Sewage associated microbes in the Hudson River Estuary: distribution, sources, and ecological connections

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Over the last decade, expanded monitoring has provided insights into HRE bacterial water quality

Riverkeeper’s HRE sampling program:

- 75 testing sites
- 5000 samples processed
- *Enterococcus* ("Entero"): a fecal indicating bacteria (FIB)

DATA & REPORTS AVAILABLE ONLINE: www.riverkeeper.org/water-quality
Enterococcus as an indicator

Enterococcus is one of many genera in fecal material

**Ubiquitous**, but not dominant (<1% gut consortia), in healthy human GI tract.
Enterococcus as an indicator

Low abundance in most water samples:
- At EPA Beach Action Value (60/100ml), there will be <1 Enterococcus cell per $10^6$-$10^7$ microbes

As an indicator we rely on Enterococcus:
- coupling to other sewage associated microbes and pathogens
- short persistence (indicates recent input)
- absence in pristine waterways
Microbial Pathway

Food

Fecal microbes (Enterococcus)

Sewage input (WWTP + CSO)

Exposure via water contact

Natural System (Transport, dilution, & decay)
Management Pathway

Natural System
A (Transport, dilution, & decay)

Waterway monitoring

Notification, education, & forecasting

mitigation
Patterns of FIB distribution observed in HRE monitoring data:

1) Despite recent improvements in HRE water quality, large % of collected samples were unacceptable relative to the EPA Beach Action Value (BAV) for Enterococcus:

   – 23% of HRE samples
   – 72% of citizen tributary samples
   – 48% of NYC access point samples

There is still a lot of work to be done to improve regional water quality and better understand patterns in monitoring data...
Patterns of FIB distribution observed in HRE monitoring data:

1) Despite recent improvements in HRE water quality, large % of collected samples were unacceptable relative to the EPA Beach Action Value (BAV) for Enterococcus:

2) Contamination patterns are found to vary temporally, with weather as a major controlling factor. Rainfall increases unacceptable conditions:

- Do fecal microbes or microbes of concern also increase?
- Are CSOs the only source of FIB during rain?
Antibiotic resistant bacteria (ARB) abundances were correlated with Entero in Flushing Bay...

Both ARB and FIB increased significantly after rain...

% of resistant isolates identified as Enterobacteriaceae (by 16S rRNA sequencing) was significantly higher following rain (21%) compared to “dry” conditions (5%).

CSO event sampling in Flushing Bay

November 2014 storm event
CSO event sampling in Flushing Bay

- Greater than two order of magnitude increase in *Entero* and ARB detected during CSO flow.

- The 4 most abundant ARB isolates (Shigella, Acinetobacter, Aeromonas, Pseudomonas) from CSO discharge were also the most abundant ARB genera detected in Flushing Bay water.
Fecal microbes are not deposited directly in waterway during rain...
Fecal microbes are not deposited directly in waterway during rain...
Source mixing and succession occur in sewage delivery system.

Fecal microbes + Infrastructure microbes

- Enterococcus
- Other fecal

Sewage associated microbes vs Natural System vs Polluted waterway

Infrastructure

Fecal → Entero
Comparison of the Microbial Community Structures of Untreated Wastewaters from Different Geographic Locales

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Fecal microbes

+ Infrastructure microbes

Sewage input

Used next generation DNA sequencing of wastewater and fecal material (human and animal) to identify taxonomic groups of bacteria that represent the “fecal core” and “sewage infrastructure core” microbiomes.

Microbial signature approach has been used to examine influence of infrastructure & fecal microbes on waterways:

4 sewage infrastructure “core” genera:
Acinetobacter, Aeromonas, Arcobacter, Trichococcus.

Fecal “core” includes genera from 7 families:
Bacteroidaceae, Porphyromonadaceae, Clostridiaceae, Lachnospiraceae, Ruminococcaceae, Rikenellaceae, Prevotellaceae
The fecal (27%) and infrastructure (33%) “core” can together represent the majority of DNA sequences from CSO discharge into Flushing Bay (22,000 sequences in total).

In contrast the FIB *Enterococcus* represented less than 0.04% of these CSO sequences.

The [Entero] and % fecal core representation are correlated in Flushing Bay monitoring samples.
Fecal microbes + Infrastructure microbes → Sewage input

Fecal core max:
- CSO 27%
- Wet 10%
- Dry 0.5%

Infrastructure core max:
- CSO 33%
- Wet 23%
- Dry 14%

Kruskal Wallis p<0.001

% representation of fecal core

O’Mullan&Juhl prelim

% representation of infrastructure core

Kruskal Wallis p<0.001

O’Mullan&Juhl prelim
However, not all locations have CSOs and sewage may not be the only source of FIB during rain...
Stormwater and wildlife may be other major sources of FIB.

Is Entero elevated in non-CSO inputs? And if so, is Entero coupled to other microbes of concern?
Stormwater can be a major source of FIB...

Median Entero levels in urban street stormwater from 8 sites in Queens were more than an order of magnitude greater than the EPA beach action value; and were significantly higher than levels in Flushing Bay even during wet weather.
Stormwater contains high levels of Entero…but preliminary data indicate very low levels of fecal microbes…

[Entero] and % representation of the fecal core are **not** correlated in stormwater.

Management Relevance- [Entero] in waterways may remain elevated even if CSOs are removed…

Far more people interact with the stormwater in streets…public health consequences and management?
Initial conclusions

• Enterococcus and other groups of sewage associated bacteria are correlated in the urban waterways and increase following rain.

• CSOs are a large source of Entero and other sewage associated bacteria following rainfall, but stormwater (and wildlife) can be as well...

• DNA sequencing for community signatures can be used to assess sewage/fecal impact and perhaps to untangle sources...

• Preliminary data suggests that while stormwater carries a large Entero concentration it does not carry many fecal bacteria.
3) Water quality varies at small spatial scales- adjacent sites can have very different conditions

% of samples unacceptable (red) relative to EPA-BAV:

Beacon Harbor
61
82

Newburgh Launch
39
Patterns of FIB distribution observed in HRE monitoring data:

3) Water quality varies at small spatial scales- adjacent sites can have very different conditions.

4) Contamination is greater near-shore, especially where tributaries mix, and in tributaries compared to the Hudson mid-channel.

Why are mid-channel locations less impacted?

![Diagram showing BAV percentages for different site types.]

- **Estuary Mid-Channel**: BAV = 18/82
- **Tidal Tributaries**: BAV = 36/64
Understanding the variable spatial distribution requires inputs and dynamics within waterways to be explored.
We assume that (1) input location, combined with (2) transport, dilution & decay functions will explain waterway distribution.

**Inputs tend to be concentrated along the shoreline**

*Sewage inputs ( ) Transport, dilution + decay*
CSOs have large particle load, with aggregate settling velocities from 2m/hr to 30m/hr. (Fugate & Chant, 2006)
Particle association of sewage indicators:

Importance: particle association can alter transport

(Suter et al. 2011) across six HRE sites:
- 23.8% ± 15.0% of bacteria were particle attached
- 52.9% ± 20.9% of sewage indicators were particle attached

- Larger % of Enterococci attached to particles (>3μm) as compared to the total bacterial community.
- Particle attached cells have a greater sinking rate than free living cells.

Spatial surveys detected high Entero concentrations in HRE sediment

-Sites with high water column [Entero] also have elevated sediment [Entero]

-Sediment [Entero] often an order of magnitude greater than in water (by mass)

Spearman r = 0.818; p = 0.003
Sediment acts as an environmental reservoir

- 90% reduction rates in sediment incubations ranged from weeks to months.

- Longest persistence observed with high organic content and low temperature.
10 wading trials were conducted near the Piermont Pier, with water column sampling before and after wading; 9 of 10 trials resulted in elevated Enterococci concentrations.
2 related hypotheses:

1) Sediment resuspension, as a source of microbes to water, is more important in shallow environments (near shore, tribs).

2) There is a “core” sediment microbial community & DNA signatures will allow the contribution of sediment-water interactions to be better quantified.
Ecological connections are far more complicated than water column transport, dilution, & decay....
As our understanding of microbial pathways become more complete, our approach to the management pathways should also evolve.
Conclusions:

- As indicator organisms travel through a system they become coupled, or de-coupled, with microbes associated with various components of that system (e.g. sewage, sediment, etc).

- Understanding microbial community composition in various components of the constructed and natural system can help to untangle the indicator interactions.

- Better understanding these complex microbial interactions can allow for optimization of the management process.
Management Implications:

– Entero is very useful in providing a framework to understand bacterial water quality; the addition of other tools (e.g. DNA signatures and source tracking) can help us to better understand water quality dynamics and better mitigate contamination sources.

– WTTP and CSOs are not the only inputs of FIB or bacterial contaminants; Exploration of other sources (e.g. stormwater and wildlife) and connections (sediment-water interactions) should be increased.
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