

Supplementary Information

Towards geospatially-resolved public-health surveillance via wastewater sequencing

Supplementary Figure 1: A) Overlap between identified bacterial families in metatranscriptomic data by different classification approaches. B) The top 25 most abundant genera across the different taxonomic classification approaches. The relative abundance values are on the scale of 0 to 1 for Kraken2/Bracken and Xtree and 0 to 100 for MetaPhlAn4 (the default output of the software). Error bars represent standard deviations from mean. Source data are provided as a Source Data file.

Supplementary Figure 2: Results from the Microbiome Association Study illustrating bacterial expression and different wastewater-associated features. Volcano plots depicting the number of associated features and direction therein for different variables. X-axis is the beta-coefficient from the variable listed at the top of the plot. Y-axis is negative \log_{10} BY-adjusted p-value (i.e., q-value). Source data are provided as a Source Data file.

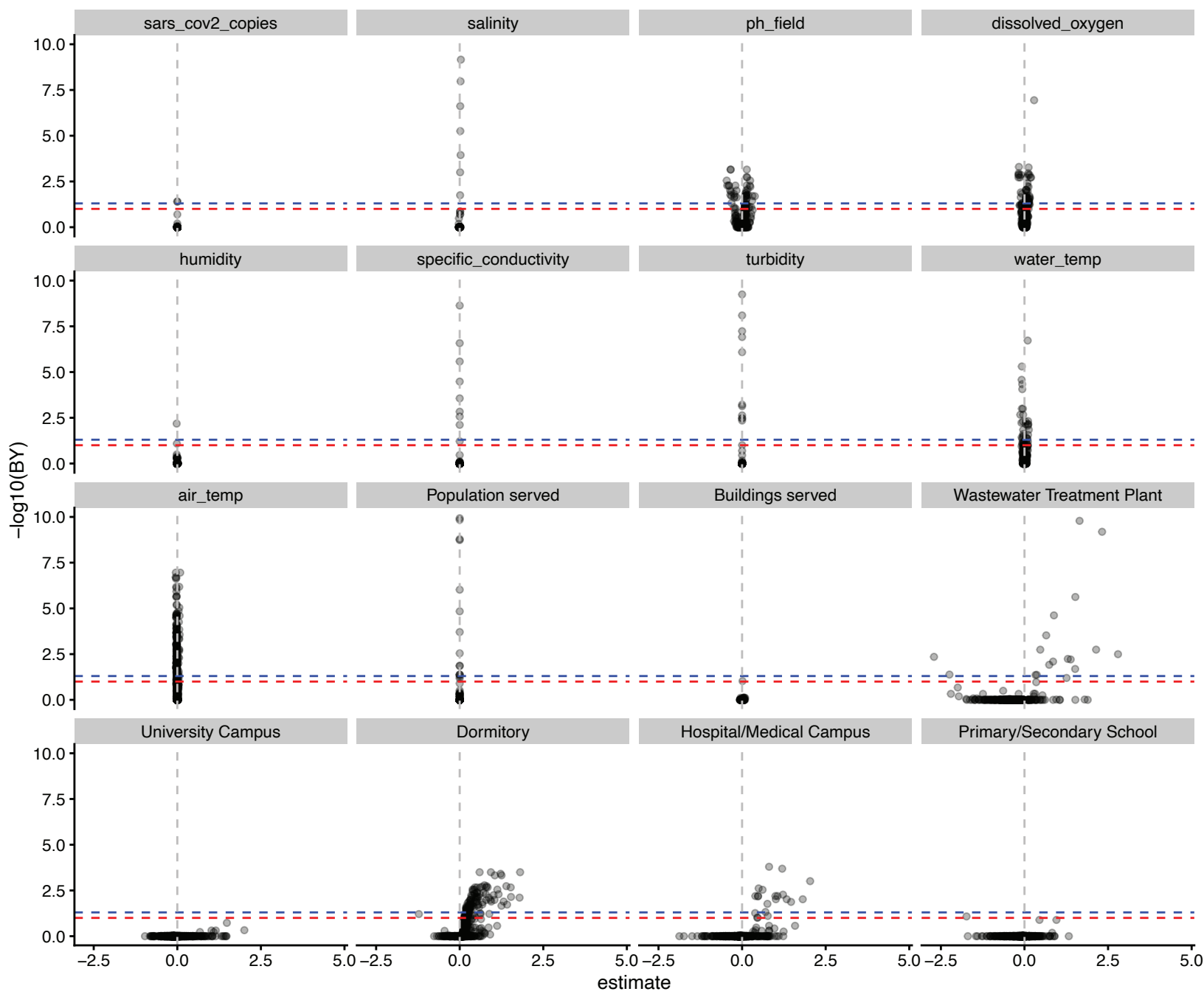
Supplementary Figure 3: The relative abundance of putative, low abundance pathogens bacteria as identified by A) Kraken2 and B) XTree. Heatmaps are hierarchically clustered. HOSP = hospital, DORM = dormitory, SCHOOL = primary/secondary school, WWTP = wastewater treatment plant, UC = university campus. Source data are provided as a Source Data file.

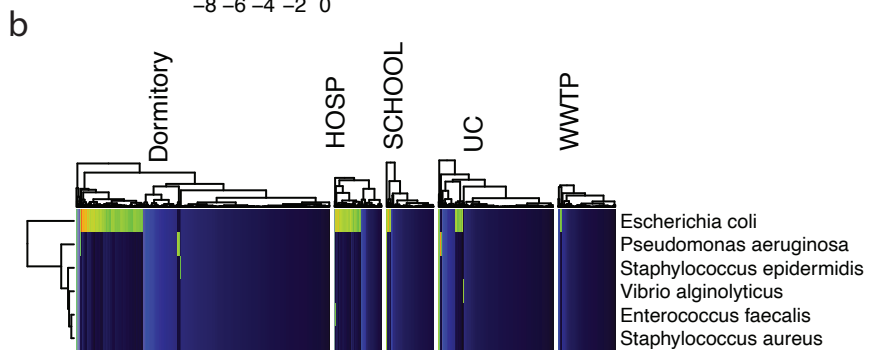
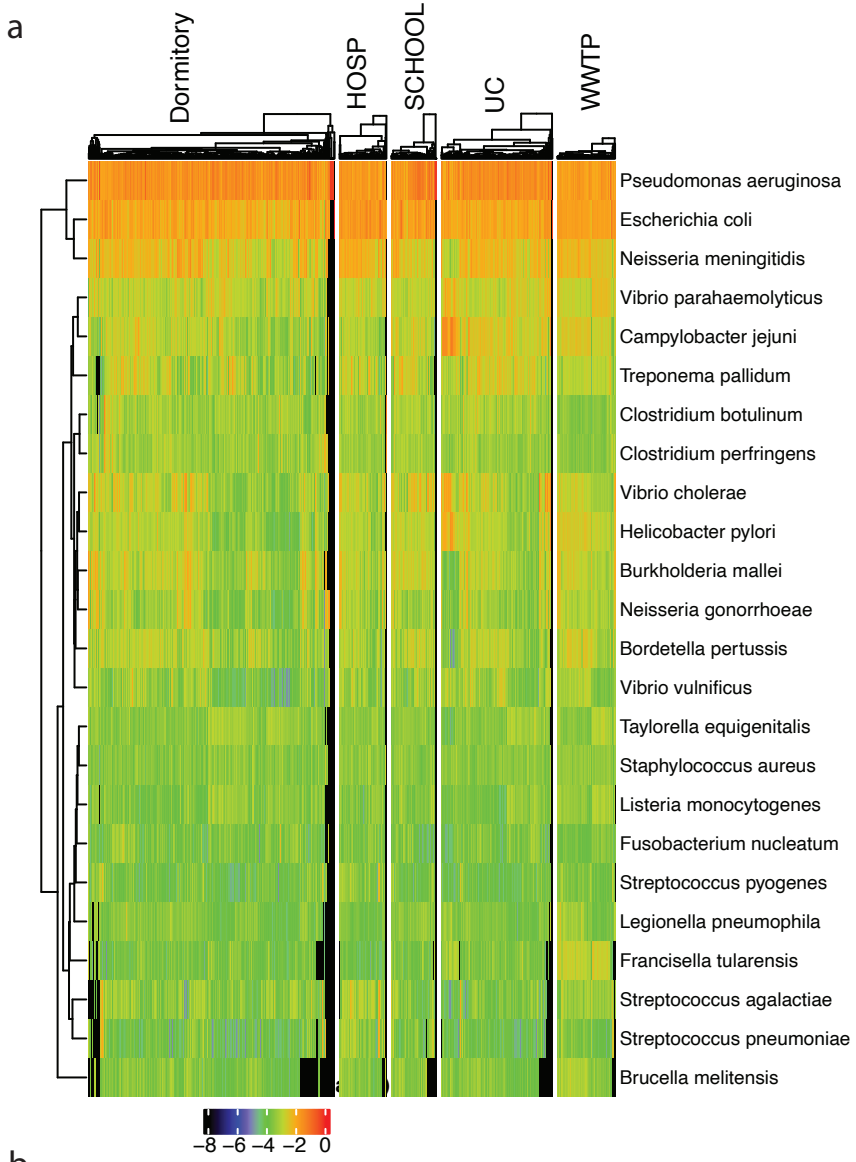
Supplementary Figure 4: Viral identification beta diversity, modeled after the bacterial beta diversity analysis in Figure 3. A) The top panel is the viral-species-specific beta diversity (bray curtis distance) between all samples within a given site. The line underneath corresponds to the average beta diversity at each associated site. The colored blocks on the bottom are vertically aligned with the population size points and the beta diversity dotplots, and colors correspond to the different sampling location types indicated in the legend. B) The intersections between different location types, with the bars indicating the intersection size and the black dots indicating the sites underneath being compared. These bars are vertically aligned with the middle and top panels, which show the relative abundance and prevalence, respectively, of all bacterial species represented by each bar. Source data are provided as a Source Data file.

Supplementary Figure 5: Results from the Microbiome Association Study illustrating viral family abundance and different wastewater-associated features. Volcano plots depicting the number of associated features and direction therein for different variables. X-axis is the beta-coefficient from the variable listed at the top of the plot. Y-axis is negative \log_{10} BY-adjusted p-value (i.e., q-value). Source data are provided as a Source Data file.

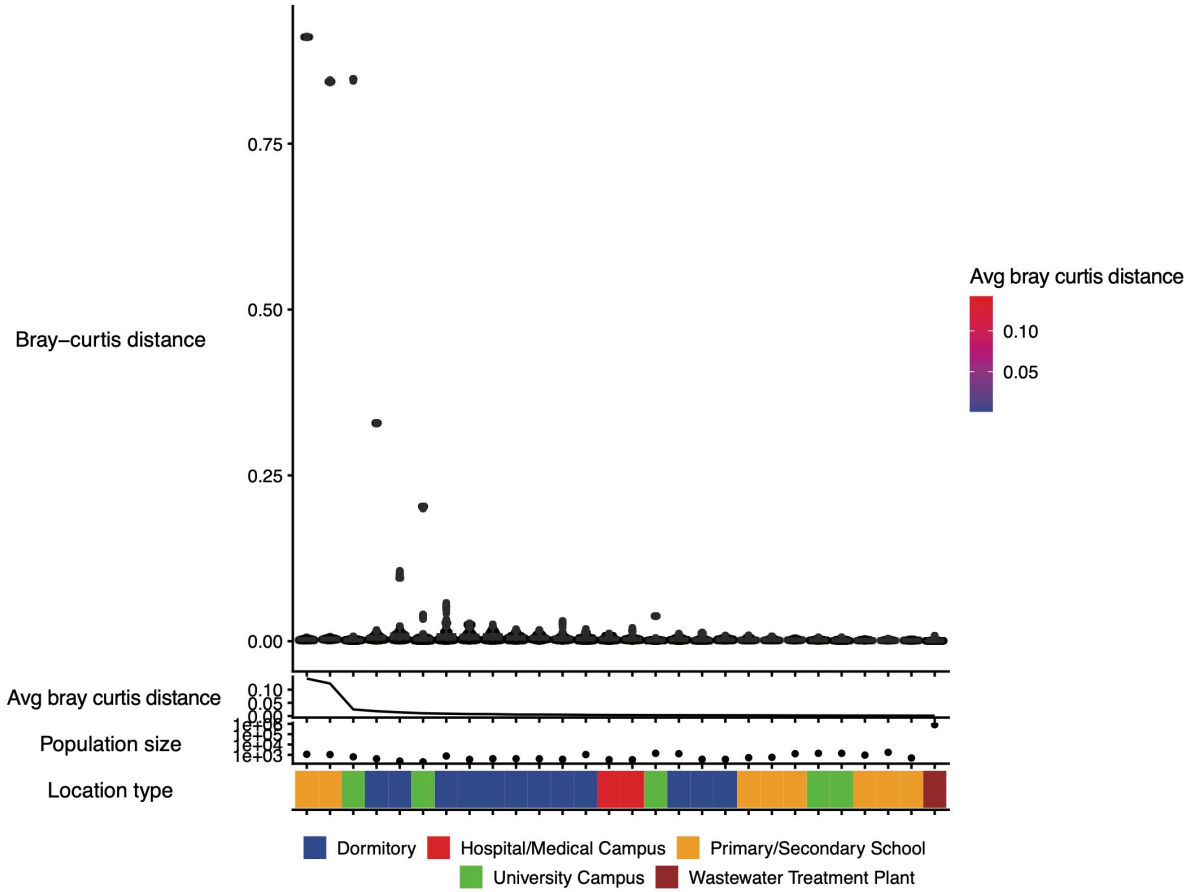
Supplementary Figure 6: The prevalence of different pathogen-associated genes in wastewater. Pathogen-associated genes are derived from the PathFinder database (see *Methods*). Source data are provided as a Source Data file.

Supplementary Figure 7: The top specific ARGs for different antibiotics across all sample types. Asterisks correspond to if a given ARG was enriched (in terms of \log_{10} observations per 10k reads) in hospital wastewater when compared to all other sites according to the adjusted p-value on a t-test. P-values were adjusted by the Benjamini-Yekutieli procedure. For an asterisk to be present, an antibiotic class must have been enriched in all four comparisons (e.g., hospitals vs dormitories, hospitals vs the university campus, hospitals vs primary schools, and hospitals vs the wastewater treatment plant). Source data are provided as a Source Data file.

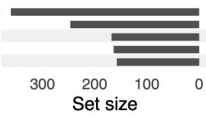
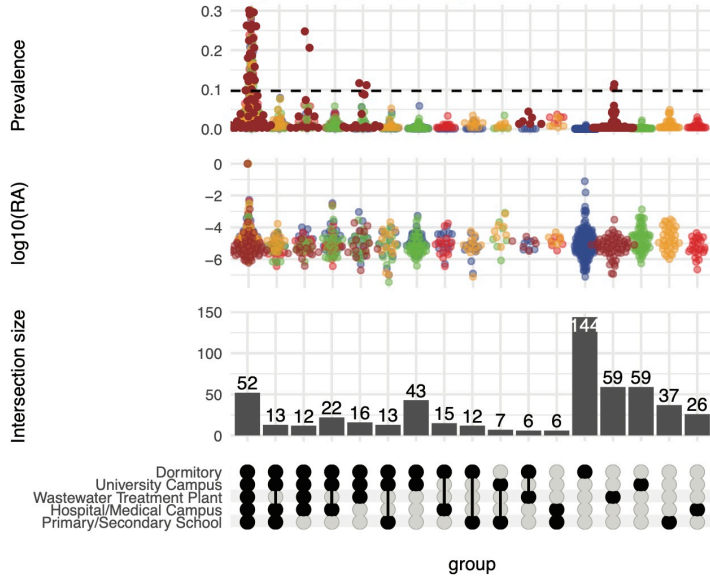


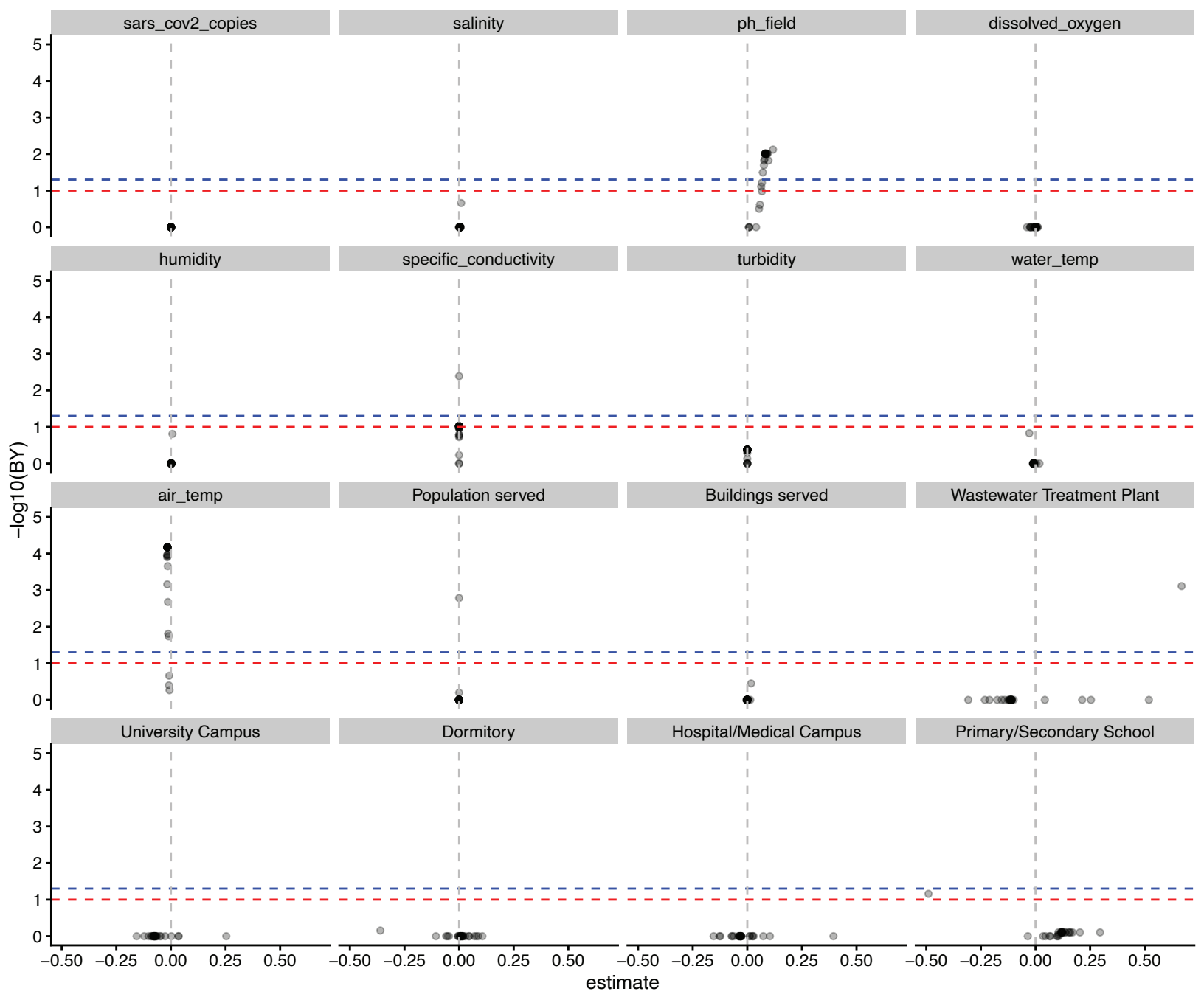


a



b





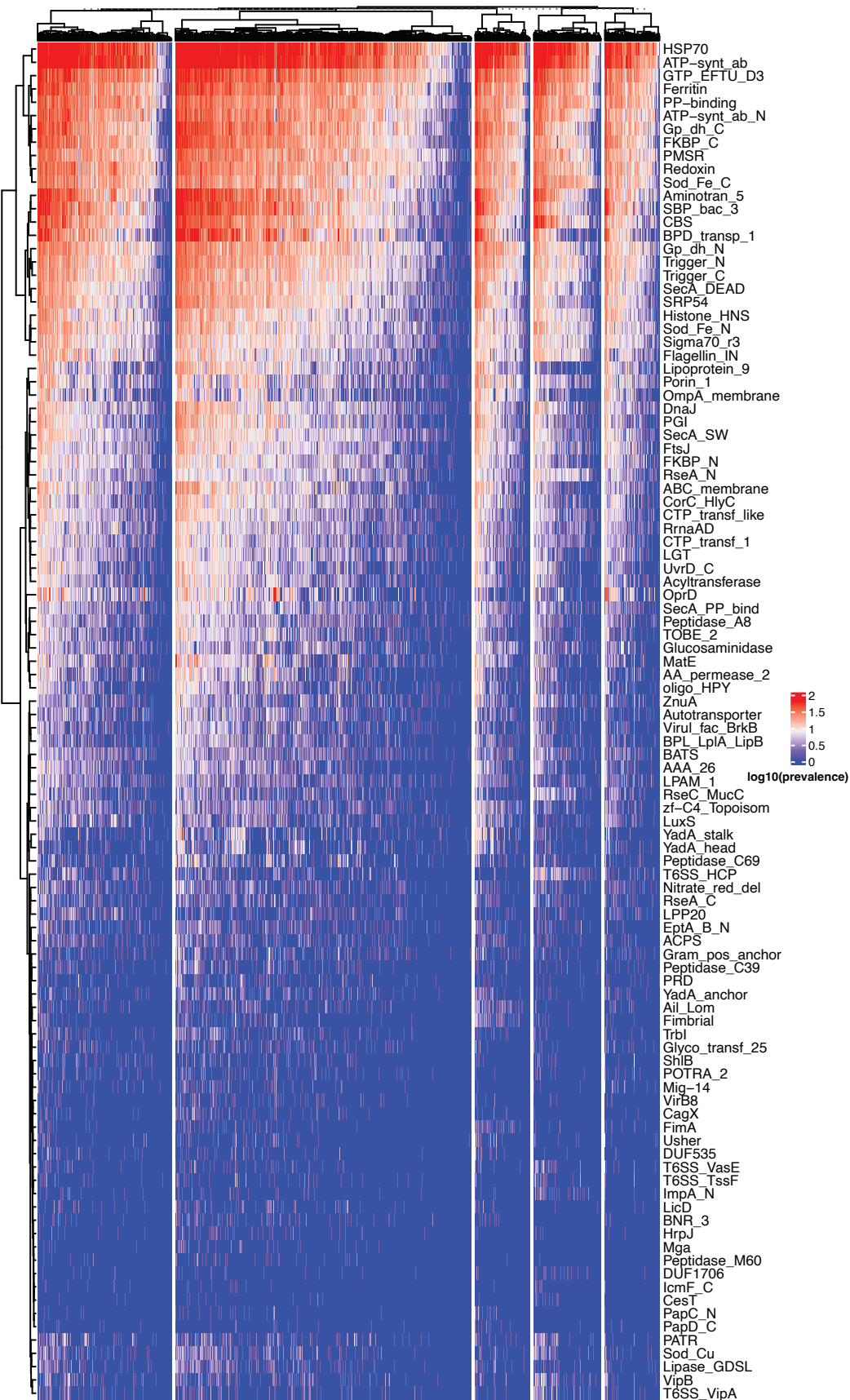
University Campus

Dormitory

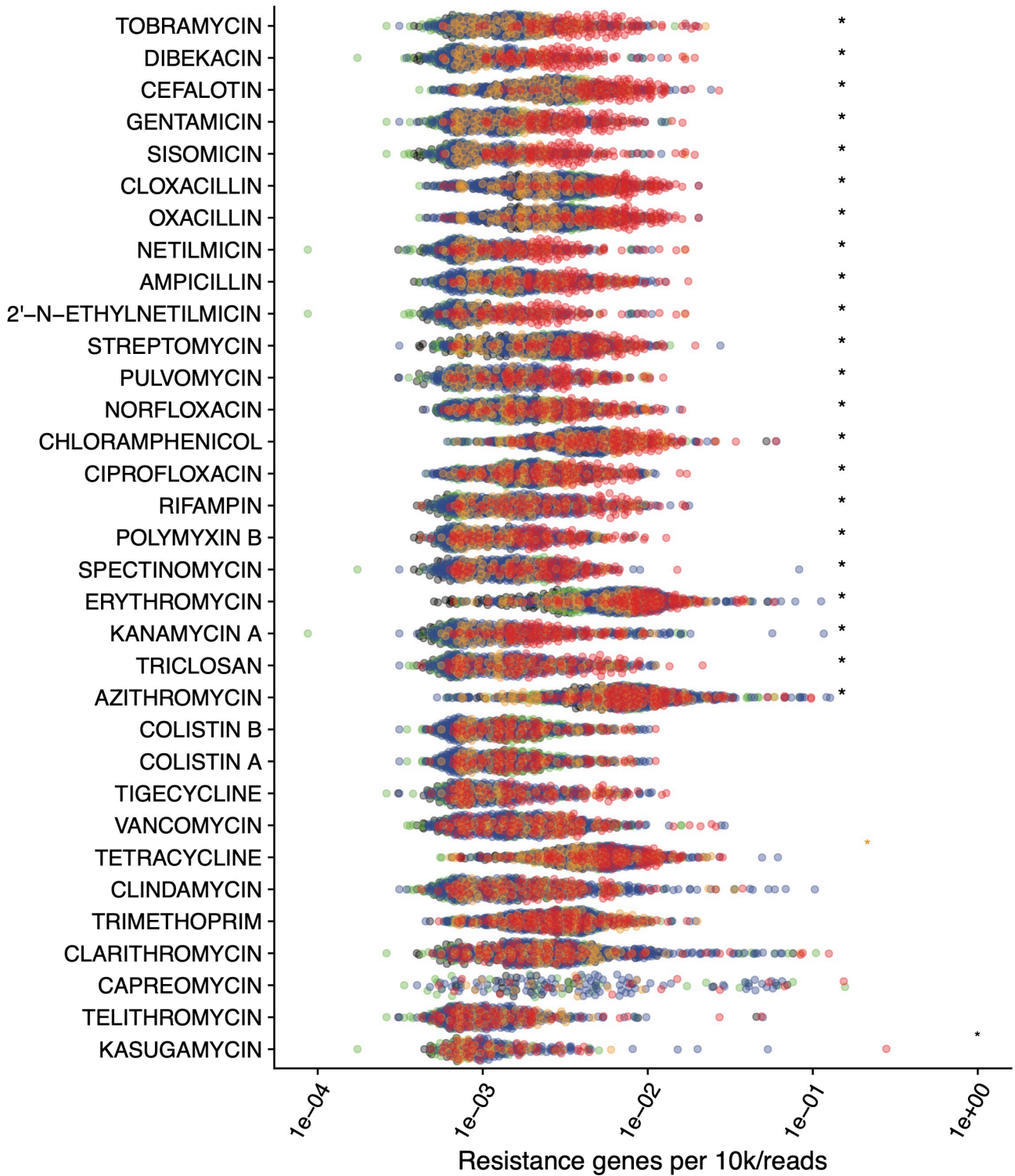
Hospital/Medical Campus

Wastewater Treatment Plant

Primary/Secondary School



Antibiotic



Location Type ● Hospital/Medical Campus ● Dormitory ● University Campus ● Primary/Secondary School ● Wastewater Treatment Plant