the recent past the public has become increasingly alarmed by new scientific findings of connections between the overuse of antibiotics in both medicine and the agriculture–agrifood industry and the environmental emergence and spread of antibiotic-resistant bacteria (Nikiforuk 1996; Feinmen 1998; Levy 1998; Khachatourians 2013). Microbial resistance to antibiotics is on the rise. With fewer new chemotherapeutic agents coming onto the market, the problem of microbial resistance to drugs already in use has become a crisis in health care (Jungkind et al. 1995; US Office of Technology Assessment 1995). Previous studies conducted on antibiotic resistant microbes in aerosols have been restricted to indoor hospital environments (King et al. 2013; Muzslay et al. 2013) and agricultural environments

(Liu et al. 2012; Schulz et al. 2012), where antibiotic resistant microbes are found to travel in viable state through the air.

Based on a recent study, high concentrations of *Enterococci* (indicating the presence of raw sewage) in HRE surface waters were directly related to concentrations of ampicillin and tetracycline-resistant bacteria in surface waters (Young et al. 2013). Given the high frequency of raw sewage release to HRE water (Riverkeeper 2011) and the documented transfer of surface water materials to the air in coastal regions (Dueker et al. 2011; Dueker et al. 2012a; Dueker et al. 2012b) the potential exists for aerosolization of antibiotic resistant and sewage-associated bacteria when raw sewage is present. Despite these findings, little to no research has been conducted on the presence and potential sources for antibiotic resistant bacteria in outdoor air.

To address this gap, microbial agents of concern in the air and water were quantified and identified at a highly-urbanized lower HRE waterfront site. The goals of this study were to determine if microbial agents of concern were detectable in urban air and adjacent surface waters and to make a preliminary assessment of possible sources for these agents. The outcome of this study provides a unique dataset confirming the presence of microbial agents of concern both in water and air at the highly-urbanized lower HRE waterfront. These findings highlight the potential for unique pathways of exposure that may connect people to aquatic pollution, via aerosolized microbial agents of concern.

METHODS

Study Site and Meteorology

Field sampling took place on five full days between 5 June 2013 and 4 July 2013 at the Newtown Creek Nature Walk (NCNW) (40.7368528 N, 73.9464472 W), a public park adjacent to the Newtown Creek Water Treatment Facility. Newtown Creek, once one of the busiest hubs of NYC industrial activity, is a 3.5 mile creek forming the northern and southern borders of Brooklyn and Queens has been heavily industrialized and traveled since the mid 1800's (US EPA 2010). Newtown Creek was declared an EPA Superfund site in September 2010 and is known to have frequent sewage contamination (including sewage-indicators and antibiotic resistant bacteria) of surface waters (Riverkeeper 2011; Young et al. 2013).

A portable Vantage Pro2 Plus Weather Station (Davis Instruments, Hayward, CA) was used to measure meteorological conditions including air temperature, wind speed, and relative humidity. Total aerosol particle size and concentration was measured using a stationary Met One 9012 Ambient Aerosol Particulate Profiler (Met One Instruments, Grants Pass, OR). The profiler was placed about 3 m above water level (depending on tidal height). During each aerosol sampling event, water surface salinity and temperature were measured using a ThermoScientific Orion Star Portable Multiparameter Meter (ThermoScientific, Waltham, MA).

Bacterial Aerosols

Culturable bacterial aerosol fallout was measured at the site during onshore wind conditions by exposing triplicate agar plates per media type to ambient aerosols on a

platform oriented into the wind. This sampling method (Dueker et al. 2011; Dueker et al. 2012a; Dueker et al. 2012b) is not representative of the total concentration of bacteria in the air, but has the advantage of enumerating bacteria that are viable and able to grow on the provided media at the time of exposure. Four types of media (each deployed in triplicate during each exposure event) were used in these exposures: Reasoner´s 2A agar (R2A) media (Fisher Scientific), R2A amended with ampicillin (50 mg/L) and R2A amended with tetracycline (10 mg/L) and MEI (Membrane Enterococcus Indoxyl-β-D-Glucoside) Agar (manufacturer-prepared plates, Molecular Toxicology, Inc.). R2A has commonly been used in past aerosol studies to detect a broad spectrum of bacterial species (Lighthart and Shaffer 1995; Shaffer and Lighthart 1997) and MEI Agar selects for *Enterococci*, which are bacteria used as indicators of sewage presence as per the EPA (US EPA Office of Water 2012).

Before each sampling day, 15 exposure plates were prepared for each media type using aseptic technique in a laminar flow hood. For each media type, three plates served as field controls, lab controls, 15-minute field exposures, 30-minute field exposures and 90 minute field exposures. After exposure in the field, all control and exposure plates were incubated for three days at 25 °C in the dark, then colonies growing on antibiotic-amended plates were transferred to fresh, unexposed antibiotic-amended media plates to ensure antibiotic resistance. After five days total incubation, CFUs (Colony-Forming Unit) on all incubated plates (control, exposure, and transfer plates) were counted and then picked for future molecular analyses by transferring colony material to sterile HyClone water (ThermoScientific, Waltham, MA). Bacterial aerosol fallout rate (CFU m^{-2} s^{-1}) for each exposure event was calculated using plate counts, the surface area of the

exposed petri dishes (0.0079 m^2), and the duration of exposure.

Surface Water Bacteria

Surface water was sampled by collecting water in 50-ml centrifuge tubes that were rinsed three times with sample water prior to collection. Immediately after sample collection, tubes were kept in the dark and on ice until returned to the lab. To assess culturable bacterial concentrations in surface waters, a series dilution was created at a 1:10, 1:100 and 1:1000 dilution of sample water with autoclaved and filter-sterilized HRE water. A 100 μL aliquot of each dilution was spread onto triplicate R2A, R2A+Amp, and R2A+Tet plates in a laminar flow hood. Plates were incubated for five days and CFUs enumerated and picked for molecular analysis as outlined for aerosol plates above.

The presence of sewage-associated bacteria was inferred from the presence of the indicator organism *Enterococcus* in surface waters and aerosols as outlined by EPA regulations (US EPA Office of Water 2012). Colony growth on MEI selective media exposures was enumerated after aerosol exposure (exposed simultaneously with R2A plates above). After exposure, these plates were incubated in the dark at 41°C for 24 hours, after which CFUs were counted, picked for molecular analysis, and transferred to fresh MEI plates to further confirm media selectivity.

Water-surface samples were processed using the EPA-approved IDEXX Enterolert system (IDEXX Laboratories, Westbrook, ME) (Riverkeeper 2011; Suter et al. 2011). Briefly, within 6 hours of collection, surface water was diluted 1:10 using autoclaved, filter-sterilized Newtown Creek surface water, and then added to liquid media before being sealed in a Quanti-tray (IDEXX) and being incubated in the dark at

41°C for 24 hours. After incubation, growth of *Enterococci* was confirmed and quantified by exposing the Quanti-tray to UV light and recording the number of wells fluorescing blue. This resulted in the most probable number (MPN) of *Enterococci* per ml of sample water.

Molecular Analyses for Taxonomic Identification of Resistant Bacteria

To gain a preliminary understanding of the types of antibiotic resistant bacteria present in waterfront aerosols and surface waters, colonies were picked from at least one antibiotic-amended, aerosol-exposed and water surface media plate per sample date and suspended in 50 μL of HyClone sterile water. This material was then boiled for five minutes to lyse cells and frozen until polymerase chain reaction (PCR) analysis was performed.

The 16S rRNA gene from DNA of lysed cells was amplified using universal bacterial primers 8F (5′-AGRGTTTGATCCTGGCTCAG-3′) and 1492R (5′-CGGCTACCTTGTTACGACTT-3′) (Teske et al. 2002). Thermocycling conditions consisted of 35 cycles: 45s denaturation at 94°C, 45s of annealing at 55 ° C, and one min elongation at 72 ° C. After PCR, gel electrophoresis was performed on PCR products to ensure correct length of amplified fragments and that controls did not amplify. Amplifications were then sent for single-pass Sanger sequencing to SeqWright Inc. (Houston, TX). The sequence output files were edited using the Geneious software package (www.geneious.com), exported in FASTA format and uploaded to the Ribosomal Database Project (RDP) webserver (http://rdp.cme.msu.edu/) for alignment and taxonomic classification to the level of genus with 80% confidence unless otherwise noted.

A GenBank top-hits analysis (Dueker et al. 2012a) was performed on aerosol sequences to assess potential sources for these bacteria. Briefly, sequences were blasted against the GenBank database using Geneious' Megablast function, and the sequence hit with the highest bit-score was designated the top hit for each sequence. The environmental source reported for this top hit was then recorded.

RESULTS

During sampling days the mean velocity of onshore winds was very low, at 1.4 ± 0.3 m s^{-1}, the average temperature was 23 ± 1 ºC and average relative humidity was 66 ± 4% (Table 1). Average water temperature was 22 ± 0.5 ºC, and average salinity was 16.9 ± 0.4 ppt (Table 2). *Enterococci* were present in surface waters every sampling day, but only exceeded EPA standards (EPA threshold for safe water contact = 104 cells/100 ml for single-sample values) on 4 July 2013, when it exceeded the threshold by an order of magnitude at 9,104 cells/100 ml (Table 2). These high sewage-indicator concentrations followed a heavy rain event occurring late afternoon/evening of 3 July 2013 (Table 2).

Table 1. Meteorological context at Newtown Creek Nature Walk on sampling days. Mean of parameter during aerosol exposures, ± 1 standard deviation.

Sample Date	Temp. (°C)	Rel. Humidity (%)	Wind Speed (m s^{-1})	Fine Aerosols (m^{-3})	Coarse Aerosols (m^{-3})
5-Jun-13	21 ± 1.0	46 ± 4.4	1.4 ± 0.3	20,891 ± 3,877	292 ± 33
19-Jun-13	20 ± 0.7	62.1 ± 1.8	2.5 ± 0.5	57,759 ± 1,896	237 ± 16
20-Jun-13	22 ± 1.1	59 ± 3.6	1.2 ± 0.2	58,701 ± 2,557	340 ± 14
3-Jul-13	26 ± 1.0	80.1 ± 3.6	0.8 ± 0.1	32,286 ± 3,524	747 ± 94
4-Jul-13	26.4 ± 1.1	83.3 ± 4.5	1.2 ± 0.5	86,660 ± 18,759	761 ± 255

Table 2. Surface water quality at Newtown Creek Nature Walk on sampling days. Mean of parameter ± 1 standard deviation. Italics, bolded numbers in *Enterococcus* column indicate that *Enterococcus* concentrations exceeded EPA standards for single-sample values (104/100 ml).

Sample Date	Temp. (°C)	Salinity (ppt)	*Enterococci* (100 ml^{-1})	Days Since Rain (> 0.1 inch)	3-day Rain Total (inches)
5-Jun-13	20.5 ± 0.2	21.1 ± 0.5	20 ± 10	2	1.72
19-Jun-13	20.5 ± 0.5	17.1 ± 0.1	67 ± 13	1	0.29
20-Jun-13	21 ± 0.8	17.6 ± 0.2	3 ± 6	2	0.3
3-Jul-13	23.1 ± 0.4	16.4 ± 0.5	31 ± 32	2	0.92
4-Jul-13	24.9 ± 0.7	12.2 ± 0.8	**9,104 ± 4,634**	1	1.45

Microbial fallout on R2A-only (no antibiotics) plates was significantly lower at NCNW in comparison to the English Kills portion of Newtown Creek (Dueker et al. 2012a) and Louis Valentino Pier (Dueker et al. In Review) (Fig. 1). Antibiotic-resistant bacteria (Fig. 2) and sewage-indicating bacteria were detected in water (Table 2) and aerosol (Table 3) samples on each sampling day at this site. Colonies that initially grew on exposed ampicillin plates transferred to new, unexposed R2A+Amp plates with 100% success, confirming ampicillin resistance. The same was true for R2A+Tet plates that were exposed for 15 or 45 minutes, but not for 90 minute exposures. R2A+Tet plates exposed for 90 minutes had < 60% transfer success rates, suggesting that the sun was breaking down the tetracycline during 90 minute exposures. Data and colonies from 90-minute R2A+Tet exposure plates were therefore excluded from further analyses. All colonies growing transferred to new MEI plates with 100% regrowth.

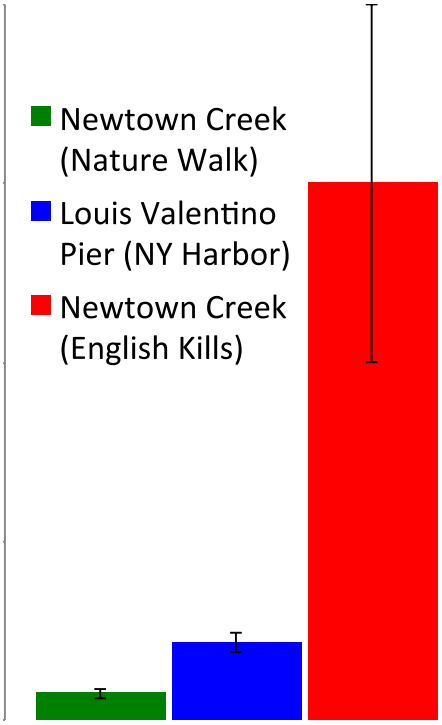

Figure 1. **Microbial fallout on R2A plates (no antibiotics) at Newtown Creek Nature Walk compared to previous studies conducted at Louis Valentino Pier (Dueker et al., In Review) and Newtown Creek English Kills (Dueker et al 2012b)**

Table 3. ***Enterococcus*** **growing on exposed MEI agar plates, with transfer plate (selection confirmation) and molecular analysis results. ND = Not Determined (sequencing not yet performed).**

Sample Date	# CFU's Detected	# Transfer Positive	Taxonomy
5-Jun-13	2	2	*Enterococcus faecalis*
19-Jun-13	1	1	ND
20-Jun-13	1	1	ND
3-Jul-13	1	1	*Enterococcus faecalis*
4-Jul-13	1	1	*Enterococcus faecalis*

The percentage of bacterial fallout made up of antibiotic resistant bacteria was significantly higher than the percentage of antibiotic resistant bacteria in the water ($p < 0.01$ for both R2A+Amp and R2A+Tet) (Figure 2). With the exception of exposures conducted on 4 July 2013, bacterial fallout and surface water concentrations were linearly related for R2A ($R^2 = 0.75$), R2A+Amp ($R^2 = 0.48$), and R2A+Tet ($R^2 = 0.69$) (Fig. 3).

Molecular analysis conducted on colonies grown on MEI plates confirmed that they were formed by *Enterococcus faecalis* (Table 3). After quality control, a total of 140 sequences from antibiotic resistant bacteria were used in analyses, 17 from aerosol exposures and 123 from surface waters. RDP classification of these sequences revealed a taxonomically diverse community of antibiotic-resistant bacteria in both water and air, with *Aeromonas, Acinetobacter*, and *Acidovorax* dominating surface water resistant

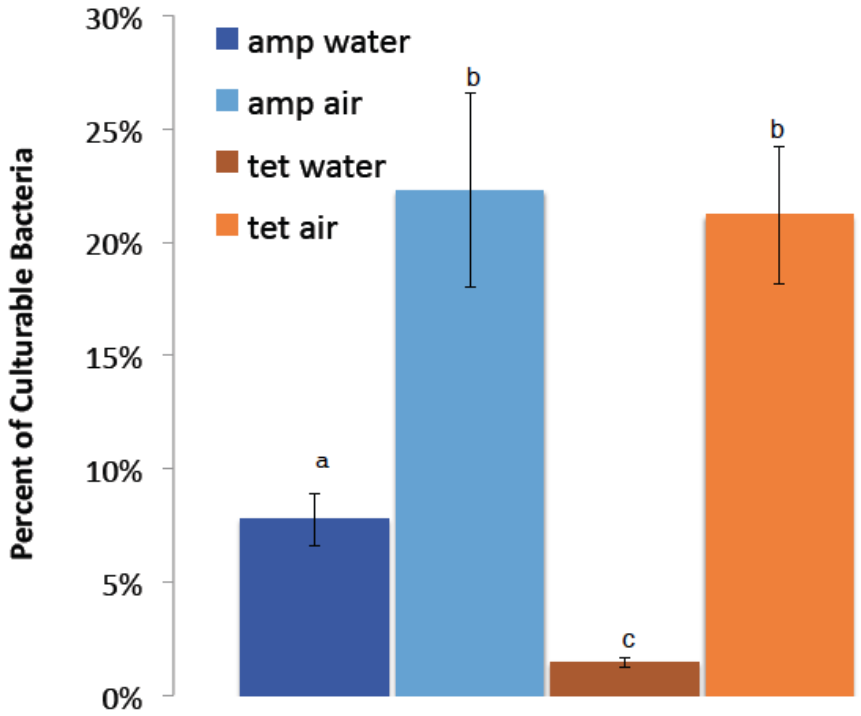

Figure 2. **Percentage of antibiotic resistance in surface water bacteria and bacterial aerosols (amp = ampicillin, tet=tetracycline). Black bars indiate standard error of geometric mean, letters above bars indicate significant difference ($p < 0.01$).**

bacteria and *Roseomonas* and *Bacillus* dominating viable resistant bacterial aerosols (Table 4). The GenBank top-hits analysis of the resistant aerosols resulted in a broad range of possible sources, including soil, water, and sewage (Table 5).

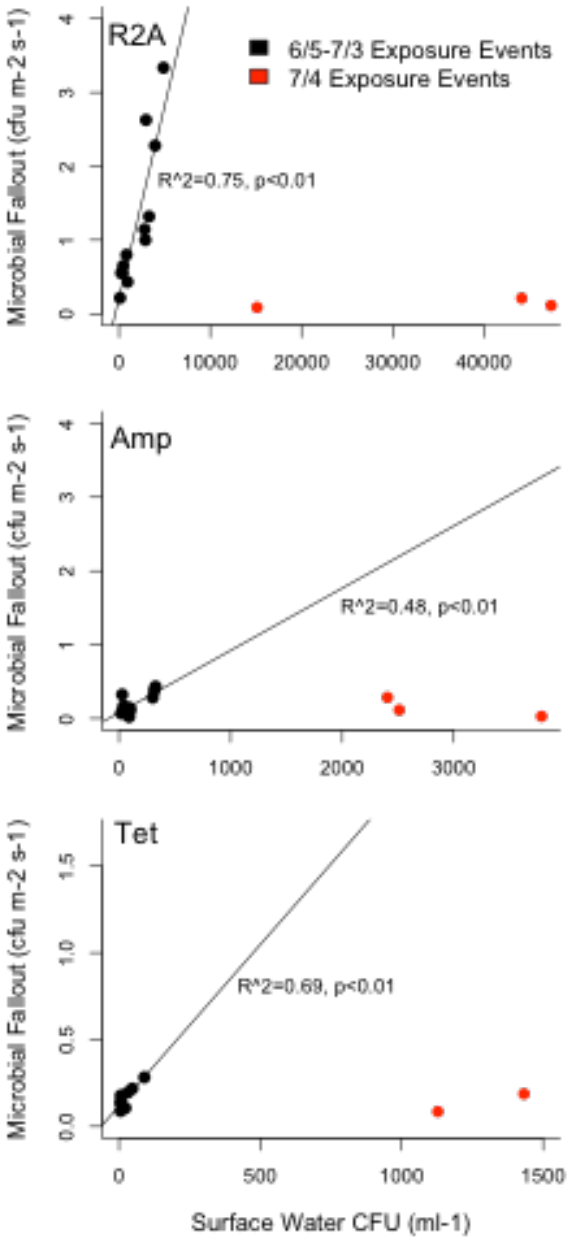

Figure 3. Microbial fallout vs. bacterial concentrations in surface waters. R^2 and p-value of linear models (excluding 4 July 2013 data) noted.

Table 4. **RDP taxonomic classification (genus level) of antibiotic-resistant bacteria in air and water at the study site**

	Aerosol	% Aerosol	Surface Water	% Surface Water
Acidovorax	1	6%	15	12%
Acinetobacter	0	0%	21	17%
Aeromonas	0	0%	33	27%
Bacillus	4	24%	0	0%
Brevundimonas	0	0%	1	1%
Burkholderia	2	12%	0	0%
Chryseobacterium	0	0%	5	4%
Citrobacter	0	0%	1	1%
Cloacibacterium	0	0%	1	1%
Comamonas	1	6%	12	10%
Enterobacter	0	0%	5	4%
Enterobacteriaceae	0	0%	2	2%
Hymenobacter	1	6%	0	0%
Klebsiella	0	0%	1	1%
Massilia	2	12%	0	0%
Ochrobactrum	0	0%	2	2%
Pseudomonas	1	6%	11	9%
Pseudorhodoferax	0	0%	1	1%
Ralstonia	0	0%	1	1%
Raoultella	0	0%	7	6%
Roseomonas	4	24%	0	0%
Sphingobacterium	0	0%	2	2%
Sphingomonas	0	0%	1	1%
Stenotrophomonas	0	0%	1	1%
Variovorax	1	6%	0	0%
total	**17**		**123**	

Table 5. Top-hits analysis of antibiotic-resistant bacterial aerosols sequenced in this study

Genus	# of sequences	Closest GenBank Hit(s) Source(s)
Acidovorax	1	human skin
Bacillus	3	soil
Bacillus	1	soil, wastewater
Burkholderia	2	insect gut
Comamonas	1	sewage sludge
Hymenobacter	1	human gastrointestinal sample
Massilia	2	soil
Pseudomonas	1	raw milk
Roseomonas	1	soil, air, water
Roseomonas	1	clean room floor
Roseomonas	2	human gastrointestinal sample
Variovorax	1	soil
total	17	

DISCUSSION

Microbial agents of concern at the HRE waterfront

This study confirmed the presence of microbial agents of concern both in water and air at the highly-urbanized NCNW waterfront. Antibiotic resistant and sewage-indicating bacteria were detected at NCNW in both air and water on each sampling day. This occurred despite the fact that bacterial fallout at this site was significantly lower than fallout at other previously-studied HRE waterfront locations (Dueker et al. 2012a; Dueker et al. In Review). This lower fallout was most likely due to differences in environmental and aerosol production conditions between sites. The Newtown Creek English Kills site is currently being remediated through underwater bubbling, which has been shown to increase aerosol particle concentrations over the waterway (Dueker et al. 2012a). Studies at Louis Valentino Pier (LVP) (Dueker et al. In Review) were conducted under much higher mean wind speeds than at the NCNW where wind speeds were < 2 m s^{-1}. Also, LVP is on a very busy harbor, with much more boat activity (and therefore aerosol production) than at NCNW. The presence of microbial agents of concern in NCNW aerosols under low fallout conditions strongly suggests the presence, and possibly higher abundance, of microbial agents of concern at HRE waterfront sites with higher fallout rates and contaminated surface waters.

Many of the resistant bacterial genera in water represented include potential pathogens, including *Aeromonas* and *Pseudomonas*. Almost all of the resistant bacteria were also in genera representing potential pathogens, including *Bacillus*, *Burkholderia*, *Comamonas*, *Massilia*, and *Roseomonas*. Both the taxonomic composition and percentages of antibiotic resistant bacteria in surface waters at this site agree with

previous research conducted by Young et al. (2013). The presence of these genera in aerosols at the waterfront indicates potential for public health impacts.

Preliminary Assessment of Antibiotic Resistant Aerosol Sources

Previous research at the HRE waterfront and Newtown Creek specifically has shown that both terrestrial and aquatic sources influence the types of viable bacteria present in aerosols (Dueker et al. 2012a; Dueker et al. In Review). Similar source potentials for microbial agents of concern were found in these aerosols, with sewage contamination strongly implicated.

Interestingly, higher percentages of the bacterial aerosols were antibiotic-resistant (~22%) compared to bacteria found in surface waters (~2-8%) at this site. This indicates different sources for antibiotic-resistant bacterial aerosols and/or selection processes occurring through the aerosolization process. Aerosolized bacterial cells experience extreme environmental stresses including desiccation and increased UV exposure. Survival of these stresses, particularly under the relatively dry conditions (66% RH) experienced during sampling, may require traits that are selected for during the aerosolization process, favoring viability of certain bacteria over others. Although determination of this is beyond the current study, it is interesting to note that the resistant aerosols detected at NCNW include *Bacillus*, which can form endospores to survive stressful environments (Leggett et al. 2012), *Pseudomonas*, which is known to survive aerosolization in indoor environments (Walter et al. 1990), and a *Roseomonas* sequence that closely matched an organism sampled previously from the Kennedy Space Center clean room floor (accession: EU705114, Vaishampayan et al., unpublished).

This study also found preliminary evidence of sewage-contaminated waters as a source for microbial agents of concern detected in the waterfront air. The presence of viable *Enterococcus faecalis* in aerosols is a strong indication of sewage influence on aerosols at the site. Given the site's proximity to the Newtown Creek Waste Water Treatment Plant, sources for these organisms include the treatment facility itself. However, these indicators were also present in surface waters every sampling day. The GenBank top hits analysis of resistant bacterial aerosols also confirmed that many of the resistant bacterial aerosols had been detected previously in aquatic and sewage-contaminated environments.

Rain is known to introduce untreated raw sewage into the water (Riverkeeper 2011) and as per previous studies there is a linear connection between sewage presence and antibiotic resistant bacteria (Young et al. 2013). Dueker et al. (in Review) found a linear relationship between culturable bacterial aerosols and culturable surface water bacterial concentrations at Louis Valentino Pier, indicating a connection between water and air quality at this site. A similar relationship was observed at NCNW, with the exception of sampling conducted on 4 July, with microbial fallout much lower than expected given the high surface water bacterial concentrations. The outlier of 4 July 2013 may be explained by the fact that it had rained for 4 consecutive days just prior to sampling (Table 2), which would cause spikes in bacterial concentrations of surface waters through subsequent CSO releases.

CONCLUSION

The outcome of this study provides a unique dataset confirming the presence of microbial agents of concern both in water and air at the highly-urbanized lower HRE waterfront. Many of the antibiotic resistant bacteria in water and air at the NCNW site were members of bacterial genera known to contain human pathogens, indicating potential public health implications for exposure to waterfront aerosols. Sources for these bacterial aerosols included terrestrial and aquatic environments. The presence of viable *Enterococcus faecalis* and other aquatic and sewage-associated antibiotic-resistant bacteria in waterfront aerosols indicate that sewage pollution may play an important role in the presence of microbial agents of concern at this site. Similarities between antibiotic-resistant bacteria found in surface waters at NCNW and Flushing Bay suggest that this study's findings may be applicable to other sewage-contaminated HRE waterfront sites. Further research into sources for these aerosolized microbial agents of concern is merited.

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