9-2021

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

Brandon Bojar
University of Wisconsin - Milwaukee

Jennifer Sheridan
University of Wisconsin - Milwaukee

Rachelle E. Beattie
Marquette University

Caitlin Cahak
Wisconsin Diagnostic Laboratories

Elizabeth Liedhegner
University of Wisconsin - Milwaukee

See next page for additional authors

Recommended Citation

Bojar, Brandon; Sheridan, Jennifer; Beattie, Rachelle E.; Cahak, Caitlin; Liedhegner, Elizabeth; Munoz-Price, L. Silvia; Hristova, Krassimira R.; and Skwor, Troy, "Antibiotic Resistance Patterns of *Escherichia coli* Isolates from the Clinic through the Wastewater Pathway" (2021). *Biological Sciences Faculty Research and Publications*. 851.
https://epublications.marquette.edu/bio_fac/851

Authors

Brandon Bojar, Jennifer Sheridan, Rachelle E. Beattie, Caitlin Cahak, Elizabeth Liedhegner, L. Silvia Munoz-Price, Krassimira R. Hristova, and Troy Skwor

International Journal of Hygiene and Environmental Health, Vol. 238 (September 2021): 113863. DOI.

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

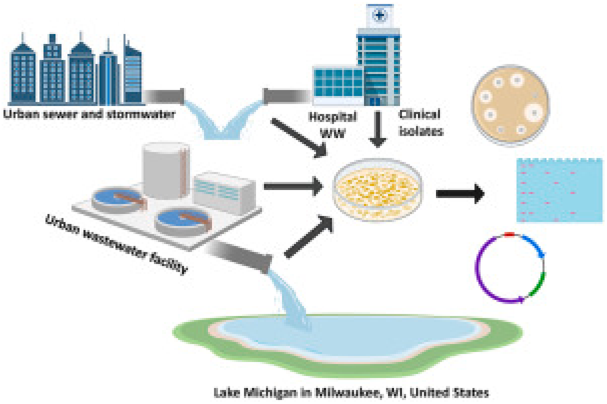
Brandon Bojar
Department of Biomedical Sciences, College of Health Sciences, University of Wisconsin - Milwaukee, Milwaukee, WI
Jennifer Sheridan
Department of Biomedical Sciences, College of Health Sciences, University of Wisconsin - Milwaukee, Milwaukee, WI
Rachelle Beattie
Department of Biological 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 (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 treatment facility – influent, pre- and post-chlorinated effluents. Antibiotic susceptibility of 201 isolates were analyzed against eleven different antibiotics, and the presence of twelve antibiotic resistant genes and type 1 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*$_{\text{CTX-M-1}}$ group was the most common extended spectrum beta-lactamase in *E. coli* from hospital wastewater (90%), although UPEC strains also encoded *bla*$_{\text{CTX-M-1}}$ group (50%) and *bla*$_{\text{TEM}}$ (100%) genes. Among tetracycline-resistant populations, tetA 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.

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 10^1–5.5 x 10^5 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

Funding

This study was supported by funds from Department of Biomedical Sciences at the University of Wisconsin – Milwaukee, WI, USA (Skwor) and Marquette University Explorer Challenge Grant, Milwaukee, WI, USA (Hristova).

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.

Acknowledgments

The authors would like to thank the staff of Milwaukee Metropolitan Sewage District and Jones Island Water Reclamation Facility for their assistance and cooperation in acquiring wastewater samples for the above study. Graphical abstract was created with BioRender.com.

References

S. Zhi *et al.* **Characterization of water treatment-resistant and multidrug-resistant urinary pathogenic *Escherichia coli* in treated wastewater.** *Water Res*. (2020)

S. Zhi *et al.* **Evidence for the evolution, clonal expansion and global dissemination of water treatment-resistant naturalized strains of *Escherichia coli* in wastewater.** *Water Res*. (2019)

E. Sib *et al.* **Antibiotic resistant bacteria and resistance genes in biofilms in clinical wastewater networks.** Int. J. *Hyg Environ. Health*. (2019)

R. Schrijver *et al.* **Review of antimicrobial resistance surveillance programmes in livestock and meat in EU with focus on humans.** *Clin. Microbiol. Infect*. (2018)

D. Salazar *et al.* **Assessment of antibiotic resistant coliforms from bioaerosol samples collected above a sewage-polluted river in La Paz, Bolivia.** *Int. J. Hyg Environ. Health.* (2020)

L. Rizzo *et al.* **Urban wastewater treatment plants as hotspots for antibiotic resistant bacteria and genes spread into the environment: a review.** *Sci. Total Environ*. (2013)

E. Paulshus *et al.* **Diversity and antibiotic resistance among *Escherichia coli* populations in hospital and community wastewater compared to wastewater at the receiving urban treatment plant.** *Water Res*. (2019)

L. Mughini-Gras *et al.* **Attributable sources of community-acquired carriage of *Escherichia coli* containing beta-lactam antibiotic resistance genes: a population-based modelling study.** *Lancet Planet Health.* (2019)

A.P. Magiorakos *et al.* **Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance.** *Clin. Microbiol. Infect*. (2012)

P. Lorenzo *et al.* **Antibiotic resistance in urban and hospital wastewaters and their impact on a receiving freshwater ecosystem.** *Chemosphere* (2018)

Y.K. Kwak *et al.* **Surveillance of antimicrobial resistance among *Escherichia coli* in wastewater in Stockholm during 1 year: does it reflect the resistance trends in the society?** *Int. J. Antimicrob. Agents* (2015)

K. Kummerer. **Antibiotics in the aquatic environment--a review--part II.** *Chemosphere* (2009)

E. Korzeniewska *et al.* **Antibiotic resistant *Escherichia coli* in hospital and municipal sewage and their emission to the environment.** *Ecotoxicol. Environ. Saf.* (2013)

R. Kaur *et al.* **Microbiology of hospital wastewater.** *Current Developments in Biotechnology and Bioengineering* (2020)

A. Karkman *et al.* **Antibiotic-resistance genes in waste water.** *Trends Microbiol.* (2018)

Y. Deng *et al.* **Resistance integrons: class 1, 2 and 3 integrons.** *Ann. Clin. Microbiol. Antimicrob.* (2015)

L.V. Chávez-Díaz *et al.* **Sediments quality must be considered when evaluating freshwater aquatic environments used for recreational activities.** *Int. J. Hyg Environ. Health* (2020)

I. Bueno *et al.* **Role of wastewater treatment plants on environmental abundance of antimicrobial resistance genes in Chilean rivers.** *Int. J. Hyg Environ. Health* (2020)

R.E. Beattie *et al.* **Survivor microbial populations in post-chlorinated wastewater are strongly associated with untreated hospital sewage and include ceftazidime and meropenem resistant populations.** *Sci. Total Environ.* (2020)

E.M. Anastasi *et al.* **Survival of *Escherichia coli* in two sewage treatment plants using UV irradiation and chlorination for disinfection.** *Water Res.* (2013)

E.M. Anastasi *et al.* **Prevalence and persistence of *Escherichia coli* strains with uropathogenic virulence characteristics in sewage treatment plants.** *Appl. Environ. Microbiol.* (2010)

S. Baron *et al.* **Aeromonas diversity and antimicrobial susceptibility in freshwater-an attempt to set generic epidemiological cut-off values.** *Front. Microbiol.* (2017)

R.E. Beattie *et al.* **Antimicrobial resistance traits of *Escherichia coli* isolated from dairy manure and freshwater ecosystems are similar to one another but differ from associated clinical isolates.** *Microorganisms* (2020)

L.J. Beversdorf *et al.* **The potential for beach sand to serve as a reservoir for *Escherichia coli* and the physical influences on cell die-off.** *J. Appl. Microbiol.* (2007)

L.A. Boczek *et al.* **Occurrence of antibiotic-resistant uropathogenic *Escherichia coli* clonal group A in wastewater effluents.** *Appl. Environ. Microbiol.* (2007)

C. Brechet *et al.* **Wastewater treatment plants release large amounts of extended-spectrum beta-lactamase-producing *Escherichia coli* into the environment.** *Clin. Infect. Dis.* (2014)

V. Calhau *et al.* **Virulence and plasmidic resistance determinants of *Escherichia coli* isolated from municipal and hospital wastewater treatment plants.** *J. Water Health.* (2014)

O. Clermont *et al.* **The Clermont *Escherichia coli* phylo-typing method revisited: improvement of specificity and detection of new phylo-groups.** *Environ Microbiol Rep.* (2013). **Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically; Approved Standard. CLSI Document M07-A10.** (2015). **Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fifth Informational Supplement. CLSI Document M100-S25.** (2015)

J. Derx *et al.* **QMRAcatch: human-associated fecal pollution and infection risk modeling for a river/floodplain environment.** *J. Environ. Qual.* (2016)

S. Dorevitch *et al.* **Health risks of limited-contact water recreation.** *Environ. Health Perspect.* (2012)

J. Engelstädter *et al.* **The evolutionary dynamics of integrons in changing environments.** *ISME J.* (2016). **Method 1603: *Escherichia coli (E. coli) i*n Water by Membrane Filtration Using Modified Membrane-Thermotolerant Escherichia coli Agar (Modified mTEC)**