

# Diversity of Antimicrobial Resistant Bacteria throughout the Wastewater Treatment Train

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## INTRODUCTION

- Wastewater is a point source for release of antimicrobial resistant bacteria to the environment, which could have negative impacts on public health and food security.
- Trends in antimicrobial resistant pathogens found in the wastewater treatment train can help design treatment stages that target their removal.
- Hypothesis:** Most identified carriers of antimicrobial resistance will be pathogenic bacteria due to selection from prior exposure (Tennstedt, Szczepanowski, Braun, Pühler, & Schlüter, 2003).

## SAMPLING SITES

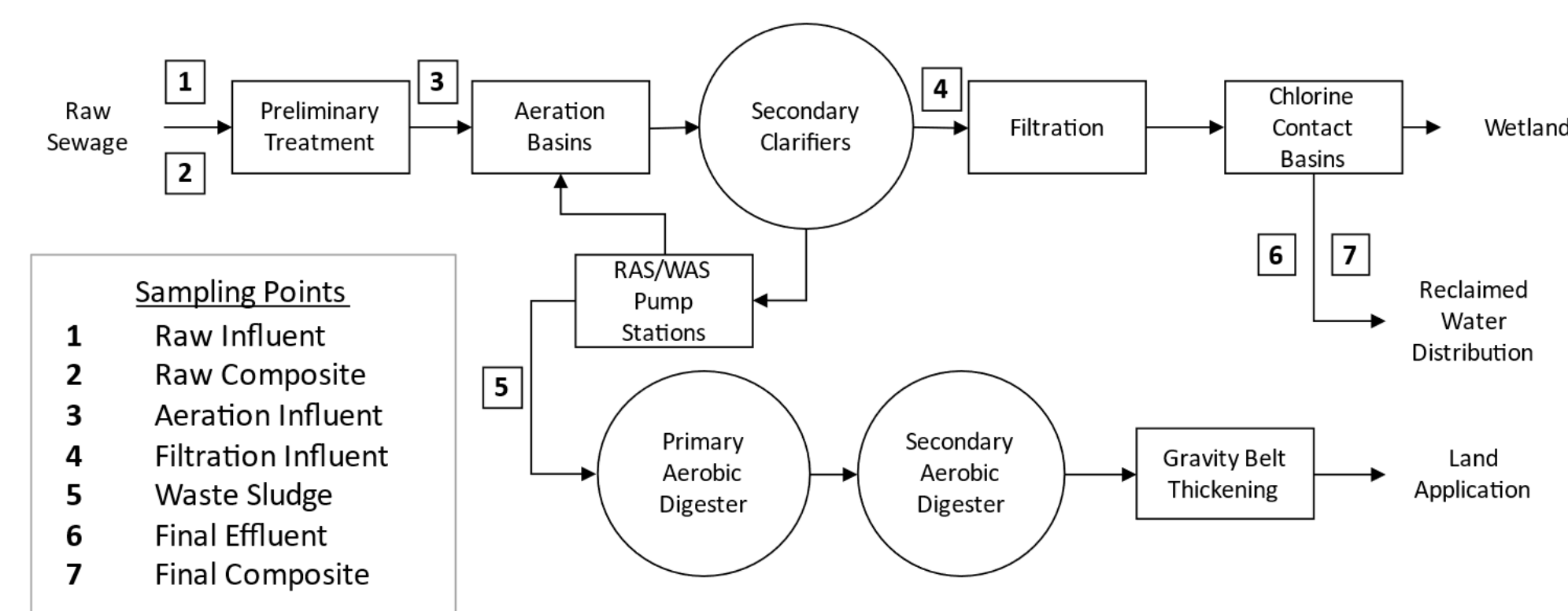


Figure 1. Sampling points in treatment train of WWTF1.

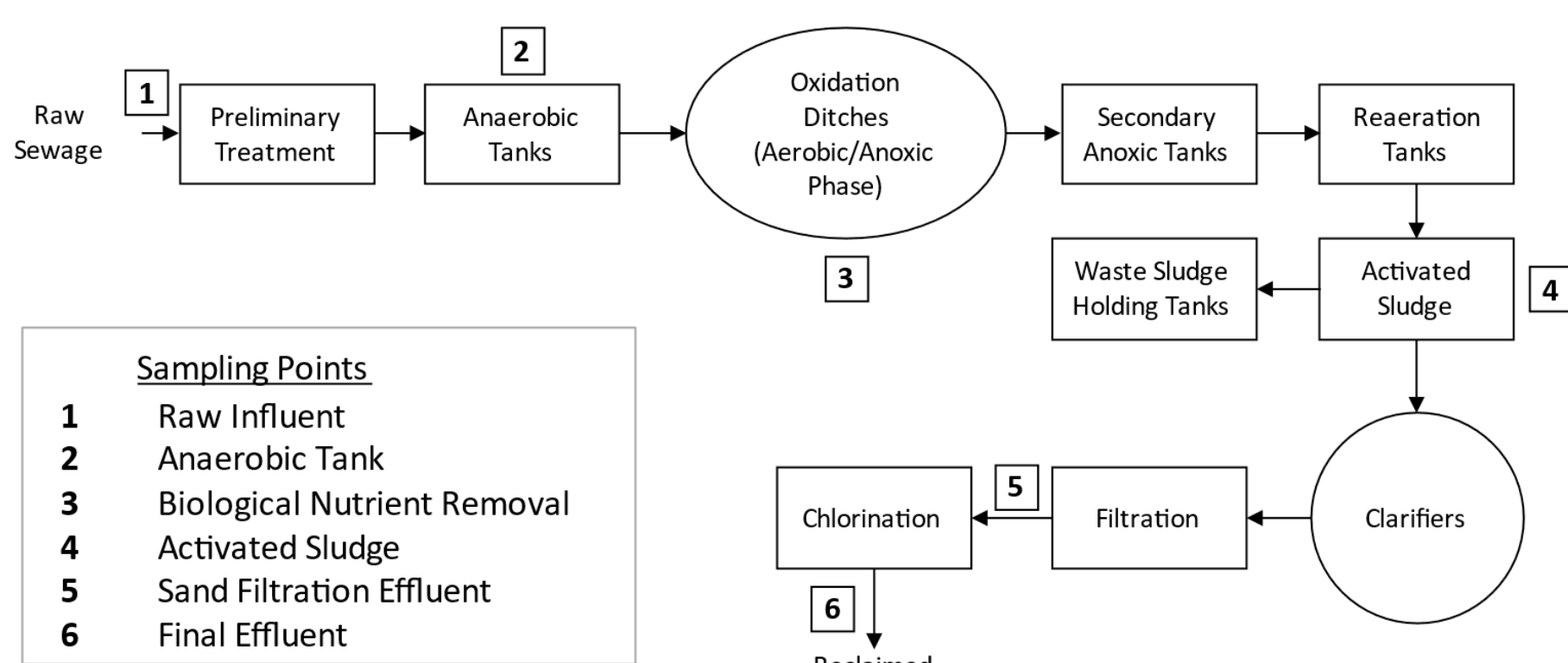


Figure 2. Sampling points in treatment train of WWTF2.

## METHODOLOGY

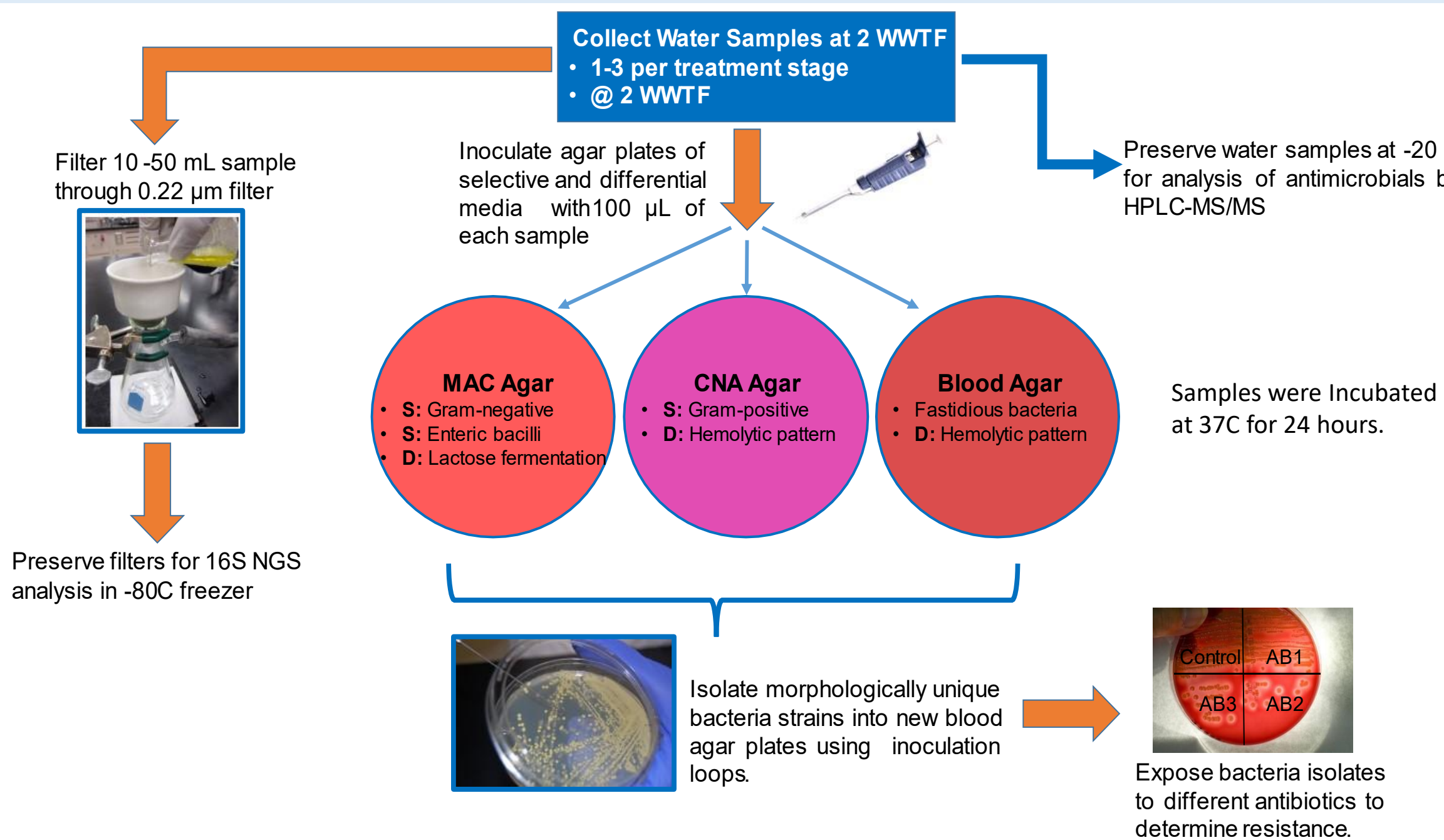


Figure 3. Summary of methods for isolation and characterization of bacteria in wastewater, and preservation of samples for molecular and chemical analysis.

## RESULTS

- Same families of bacteria were identified throughout treatment trains of WWTF1 and WWTF2 with exception of *Alcaligenaceae* for WWTF1 and *Enterobacteriaceae* for WWTF2 (Figure 4).
- Both WWTFs selected for facultatively anaerobic species during secondary treatment stages: aerobic, anaerobic, anoxic, and activated sludge stages.
- Different than WWTF2, bacteria were isolated from the effluent of WWTF1 effluent (Figure 5).
- Species isolated from filtration stages showed decreased susceptibility to low inhibitory concentrations of all tested antibiotics (Figure 6).

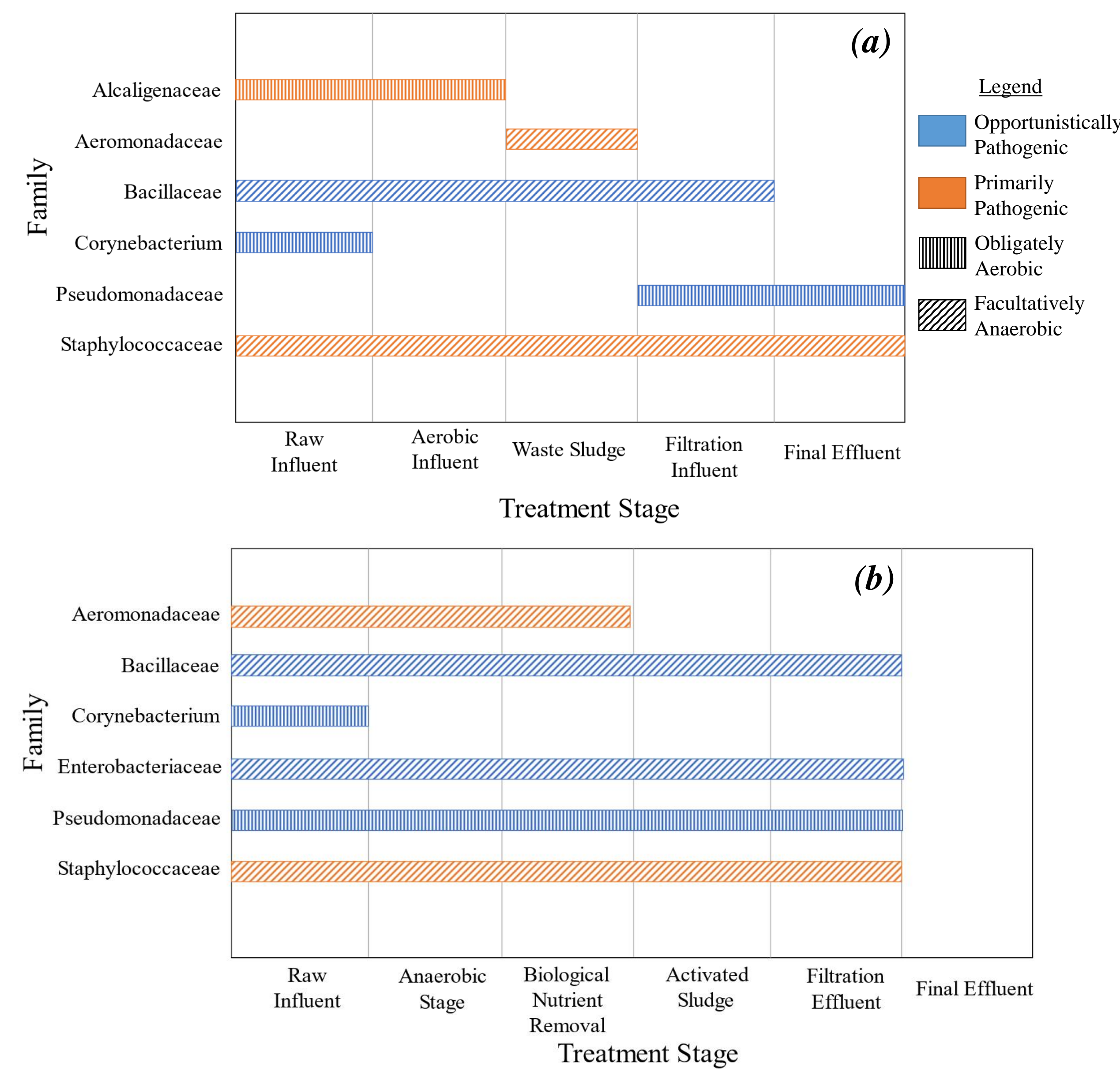


Figure 4. Occurrence of culturable bacteria throughout the treatment train of (a) WWTF1 and (b) WWTF2.

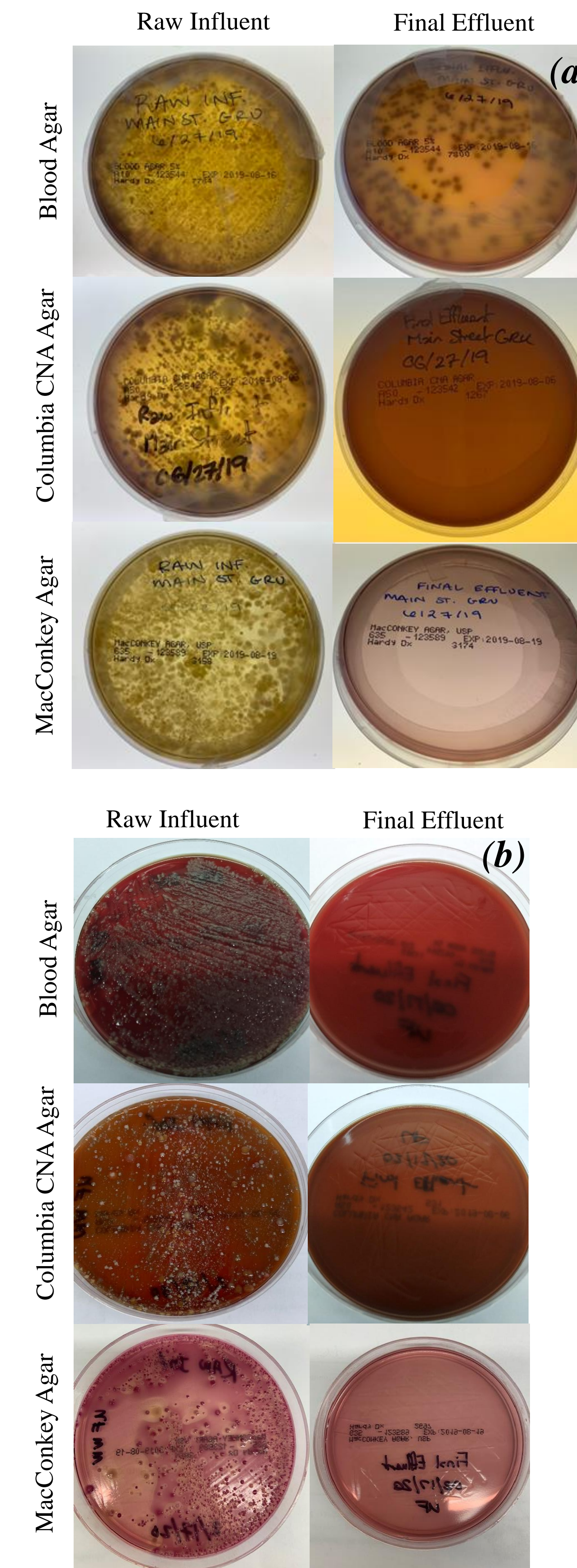


Figure 5. Microbial growth on selective and differential media inoculated with samples from (a) WWTF1 and (b) WWTF2.

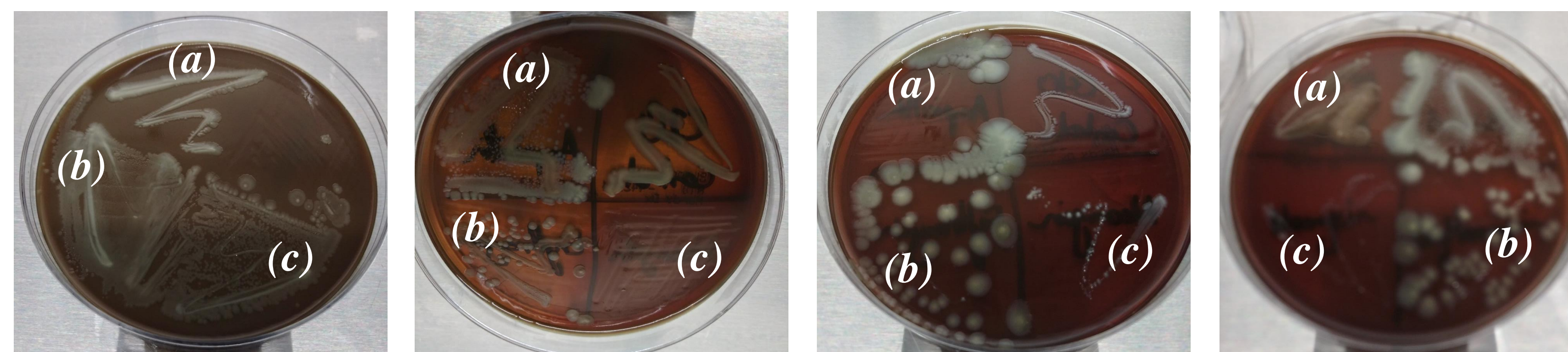


Figure 6. Resistance of selected isolated bacteria from filter effluent to (a) ampicillin, (b) erythromycin, and (c) vancomycin.

## DISCUSSION

- Identified bacterial population in both WWTFs resembles those of other investigated facilities, which have found genera from *Aeromonadaceae*, *Enterobacteriaceae*, and *Pseudomonadaceae* occurring in high abundance within treatment trains (Benami et al., 2013; Ye & Zhang, 2011).
- The absence of detected *Enterobacteriaceae* in WWTF1 could be due to small inoculating subsample used.
- Presence of bacteria in effluent water of WWTF1 could be due to contamination from collection vials.
  - Sampling was performed by the facility staff using their sampling containers.
- Greater selection for opportunistically pathogenic bacteria in WWTF2 than in WWTF1 can be attributed to inclusion of anaerobic stage, in which opportunistically pathogenic bacteria have better chance of competing with indigenous microorganisms.
- Lesser susceptibility of isolated species to ampicillin and neomycin attributed to greater transfer of genes conferring resistance to broad-spectrum antibiotics within suspended growth systems (J. Guo, Li, Chen, Bond, & Yuan, 2017).

## CONCLUSIONS & FUTURE WORK

- Wastewater treatment facilities largely select for opportunistically pathogenic, facultatively anaerobic bacteria due to their greater ability to compete against other microorganisms in highly dynamic environments.
- Preliminary results show isolated bacteria carry resistance genes for antibiotics. Ongoing HPLC-MS/MS and molecular analysis (PCR) will confirm the presence of antimicrobials and antimicrobial resistance genes (ARGs).
- Molecular analysis of bacteria in filters using the 16S/ITS amplicon kit will be used to confirm the abundance and diversity of bacteria, fungi and archaea in WWTFs.

## ACKNOWLEDGEMENTS

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## References

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