ELUCIDATING BIOFILM CONTRIBUTIONS TO WASTEWATER SURVEILLANCE SIGNAL OF BACTERIAL PATHOGENS AND ANTIMICROBIAL RESISTANCE GENES

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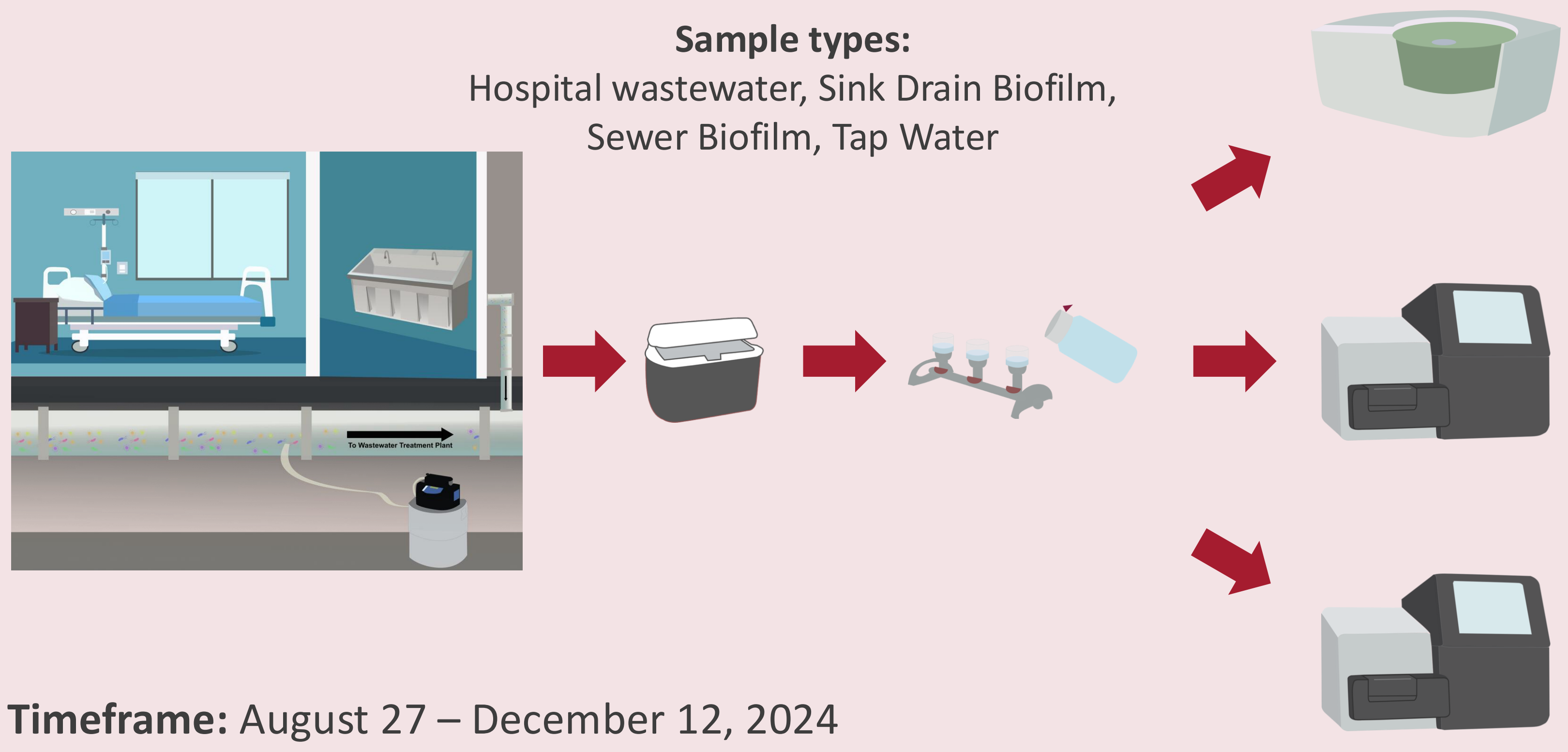
INTRODUCTION

- As wastewater-based surveillance (WBS) is increasingly used for bacterial, fungal, and antimicrobial resistance (AMR) targets, there is a need to distinguish which biomarkers identified in wastewater reflect human versus environmental inputs from biofilms.
- Delineating shared taxa between biofilms and wastewater will guide WBS practitioners on more reliably using wastewater to represent ongoing infection in communities.

METHODOLOGY



Sample types:
Hospital wastewater, Sink Drain Biofilm,
Sewer Biofilm, Tap Water

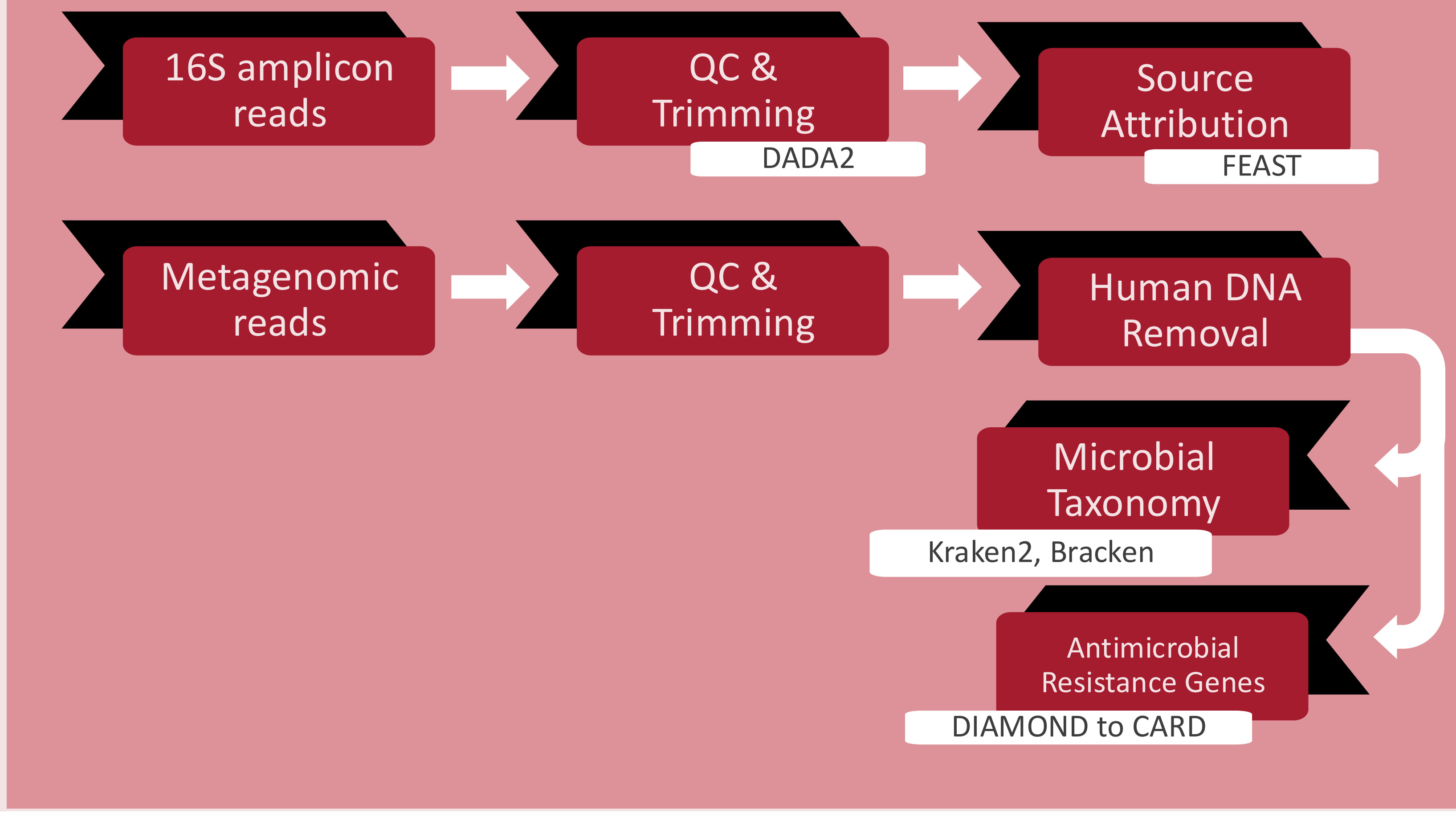
qPCR for:
16S rRNA gene

Shotgun metagenomic sequencing:
Illumina NovaSeq 6000
Respiratory Pathogen & AMR Panel (RPIP)

16S rRNA gene amplicon sequencing:
Illumina MiSeq

Timeframe: August 27 – December 12, 2024
Sites: Five Hospital Wards (MARIO, LUIGI, OSCAR, SHREK, FIONA)

BIOINFORMATIC WORKFLOW



16S amplicon reads → QC & Trimming (DADA2) → Source Attribution (FEAST)

Metagenomic reads → QC & Trimming → Human DNA Removal → Microbial Taxonomy (Kraken2, Bracken) → Antimicrobial Resistance Genes (DIAMOND to CARD)

RESULTS

SEWER & DRAIN BIOFILMS SHARE BIOFILM-ASSOCIATED PATHOGENS WITH WASTEWATER AT STABLE LEVELS

- Biofilm-associated pathogens were consistently detected in biofilms and wastewater
- Most pathogens showed temporal stability (Coefficients of Variation <50%)

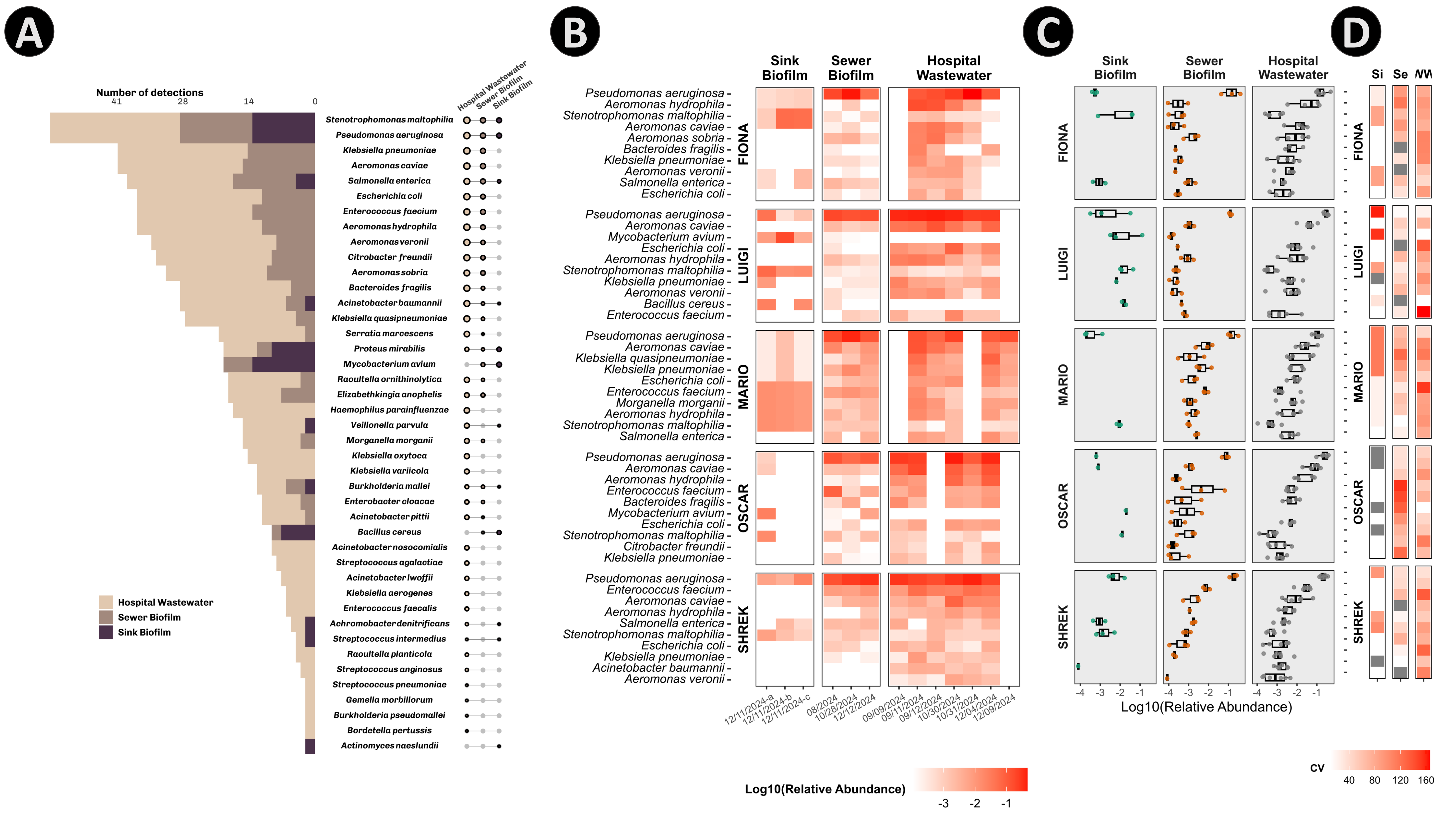


Figure 1: (A) Unique & shared taxa across sample types; (B) Relative abundance heat map of top taxa by summed relative abundance; (C) Relative abundance box plots of top shared taxa; (D) Coefficients of variation for top taxa – Si: Sink biofilm, Se: sewer biofilm, WW: wastewater

HOSPITAL WASTEWATER MICROBIOME COMPOSITION CAN BE PARTLY ATTRIBUTED TO SEWER BIOFILMS

- 16S rRNA amplicon sequencing-derived data revealed 3-24% of wastewater microbiome composition could be attributed to sewer biofilms

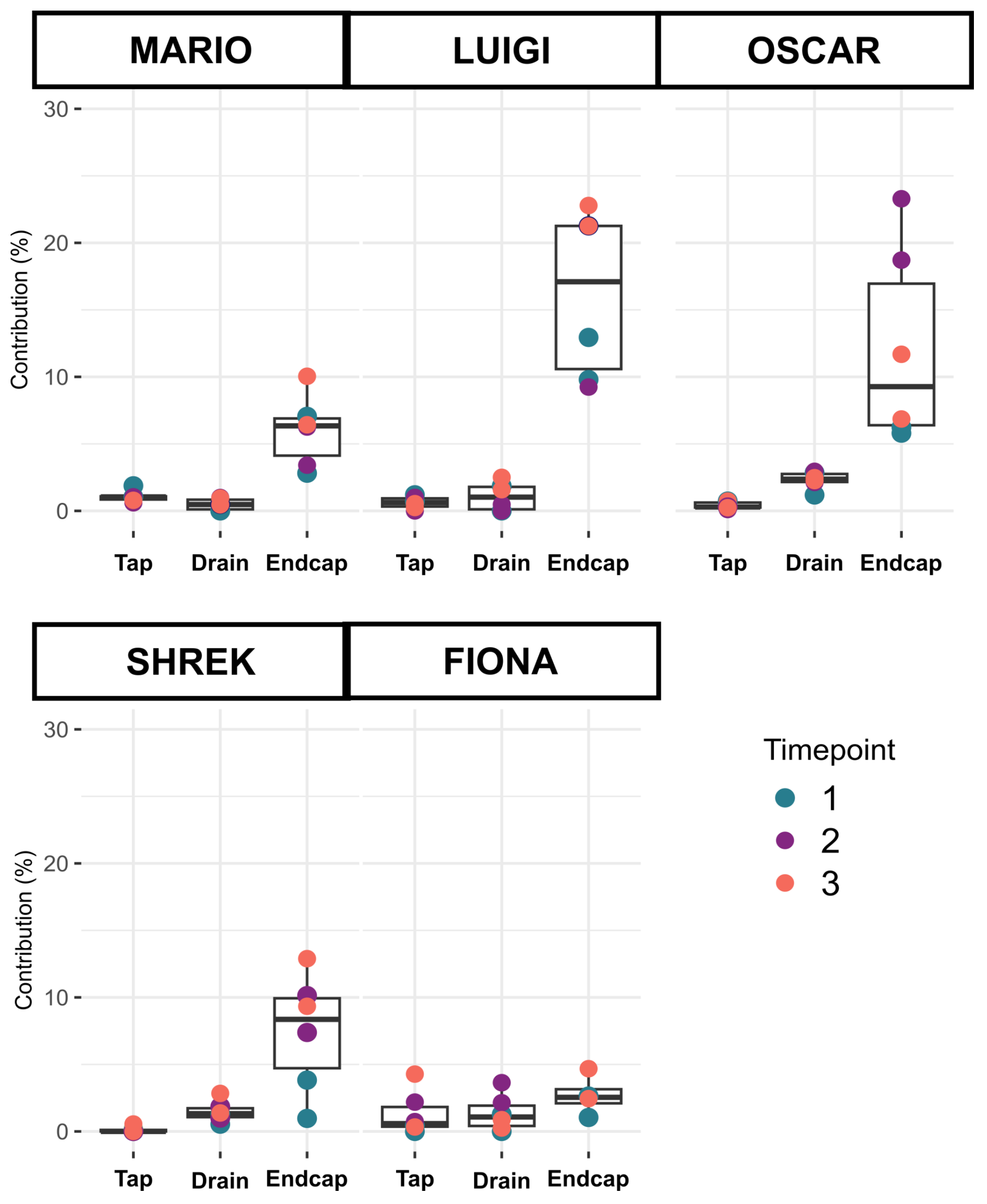


Figure 2: Percent of hospital wastewater attributed to up-sewer sources (branch sewer biofilms, sink drain biofilms , or tap water) from Fast Expectation-Maximization for Microbial Source Tracking (FEAST) analysis

BIOFILM AND WASTEWATER HAD DISTINCT AMR PROFILES

- Several vancomycin, betalactam, aminoglycoside, and multi-drug resistant genes, and *sul*1 were enriched in sewer biofilm.

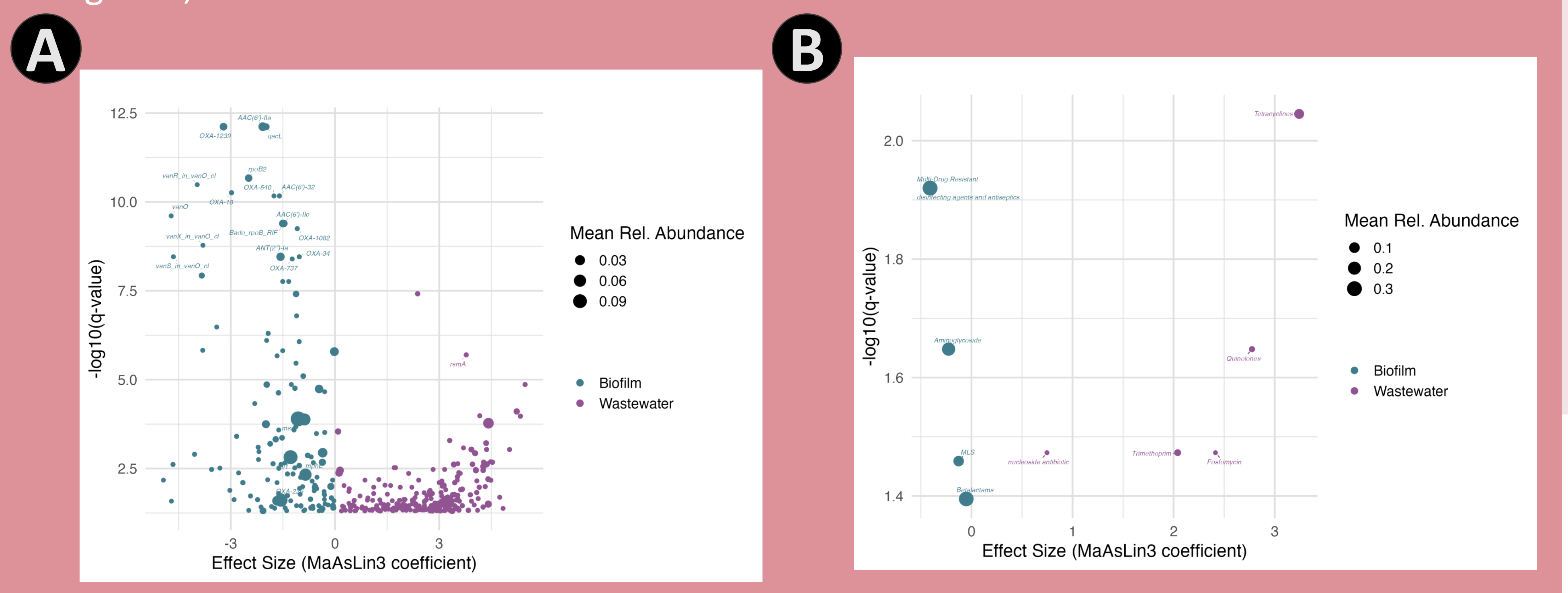


Figure 3: Volcano plots comparing microbial abundances and MaAslin3 effect sizes & significance levels between sewer biofilm and bulk wastewater for (A) ARGs and (B) ARG drug classes

CONCLUSIONS

- Biofilm composition can influence observed taxa in hospital wastewater
- Species associated with biofilm growth may require correction factors in WBS application.
- AMR profiles from wastewater may reflect biofilm-associated rather than community-derived signals.

FUTURE WORK

- Explore shared strain profiles between sink drain biofilm, sewer biofilm, and hospital wastewater microbial communities

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