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# Supplemental Material
# Metagenomic Frameworks for Monitoring Antibiotic Resistance in Aquatic Environments

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**Supplemental Material, Table S1.** Most abundant genomic elements (e.g., gene class, species, host species) for each of the index subcategories by ecosystem type. Count data and relative abundance (percent out of all genomic elements within an index subcategory) are provided for each sample (except for pathogens where only count data is shown). This data was generated using the medium-high sequence similarity threshold described in Table 2.

| **Genomic element** | **Estuary** | **Coastal ocean** | **Freshwater lake** | **Marina** | **WWTP sludge** | **WWTP effluent** | **River sediment** |
|---|---|---|---|---|---|---|---|
| **Antibiotic resistance genes (class)** | | | | | | | |
| Aminoglycoside | 0 | 0 | 0 | 0 | 0 | 3 (25.0%) | 248 (22.5%) |
| Bacitracin | 0 | 0 | 0 | 2 (50.0%) | 0 | 0 | 0 |
| Beta lactam | 0 | 0 | 0 | 1 (25.0%) | 0 | 2 (16.7%) | 2 (0.181%) |
| Chloramphenicol | 0 | 0 | 1 (50.0%) | 0 | 0 | 0 | 0 |
| Macrolide | 0 | 0 | 0 | 0 | 0 | 3 (25.0%) | 53 (4.8%) |
| Multidrug | 0 | 1 | 0 | 1 (25.0%) | 0 | 0 | 1 (0.091%) |
| Quinolone | 0 | 0 | 0 | 0 | 0 | 0 | 1 (0.091%) |
| Sulfonamide | 0 | 0 | 0 | 0 | 0 | 0 | 796 (72.1%) |
| Tetracycline | 0 | 0 | 0 | 0 | 0 | 4 (33.3%) | 0 |
| Trimethoprin | 0 | 0 | 0 | 0 | 0 | 0 | 2 (0.181%) |
| Other | 0 | 0 | 1 (50.0%) | 0 | 0 | 0 | 1 (0.091%) |
| **Metal resistance genes (class)** | | | | | | | |
| Arsenic resistance | 146 (61.9%) | 72 (75.0%) | 32 (78.0%) | 59 (43.7%) | 0 | 55 (34.3%) | 26 (59.1%) |
| Cadmium resistance | 0 | 0 | 0 | 0 | 0 | 0 | 2 (4.5%) |
| Mercury resistance | 90 (38.1%) | 24 (25.0%) | 9 (22.0%) | 76 (56.3%) | 1 (100%) | 105 (65.6%) | 16 (36.4%) |
| **Plasmids (host)** | | | | | | | |
| *Aeromonas* sp. | 0 | 0 | 0 | 0 | 0 | 8 (4.5%) | 282 (4.6%) |
| *Acinetobacter* spp. | 1 (1.6%) | 0 | 2 (20.0%) | 0 | 0 | 3 (1.7%) | 180 (3.0%) |
| *Comamonas testosteroni* | 0 | 0 | 2 (20.0%) | 0 | 0 | 7 (3.9%) | 1 (0.02%) |
| *Cupriavidus metallidurans* | 1 (1.6%) | 2 (2.0%) | 0 | 0 | 0 | 31 (17.3%) | 116 (1.9%) |
| *Edwardsiella tarda* | 0 | 0 | 0 | 0 | 0 | 0 | 2,020 (33.3%) |
| *Escherichia coli* | 1 (1.6%) | 1 (1.0%) | 1 (10.0%) | 0 | 0 | 4 (2.2%) | 1,877 (30.9%) |
| *Klebsiella pneumonia* | 0 | 1 (1.0%) | 0 | 3 (20.0%) | 0 | 3 (1.7%) | 15 (0.25%) |
| *Lactococcus* sp. | 0 | 0 | 0 | 0 | 0 | 22 (12.3%) | 1 (0.016%) |

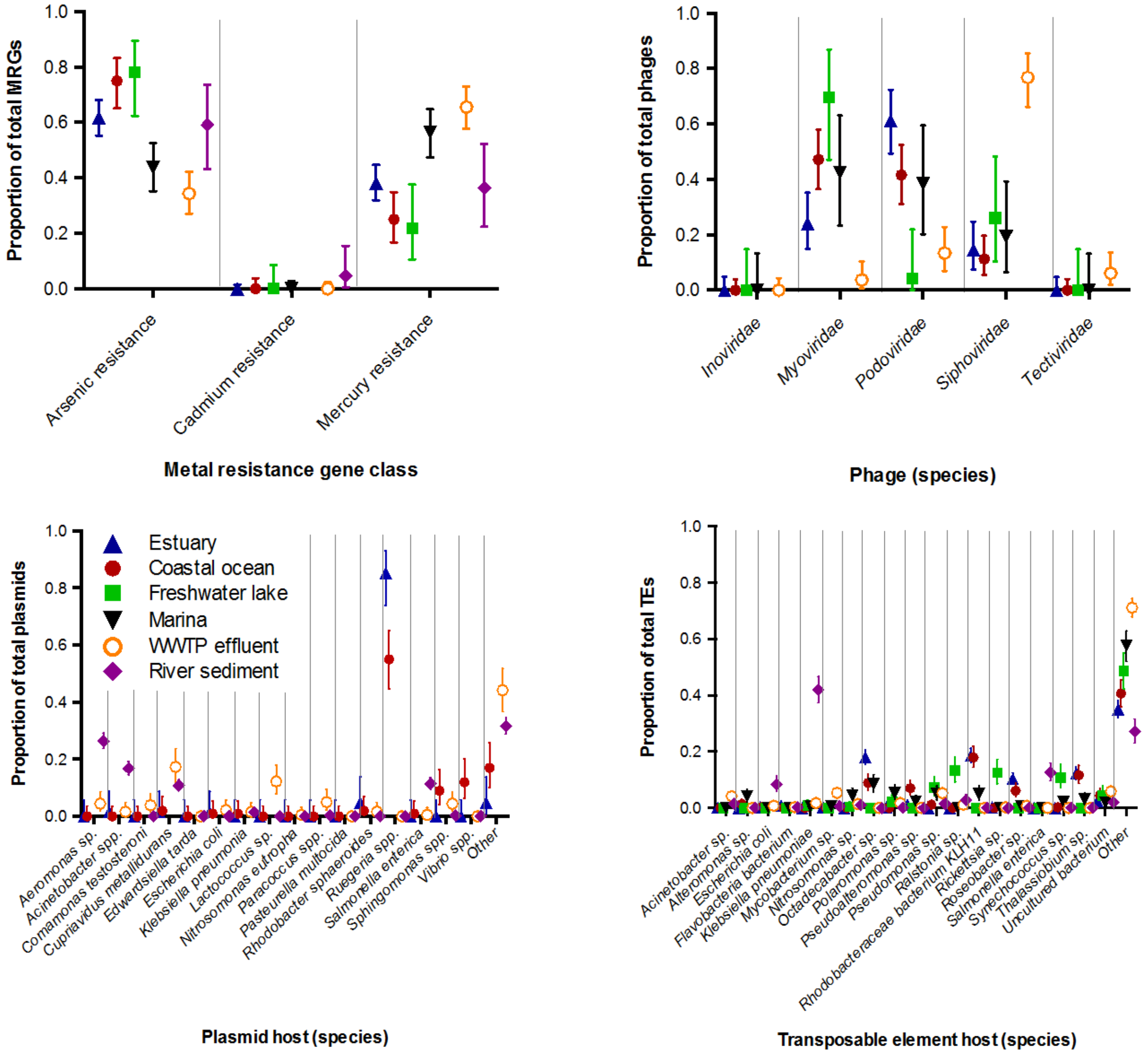
| **Genomic element** | **Estuary** | **Coastal ocean** | **Freshwater lake** | **Marina** | **WWTP sludge** | **WWTP effluent** | **River sediment** |
|---|---|---|---|---|---|---|---|
| *Nitrosomonas eutropha* | 0 | 0 | 0 | 2 (13.3%) | 0 | 1 (0.56%) | 0 |
| *Paracoccus* spp. | 0 | 0 | 0 | 0 | 0 | 9 (5.0%) | 4 (0.065%) |
| *Pasteurella multocida* | 0 | 0 | 0 | 0 | 0 | 0 | 1,113 (18.3%) |
| *Rhodobacter sphaeroides* | 3 (4.9%) | 2 (2.0%) | 0 | 0 | 0 | 3 (1.7%) | 0 |
| *Ruegeria* spp. | 52 (85.2%) | 55 (55.0%) | 0 | 4 (26.7%) | 0 | 0 | 0 |
| *Salmonella enterica* | 0 | 1 (1.0%) | 0 | 0 | 0 | 1 (0.56%) | 122 (2.0%) |
| *Sphingomonas* spp. | 0 | 9 (9.0%) | 1 (10.0%) | 0 | 0 | 8 (4.5%) | 4 (0.066%) |
| *Vibrio* spp. | 0 | 12 (12.0%) | 0 | 1 (6.7%) | 0 | 0 | 1 (0.016%) |
| Other | 3 (4.9%) | 17 (17.0%) | 4 (40.0%) | 5 (33.3%) | 0 | 79 (44.1%) | 338 (5.6%) |
| **Transposable elements (host)** | | | | | | | |
| *Acinetobacter* sp. | 2 (0.21%) | 0 | 0 | 0 | 1 (3.0%) | 33 (4.2%) | 7 (1.5%) |
| *Alteromonas* sp. | 0 | 6 (1.4%) | 0 | 14 (4.0%) | 0 | 0 | 0 |
| *Escherichia coli* | 2 (0.21%) | 2 (0.47%) | 0 | 0 | 0 | 6 (0.76%) | 38 (8.4%) |
| *Flavobacteria bacterium* | 9 (0.92%) | 0 | 0 | 0 | 10 (30.3%) | 2 (0.25%) | 1 (0.22%) |
| *Klebsiella pneumoniae* | 0 | 0 | 2 (0.80%) | 2 (0.58%) | 1 (3.0%) | 14 (1.8%) | 190 (41.9%) |
| *Mycobacterium* sp. | 4 (0.41%) | 0 | 0 | 2 (0.58%) | 1 (3.0%) | 43 (5.4%) | 3 (0.66%) |
| *Nitrosomonas* sp. | 0 | 0 | 1 (0.40%) | 15 (4.3%) | | 11 (1.4%) | 5 (1.1%) |
| *Octadecabacter* sp. | 175 (17.9%) | 38 (8.9%) | 0 | 29 (8.4%) | 0 | 0 | 0 |
| *Polaromonas* sp. | 2 (0.21%) | 0 | 5 (2.0%) | 18 (5.2%) | 0 | 14 (1.8%) | 0 |
| *Pseudoalteromonas* sp. | 11 (1.1%) | 30 (7.0%) | 0 | 8 (2.3%) | 0 | 0 | 0 |
| *Pseudomonas* sp. | 0 | 5 (1.2%) | 18 (7.2%) | 17 (4.9%) | 2 (6.1%) | 42 (5.3%) | 7 (1.5%) |
| *Ralstonia* sp. | 0 | 0 | 33 (13.2%) | 0 | 0 | 9 (1.1%) | 13 (2.9%) |
| *Rhodobacteraceae bacterium KLH11* | 181 (18.0%) | 77 (18.0%) | 0 | 17 (4.9%) | 0 | 0 | 0 |
| *Rickettsia* sp. | 2 (0.21%) | 0 | 31 (12.4%) | 0 | 0 | 2 (0.25%) | 0 |
| *Roseobacter* sp. | 101 (10.4%) | 26 (6.1%) | 0 | 2 (0.58%) | 0 | 6 (0.76%) | 0 |
| *Salmonella enterica* | 0 | 0 | 0 | 0 | 0 | 0 | 57 (12.6%) |
| *Synechococcus* sp. | 0 | 1 (0.23%) | 27 (10.8%) | 7 (2.0%) | 0 | 0 | 0 |
| *Thalassiobium* sp. | 120 (12.3%) | 50 (11.7%) | 0 | 10 (2.9%) | 0 | 0 | 0 |
| Uncultured bacterium | 24 (2.5%) | 19 (4.4%) | 11 (4.4%) | 6 (1.7%) | 0 | 47 (5.9%) | 9 (2.0%) |
| Other | 342 (35.1%) | 174 (40.7%) | 121 (48.6%) | 199 (57.5%) | 18 (54.5%) | 564 (71.1%) | 123 (27.1%) |

| Genomic element | Estuary | Coastal ocean | Freshwater lake | Marina | WWTP sludge | WWTP effluent | River sediment |
|---|---|---|---|---|---|---|---|
| **Phages** | | | | | | | |
| *Inoviridae* | 0 | 0 | 0 | 0 | 0 | 0 | 3 (50.0%) |
| *Myoviridae* | 18 (24.0%) | 42 (47.2%) | 16 (69.6%) | 11 (42.3%) | 1 (33.3%) | 3 (3.7%) | 0 |
| *Podoviridae* | 46 (61.3%) | 37 (41.6%) | 1 (4.3%) | 10 (38.5%) | 0 | 11 (13.4%) | 3 (50.0%) |
| *Siphoviridae* | 11 (14.6%) | 10 (11.2%) | 6 (26.1%) | 5 (19.2%) | 2 (66.7%) | 63 (76.8%) | 0 |
| *Tectiviridae* | 0 | 0 | 0 | 0 | 0 | 5 (6.1%) | 0 |
| **Pathogens** | | | | | | | |
| *Acinetobacter calcoaceticus* | 0 | 0 | 0 | 0 | 0 | 2 | 0 |
| *Acinetobacter lwoffii* | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| *Aeromonas caviae* | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| *Ralstonia pickettii* | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| *Salmonella enterica* | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| *Staphylococcus epidermidis* | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| *Yersinia pestis* | 0 | 1 | 0 | 0 | 0 | 0 | 0 |

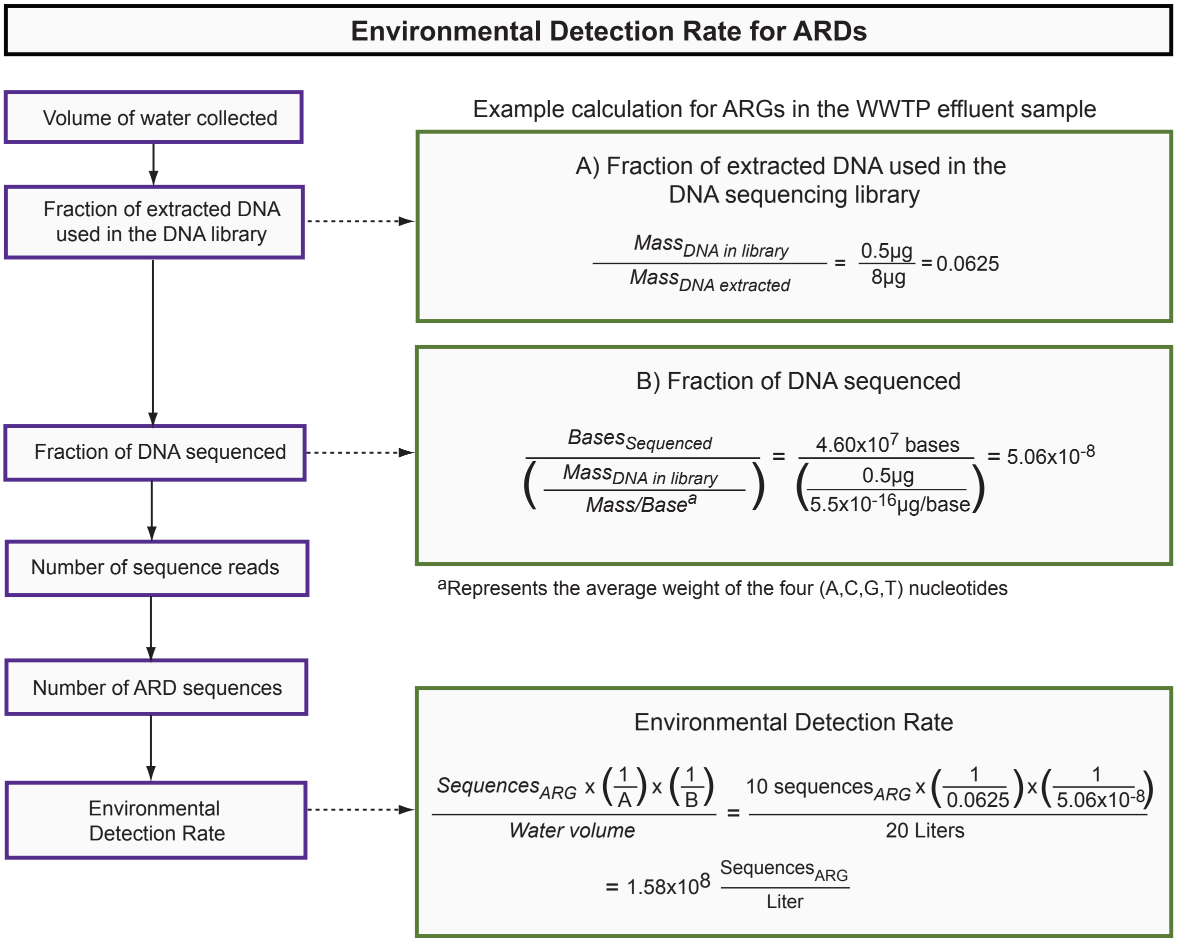
**Supplemental Material, Table S2.** Pearson's correlation coefficients (r) for multivariate analysis of the index subcategories. The values for each sequence similarity threshold (low, medium-low, medium-high, high) are shown for each comparison.

| **Index subcategory** | **ARG** | **MRG** | **Plasmid** | **TE** | **Phage** | **Pathogen** |
|---|---|---|---|---|---|---|
| ARG | | | | | | |
| MRG | -0.36, -0.31, -0.16, ND | | | | | |
| Plasmid | 0.98***,0.94***, 0.90***, 0.85*** | -0.31, -0.34, -0.07, ND | | | | |
| TE | 0.48, -0.01, -0.78***, -0.71*** | 0.26, -0.07, -0.17, ND | 0.58*,0.23, -0.87***, -0.77*** | | | |
| Phage | -0.52*, -0.45, -0.36, -0.19 | -0.57*, -0.47, -0.07, ND | -0.59*, -0.56*, -0.39, -0.17 | -0.82***, -0.55*, -0.02, 0.11 | | |
| Pathogen | -0.07, -0.06, -0.09, -0.09 | 0.21, 0.09, 0.01, ND | 0.01, 0.04, -0.06, -0.15 | 0.31, 0.35, -0.03, -0.15 | -0.22, -0.13, -0.18, -0.09 | |

Abbreviations: ARGs, antibiotic resistance genes; MRGs, metal resistance genes; ND, non-detect; TE, transposable elements. *p<0.01. **p<0.001. ***p<0.0001.



**Supplemental Material, Figure S1.** Relative abundance of the genomic elements composing the index subcategories. The number of sequences representing each genomic element in a given ecosystem was normalized to the total number of sequences for the respective index subcategory in that ecosystem. Bars represent 95% confidence intervals. A similar analysis was performed for antibiotic resistance genes (ARGs) and pathogens, but the sample size was sufficient to carry out the significance test for ARGs in the river sediment sample only. Ecosystems that are absent for a subcategory plot also had insufficient sample sizes. Abundances were generated using the medium-high sequence similarity threshold described in Table 2.



**Supplementary Material, Figure S2.** Mathematical framework for estimating the environmental detection rate of antibiotic resistance determinants (ARDs) in metagenomic samples using next generation sequencing. The environmental detection rate reflects the number of ARD-related sequences per liter of water filtered. An example calculation is shown for antibiotic resistance genes (ARGs) in the WWTP effluent sample (see Table 1 for description). The equations correct for the fraction of extracted DNA used as input for the DNA sequencing library (variable A) and the fraction sequenced (variable B).