



Analysis of *Salmonella*, *E. coli*, and *Enterococcus* isolates from the Upper Oconee Watershed, GA

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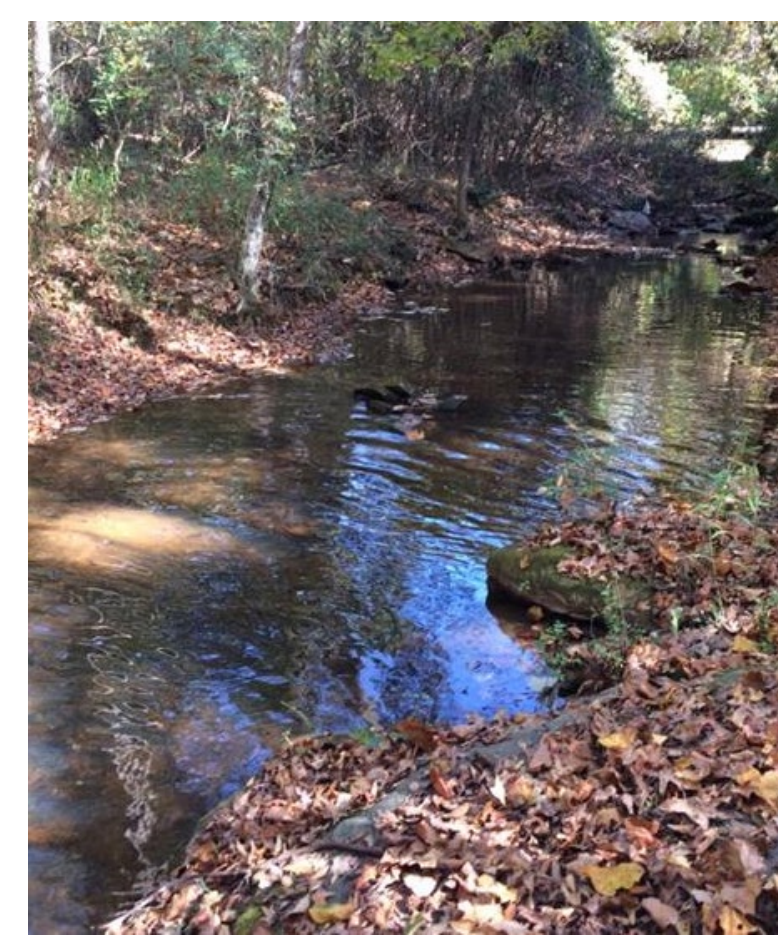
Abstract

As surface water is a receptacle for antimicrobial resistant bacteria from human and animal sources and a vehicle for their dissemination, understanding antimicrobial resistance (AR) in water environments and minimizing its spread is crucial. Water samples were collected from the rivers and streams within the Upper Oconee Watershed, GA, and then filtered and enriched for the isolation of *Salmonella*, *E. coli*, and *Enterococcus*. Bacterial isolates were confirmed, typed, and characterized for AR phenotype and AR gene content using biochemical testing, susceptibility testing, PCR, PFGE, and WGS. Analysis of this data demonstrated the prevalence and diversity of these bacteria and AR genes they harbor. Resistant bacteria that are of public health concern were frequently recovered from water environments.

Summary of Results

- 1,052 water samples were collected from 21 sampling events (2015 Winter to 2020 Winter)
- 70.1%, 99.5%, and 98.9% of the samples were positive for *Salmonella*, *E. coli*, and *Enterococcus*, respectively
- 1,796 *Salmonella*, 1,103 *E. coli* and 1,228 *Enterococcus* isolates were recovered in total
- From the 15 samplings that were fully analyzed, AR was seen in 3.8% (54/1,424) of *Salmonella*, 7.0% (60/853) of *E. coli*, and 95.6% (947/991) of *Enterococcus*

Methods



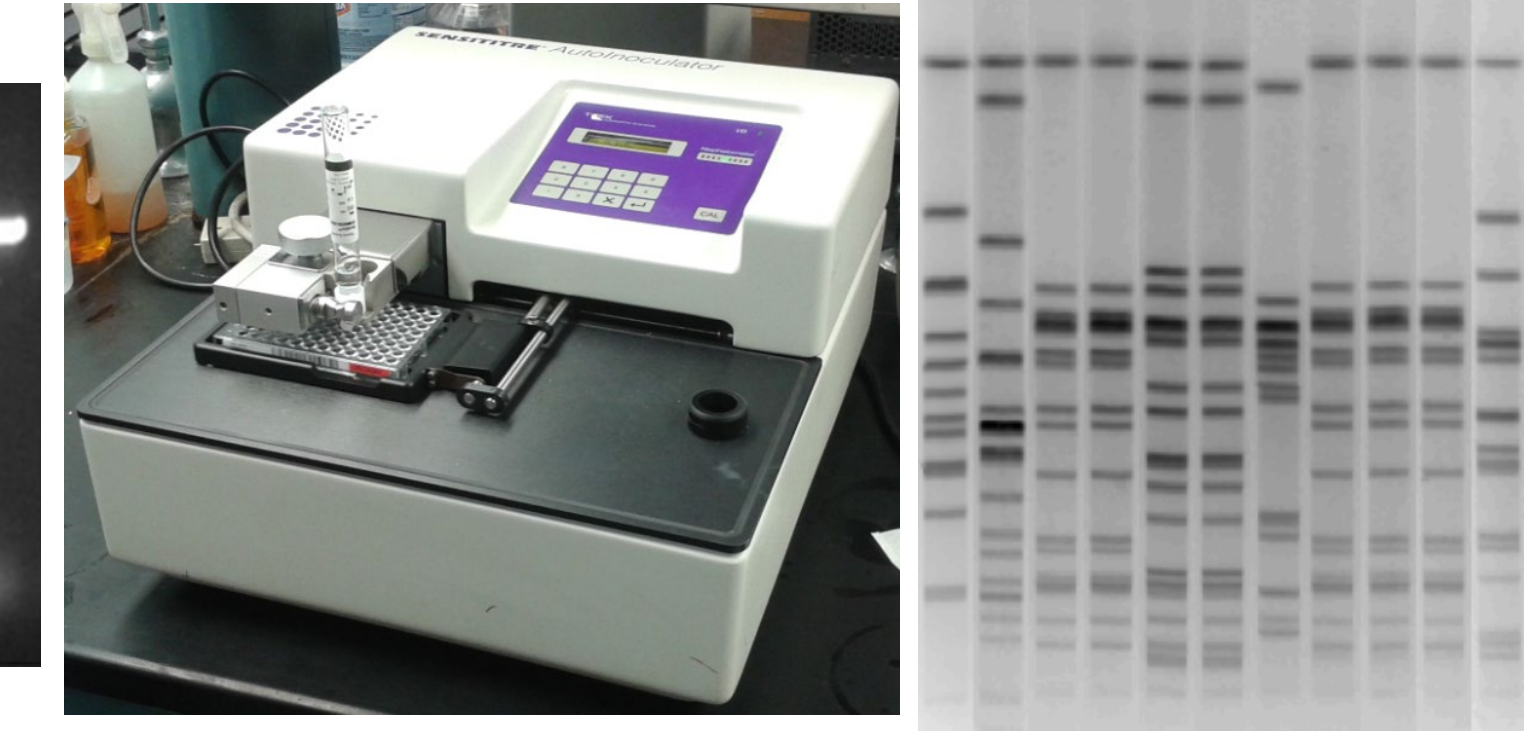
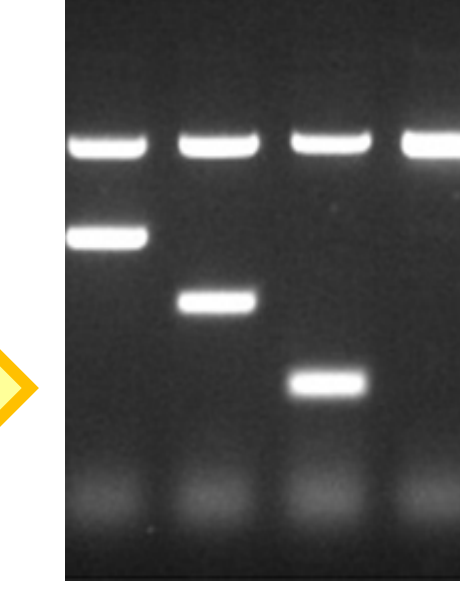
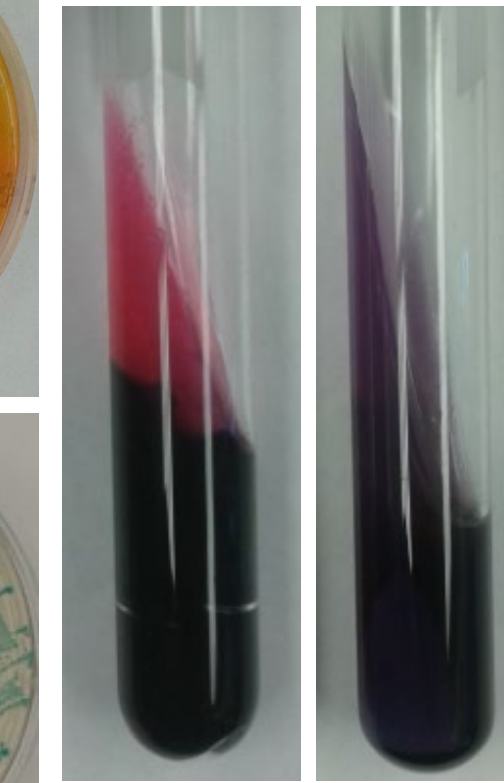
Water sampling from rivers and streams



Filtration of 1L water samples



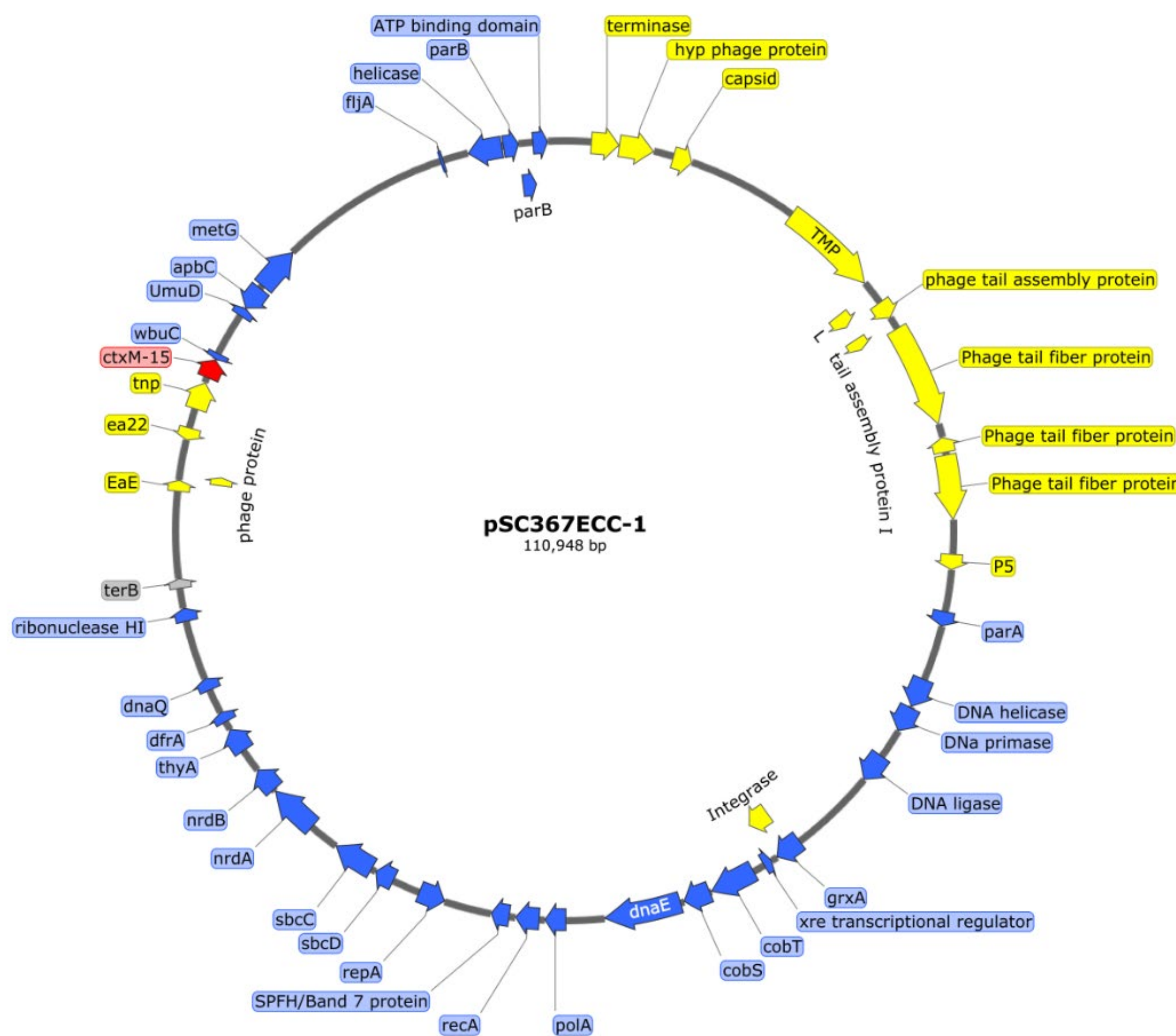
Isolation of bacteria



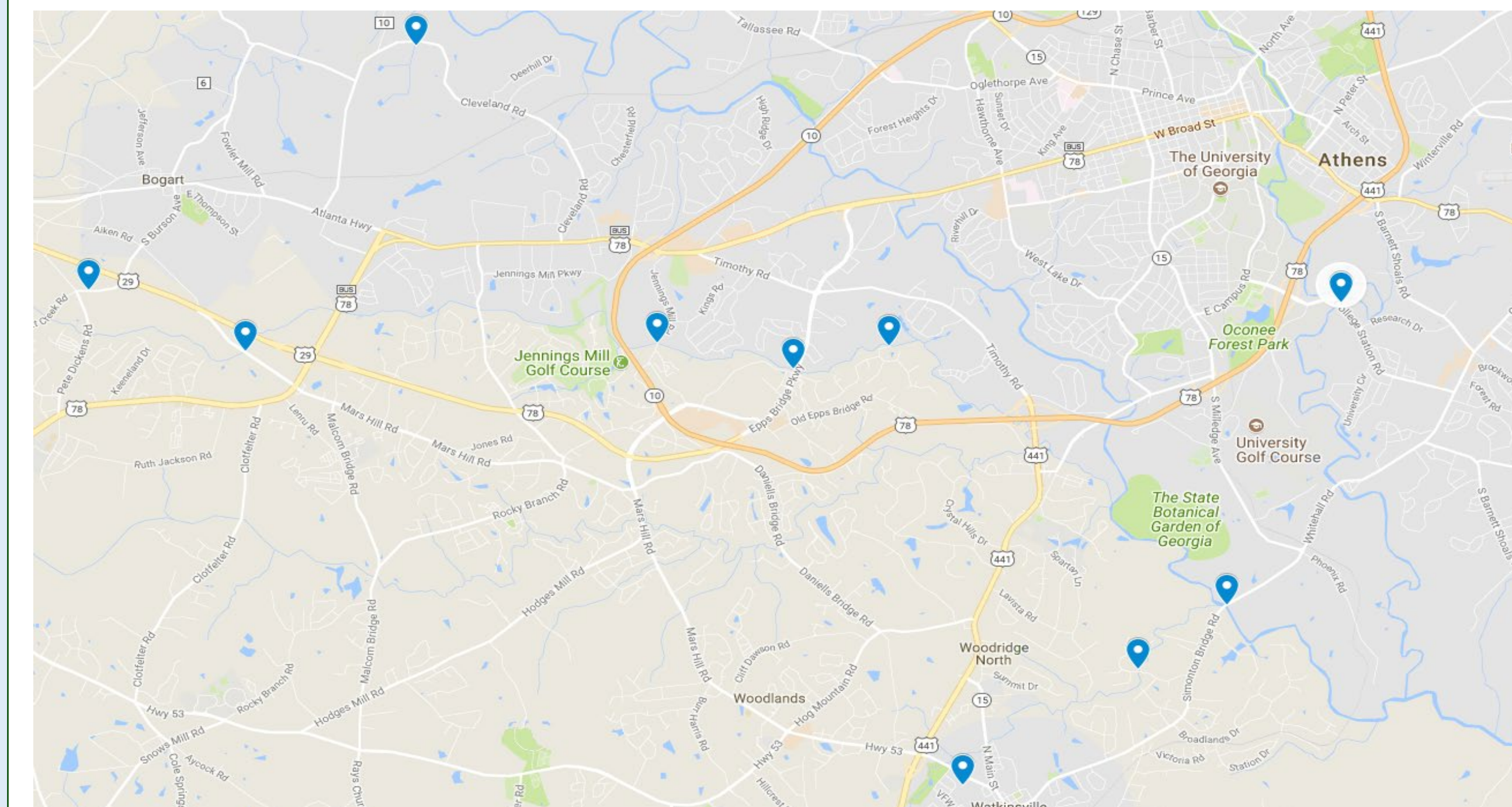
Identification and characterization of bacteria: virulence gene PCR, antimicrobial susceptibility testing, pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST), whole genome sequencing (WGS), etc.

Resistance to new drugs in *Enterococcus*. Daptomycin and tigecycline are fairly new antimicrobial drugs approved by FDA in 2003 and 2005, respectively. They are often used as the last resort drugs against Gram positive bacteria and not authorized for use in animals. However, daptomycin and tigecycline resistance was seen in 8.5% (84/991) and 8.0% (79/991) of the environmental isolates.

MDR *Enterococcus*. 84 out of 991 *Enterococcus* isolates (8.5%) were multidrug resistant (MDR: resistant to 3 or more antimicrobial drugs). The dendrogram (below) shows the genetic mechanisms of these MDR isolates. Black: resistance or presence; other colors: susceptibility or absence



Emergence of an epidemic urinary tract infection-associated *E. coli*. It is the first time an extended-spectrum beta-lactamase (ESBL)-producing *E. coli* ST131 was isolated from environmental water in the U.S. This isolate contained *bla*_{CTX-M-15} gene, which confers resistance to third-generation cephalosporins, on a very rarely reported family of phage-like plasmids. The isolate was recovered from a site near a hospital.



Isolation of multidrug resistant *Salmonella* serotype Oranienburg. 27 isolates were recovered from 10 sites (MIDO 301, 601, 604, 608, 613, 617, 707, 712, 719; NORO 605) during the 2017 Fall collection. They were clones with the same phenotypic and genotypic profiles. They were resistant to 10 antimicrobial drugs (amoxicillin/clavulanic acid, ampicillin, cefoxitin, ceftiofur, ceftriaxone, chloramphenicol, gentamicin, streptomycin, sulfisoxazole, and tetracycline). This indicates a common source of contamination.

Acknowledgements

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Publications

- Cho S, Hiott LM, Barrett JB, et al. (2018) Prevalence and characterization of *E. coli* isolated from the Upper Oconee Watershed in Northeast Georgia. PLoS ONE 13: e0197005.
- Cho S, Nguyen HAT, McDonald JM, et al. (2019) Genetic characterization of antimicrobial resistant *E. coli* isolated from a mixed-use watershed in Northeast Georgia, USA. Int J Environ Res Public Health 16: 3761.
- Cho S, Gupta SK, McMillan EA, et al. (2020) Whole genome sequence analysis of multidrug resistant *E. coli* isolated from surface water in Northeast Georgia, USA, including a ST131 epidemic strain with a phage-like plasmid encoding a *bla*_{CTX-M-15} extended spectrum beta-lactamase gene. Microb Drug Resist. 26: 447-455.
- Cho S, Hiott LM, McDonald JM, et al. (2020) Diversity and antimicrobial resistance of *Enterococcus* from the Upper Oconee watershed, Georgia. J Appl Microbiol. 128: 1221-1233.
- Cho S, Jackson CR, Frye JG (2020) The prevalence and antimicrobial resistance phenotypes of *Salmonella*, *E. coli* and *Enterococcus* sp. in surface water. Lett Appl Microbiol. 71: 3-25.
- Cho S, Barrett JB, Frye JG, et al. (2020) Antimicrobial resistance gene detection and plasmid typing among multidrug resistant enterococci isolated from freshwater environment. Microorganisms 8: 1338.
- Cho S, Hiott LM, Woodley TA, et al. (submitted) Evaluation of a new chromogenic agar for the detection of environmental *Enterococcus*.
- Cho S, Hiott LM, House SL, et al. (in preparation) Prevalence, diversity, and antimicrobial resistance of *Salmonella* isolated from a mixed use watershed, Georgia.

