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Antibiotic Resistance Patterns of *Escherichia coli* Isolates from the Clinic through the Wastewater Pathway

Brandon Bojar

Department of Biomedical Sciences, College of Health Sciences, University of Wisconsin - Milwaukee, Milwaukee, WI

Jennifer Sheridan

Department of Biomedical Sciences, College of Health Sciences, University of Wisconsin - Milwaukee, Milwaukee, WI

Rachelle Beattie

Department of Biological Sciences, Marquette University, Milwaukee, WI

Caitlin Cahak

Wisconsin Diagnostic Laboratories, Milwaukee, WI

Elizabeth Liedhegner

Department of Biomedical Sciences, College of Health Sciences, University of Wisconsin - Milwaukee, Milwaukee, WI

L. Silvia Munoz-Price

Department of Medicine, Medical College of Wisconsin, Milwaukee, WI

Krassimira R. Hristova

Department of Biological Sciences, Marquette University, Milwaukee, WI

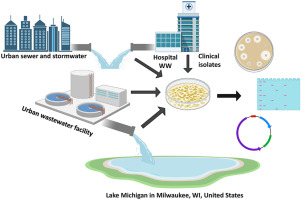
Troy Skwor

Department of Biomedical Sciences, College of Health Sciences, University of Wisconsin - Milwaukee, Milwaukee, WI

# Abstract

[Antimicrobial resistance](https://www.sciencedirect.com/topics/medicine-and-dentistry/antibiotic-resistance) (AMR) remains one of the leading global health threats. This study compared antimicrobial resistance patterns among *E. coli* isolates from clinical uropathogenic *Escherichia coli* (UPEC) to hospital wastewater populations and throughout an [urban wastewater](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/urban-wastewater) treatment facility – influent, pre- and post-chlorinated effluents. [Antibiotic susceptibility](https://www.sciencedirect.com/topics/medicine-and-dentistry/antibiogram) of 201 isolates were analyzed against eleven different [antibiotics](https://www.sciencedirect.com/topics/pharmacology-toxicology-and-pharmaceutical-science/microbicides), and the presence of twelve antibiotic resistant genes and type 1 [integrase](https://www.sciencedirect.com/topics/medicine-and-dentistry/integrase) were identified. AMR exhibited the following pattern: UPEC (46.8%) > hospital wastewater (37.8%) > urban post-chlorinated effluent (27.6%) > pre-chlorinated effluent (21.4%) > urban influent wastewater (13.3%). However, multi-drug resistance against three or more antimicrobial classes was more prevalent among hospital wastewater populations (29.7%) compared to other sources. *E. coli* from wastewaters disinfected with chlorine were significantly correlated with increased trimethoprim-sulfamethoxazole resistance in *E. coli* compared to raw and treated wastewater populations. *bla*CTX-M-1 group was the most common extended spectrum beta-lactamase in *E. coli* from hospital wastewater (90%), although UPEC strains also encoded *bla*CTX-M-1 group (50%) and *bla*TEM (100%) genes. Among tetracycline-resistant populations, [tetA](https://www.sciencedirect.com/topics/medicine-and-dentistry/trientine) and *tetB* were the only resistance genes identified throughout wastewater populations that were associated with increased phenotypic resistance. Further characterization of the *E. coli* populations identified phylogroup B2 predominating among clinical UPEC populations and correlated with the highest AMR, whereas the elevated rate of multi-drug resistance among hospital wastewater was mostly phylogroup A. Together, our findings highlight hospital wastewater as a rich source of AMR and multi-drug resistant [bacterial populations](https://www.sciencedirect.com/topics/immunology-and-microbiology/bacterial-population).

# Graphical abstract



# Introduction

Antimicrobial resistance (AMR) poses one of the largest global public health threats claiming up to 700,000 deaths worldwide annually (WHO, 2019). A major driver of AMR is the prevalent use of antibiotics throughout healthcare and agriculture (Kummerer, 2009), which leads to increased antibiotic deposition in wastewater. This widespread antibiotic use contributes to selective pressure for the transfer and acquisition of antibiotic resistant genes (ARGs) (Karkman et al., 2018; Rizzo et al., 2013). In particular, hospital wastewater remains a hotspot of antibiotic resistant bacterial populations due to contaminating levels of sub-inhibitory concentrations of antibiotics (Verburg et al., 2019) and pathogenic organisms (Paulshus et al., 2019; Sib et al., 2019). In addition, wastewater, from both hospital and urban sources, contains other stressors such as heavy metals and biocides, which have been implicated in accelerated mutation rates and horizontal gene transfer (HGT) among bacterial populations, including *Escherichia coli* (Pal et al., 2015; Seiler and Berendonk, 2012). Thus, when hospital wastewater merges with municipal streams, favorable niches for the acquisition, retention, and transfer of ARGs are created due to the high concentration of bacteria, sub-inhibitory concentrations of antibiotics, and the presence of other stressors (i.e. heavy metals) resulting in antibiotic resistant bacterial populations (Karkman et al., 2018; Lorenzo et al., 2018). Importantly, these antibiotic resistant bacterial populations are capable of surviving the wastewater treatment process and are commonly found in receiving bodies of water (Kappell et al., 2015; Skwor et al., 2020) where they can further cause recreational waterborne illnesses (Dorevitch et al., 2012; Graciaa et al., 2018a; Søraas et al., 2013).

One bacterial population prevalent in wastewater is *Escherichia coli*, which is commonly used as a fecal pollution indicator in environmental waters (Brechet et al., 2014; Lenart-Boron et al., 2020). Due to an abundance of genetic diversity, *E. coli* populations can be phylogenetically clustered into phylogroups partially coincide different ecological niches and propensities to cause disease. For example, commensal populations residing in a symbiotic relationship in warm-blooded animals are typically comprised of phylogroup A (Stoppe et al., 2017), whereby other phylogroups can cause clinical manifestations ranging from mild gastroenteritis to life-threatening sepsis caused by extraintestinal pathogenic *E. coli* (ExPEC) (Jang et al., 2017). Most ExPEC strains belong to phylogroups B2 and D, whereby intestinal pathogenic strains typically belong to phylogroups B1, D or A (Sarowska et al., 2019). Phylogrouping *E. coli* populations in wastewater allows for improved understanding of the bacterial source and can further predict linkages between source and resistance patterns.

Coliforms, such as *E. coli*, are prevalent in both warm-blooded animals, including humans, and the environment and can be used in AMR monitoring programs to assess antibiotic resistance patterns (Schrijver et al., 2018; Stelling et al., 2005). One rationale for the use of *E. coli* to monitor resistance is its identification as both a major donor and recipient of ARGs mediated through horizontal gene transfer (Young, 1993). Thus, assessing the antimicrobial resistance profiles of *E. coli* populations allows comparison between sources to aid in identifying potential hot spots of antibiotic resistance acquisition, like hospital and urban wastewater. Identifying these resistance profiles also aids in determining if clinical resistance patterns survive throughout the wastewater process which can lead to environmental release of resistant bacteria into receiving aquatic ecosystems. Given that heterogeneity of resistance patterns globally are influenced by geographic region and the Human Development Index (Hendriksen et al., 2019a), it is critical to identify AMR patterns within connected sites. To address this, our study compared AMR profiles among clinical uropathogenic *E. coli* (UPEC), hospital wastewater, and throughout different stages of wastewater treatment plant in an urban city. We also compared phylogroups to resistance profiles to identify a potential common source for the resistant populations.

# Section snippets

## Isolation of *E. coli* strains

Wastewater samples were acquired weekly for three straight weeks (October 5th, 12th, and 18th) from Jones Island Water Reclamation Facility (Supplemental Figure 1; Milwaukee, WI, USA: 43.02044, −87.89905) and two continuous weeks from two separate outflow locations (October 2nd and 11th) at the Medical College of Wisconsin (Supplemental Figure 1; Milwaukee, WI, USA: 43.04355, −88.02139) in October 2018 by plant technicians. Water samples were immediately placed on ice until further processed in

## Comparing antimicrobial resistance of *E. coli* UPEC, and isolates from hospital and urban wastewater

To aid in assessing the health risk associated with wastewater, we compared antibiotic resistance patterns among UPEC isolates (n = 77) to both hospital (n = 37) and combined (both stormwater runoff and sanitary sewage) urban wastewater (n = 30) *E. coli* isolates. All isolates were acquired from the Milwaukee, WI metropolitan area (population of 1,572,245). Total *E. coli* colonies acquired from hospital wastewater over the period of the study ranged from 4.0 x 101–5.5 x 105 CFU/100 ml compared to

# Discussion

AMR is a leading public health concern and both municipal and hospital wastewaters have been identified as point sources of ARGs and AMR bacterial contaminants to the recipient aquatic environments. Global metagenomics analysis of wastewater has not only highlighted the diversity of ARGs associated with AMR, but also highlighted the variability between geographical regions, dependent on the Human Development Index (Hendriksen et al., 2019b). Additionally, the gut microbiomes of urban

# Conclusions

This study analyzed antimicrobial resistance patterns from directionally connected sites from clinical strains (UPEC) to hospital wastewater through an urban wastewater treatment processes concluding with the final effluent entering the recipient aquatic ecosystem. Our findings identify hospital wastewater effluent *E. coli* populations with the highest MDR patterns, as well as encoding similar ARGs and *int1* compared to UPEC populations*.* The highest prevalence of AMR existed within UPEC

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# Declaration of competing interest

The authors declare no actual or potential conflict of interest with other people or organizations that might influence the associated research.

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