2016

Microbial Sampling of Major Bodies of Water in Rochester, NY

Laura Moore
St. John Fisher College, lam04586@students.sjfc.edu

Daniel Zimmerman
St. John Fisher College, djz03192@students.sjfc.edu

J. A. Concha
Rochester Institute of Technology

N. Raqueño
Rochester Institute of Technology

Fernando Ontiveros
St. John Fisher College, fontiveros-llamas@sjfc.edu

See next page for additional authors

Publication Information
Moore, Laura; Zimmerman, Daniel; Concha, J. A.; Raqueño, N.; Ontiveros, Fernando; and Herman, Maryann, "Microbial Sampling of Major Bodies of Water in Rochester, NY" (2016). *Undergraduate External Publications.* Paper 9.
https://fisherpub.sjfc.edu/undergraduate_ext_pub/9
Please note that the Publication Information provides general citation information and may not be appropriate for your discipline. To receive help in creating a citation based on your discipline, please visit http://libguides.sjfc.edu/citations.

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Abstract

Public health concerns from wastewater treatment and agricultural runoff are an issue locally in Rochester, NewYork. In fact, many closings of Ontario Beach have been attributed to pollution or the threat of microbial contamination. Antibiotic resistance is a major issue that has become more prevalent in society, antibiotic resistant human pathogenic bacteria can overcome normal types of medicinal therapy, which cannot only lead to increased mortality but also increases in illness and cost of care. Additionally, antibiotic resistant plant pathogens can impact agriculture. In this study, bacterial species from the Lake Ontario embayment collected over the past three years were analyzed. Bacteria that were known to be human or plant pathogens were selected to evaluate for resistance to commonly used antibiotics using the Kirby Bauer disk diffusion assay. A few species exhibited resistance, preliminary results will be shown.

Keywords

fsc2016

Disciplines

Biology

Comments

Presented at the Tri-Beta Northeast-3 District Conference in Latrobe, Pennsylvania, 2016.

Authors

Laura Moore, Daniel Zimmerman, J. A. Concha, N. Raqueño, Fernando Ontiveros, and Maryann Herman

Microbial Sampling of Major Bodies of Water in Rochester, NY

L. Moore*, D. Zimmerman*, J.A. Concha†, N. Raqueño†, F. Ontiveros PhD*, M. Herman PhD*

*Biology Department, St. John Fisher College, Rochester, NY 14618

†Rochester Institute of Technology, 1 Lomb Memorial Dr., Rochester, NY 14623

Abstract

Public health concerns from wastewater treatment and agricultural runoff are an issue locally in Rochester, New York. In fact, many closings of Ontario Beach have been attributed to pollution or the threat of microbial contamination. Antibiotic resistance is a major issue that has become more prevalent in society, antibiotic resistant human pathogenic bacteria can overcome normal types of medicinal therapy, which cannot only lead to increased mortality but also increases in illness and cost of care. Additionally, antibiotic resistant plant pathogens can impact agriculture. In this study, bacterial species from the Lake Ontario embayment collected over the past three years were analyzed. Bacteria that were known to be human or plant pathogens were selected to evaluate for resistance to commonly used antibiotics using the Kirby Bauer disk diffusion assay. A few species exhibited resistance, preliminary results will be shown.

Introduction

Increasing levels of antibiotic resistance has become a growing concern worldwide [Figure 1]. Wastewater is a major potential source for entry of antibiotic resistant bacteria into local waterways and antibiotic resistant bacteria have been found in high concentrations in water discharged from wastewater treatment plants [1].

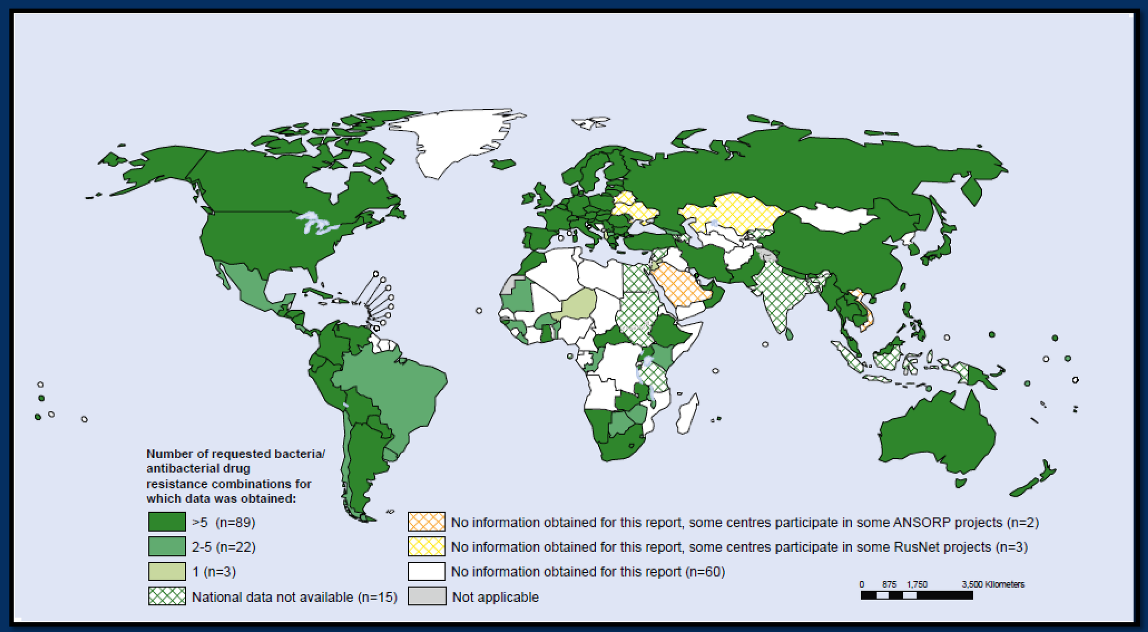

Figure 1. Availability of data on resistance for selected bacteria-antibacterial drug combinations, 2013 [2].

In Rochester, New York many closings of local beaches have been attributed to pollution or the threat of microbial contamination. Several local beaches are among the nation's top repeat offenders for consistently displaying high bacterial counts that exceed safety levels [3].

Human activities lead to the creation of an urban water cycle, where bacteria can be moved from unclean water habitats, like wastewater, to clean water environments like spring water or local lakes and ponds, eventually reaching humans. Also, bacteria can transfer mobile genetic elements coding for antibiotic resistance between species found in different types of water, soil and humans, as depicted in Figure 2 [4].

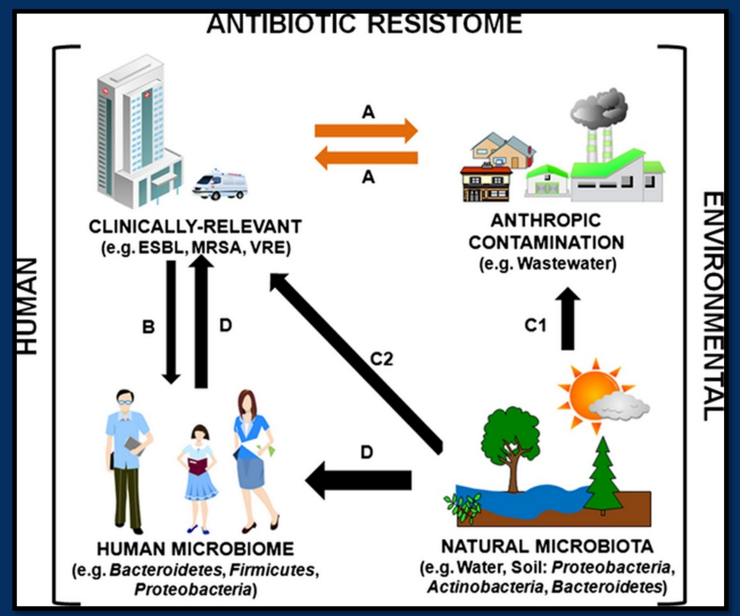

Figure 2. This figure depicts the proposed relationship between the environmental and human antibiotic resistome. Antibiotic resistance determinants can be transferred in many different ways, including between commensal species on humans, clinical settings, wastewater, and the natural environment [4].

The objective of this work was identify pathogenic bacteria in local bodies of water and test these bacterial strains for resistance against five standard antibiotics.

Materials and Methods

Bacterial Strains tested:

- All strains were isolated 2013-2015 from fresh water lakes, ponds, and rivers in Rochester, NY (Figure 3).
- Sequencing of 16S ribosomal DNA was used to identify bacterial isolates.
- Animal and plant pathogens were selected for initial antibiotic resistance testing.

Figure 3. Locations of water sampling in Rochester, NY.

Test Compounds:

- Five common antibiotics were tested with each bacterial strain (Ampicillin 10µg, Ciprofloxacin 5µg, Erythromycin 15µg, Gentamycin 10µg, Sulfamethoxazol 23.75µg).

Disc Diffusion Assay [5]

- Liquid cultures were incubated at 21°C for 16-18 hours (grown to concentrations 10^7-10^8 cfu/mL).
- Spread plates on Mueller-Hinton agar were made from bacterial suspensions and incubated with antibiotic disks at 21°C for 18-24 hours (Figure 4).
- Three independent experiments, each with three replicates, were performed and the zones of inhibition (mm) expressed in mean values. Degree of antibiotic resistance (susceptible, intermediate, or resistant) was determined for each antibiotic-bacterial isolate pair.

Results

- All bacterial pathogens tested were susceptible to gentamycin.
- Most species were susceptible to ciprofloxacin and sulfamethoxazole and none exhibited resistance.
- Only two species were resistant to erythromycin but the majority exhibited intermediate resistance.
- Over half of the bacteria tested exhibited intermediate or full resistance to ampicillin.

	Antibiotic				
	GEN	CIP	AMP	SXT	ERY
Gram Positive Isolates					
Kocuria rhizophila	21.7 (S)	33.5 (S)	18.2 (R)	15.5 (I)	18.0 (I)
Staphylococcus epidermidis	27.2 (S)	34.2 (S)	22.0 (R)	16.7 (S)	20.0 (I)
Bacillus pumilus (SN1)	30.2 (S)	32.0 (S)	39.7 (S)	38.2 (S)	24.2 (S)
Bacillus pumilus (SN99)	27.7 (S)	33.3 (S)	17.5 (S)	22.3 (S)	16.8 (I)
Gram Negative Isolates					
Pseudomonas oryzhabitans (SN102)	19.5 (S)	22.8 (S)	13.5 (R)	12.5 (I)	16.2 (I)
Pseudomonas oryzhabitans (SN108)	24.7 (S)	33.8 (S)	18.2 (S)	14.5 (I)	15.3 (I)
Pseudomonas aeruginosa	24.8 (S)	35.2 (S)	18.3 (S)	13.8 (I)	18.8 (I)
Xanthomonas oryzae	31.7 (S)	37.0 (S)	23.0 (S)	17.0 (S)	20.5 (I)
Pseudomonas oleovorans	24.5 (S)	26.2 (S)	17.5 (S)	13.8 (I)	15.7 (I)
Acinetobacter junii	18.7 (S)	20.5 (I)	11.5 (R)	11.8 (I)	15.5 (I)
Acinetobacter lwoffi (SN85)	19.5 (S)	23.0 (S)	16.2 (I)	25.7 (S)	15.8 (I)
Acinetobacter lwoffi (SN86)	19.8 (S)	24.8 (S)	16.0 (I)	17.5 (S)	19.2 (I)
Acinetobacter lwoffi (SN51)	21.2 (S)	41.0 (S)	10.8 (R)	34.3 (S)	18.5 (I)
Acinetobacter lwoffi (SN53)	24.0 (S)	40.5 (S)	11.5 (R)	36.0 (S)	16.2 (I)
Pantoea agglomerans (SN55)	22.2 (S)	34.0 (S)	13.0 (R)	29.8 (S)	13.7 (R)
Pantoea agglomerans (SN56)	22.3 (S)	35.3 (S)	15.2 (I)	35.8 (S)	13.3 (R)
Pantoea agglomerans (SN59)	24.2 (S)	38.8 (S)	14.5 (I)	35.3 (S)	14.8 (I)

Table 2. Efficacy of common antibiotics against human and plant pathogens. Zones of inhibition were measured in millimeters. Antibiotics are denoted as: Ampicillin (AMP), Ciprofloxacin (CIP), ERY (Erythromycin), GEN (Gentamycin), SXT (Sulfamethoxazol). Initials S, I, R refer to degree of antibiotic resistance (S = Sensitive, I = Intermediate, R = Resistant).

Discussion and Future Directions

This research provides evidence that some pathogenic bacterial species found in local bodies of water are resistant to common antibiotics. This could have a major impact on society if humans, plants, or other bacteria are exposed to these resistant isolates. However, the majority of the species were sensitive partially resistant to most of the antibiotics tested.

Research is ongoing to:

- Complete characterization of all pathogens isolated from Rochester waterways (2015 isolates).
- Compare similar species of bacteria across different locations and collection dates to determine any difference in antibiotic resistance.
- Expand testing to non-pathogenic species in the collection.

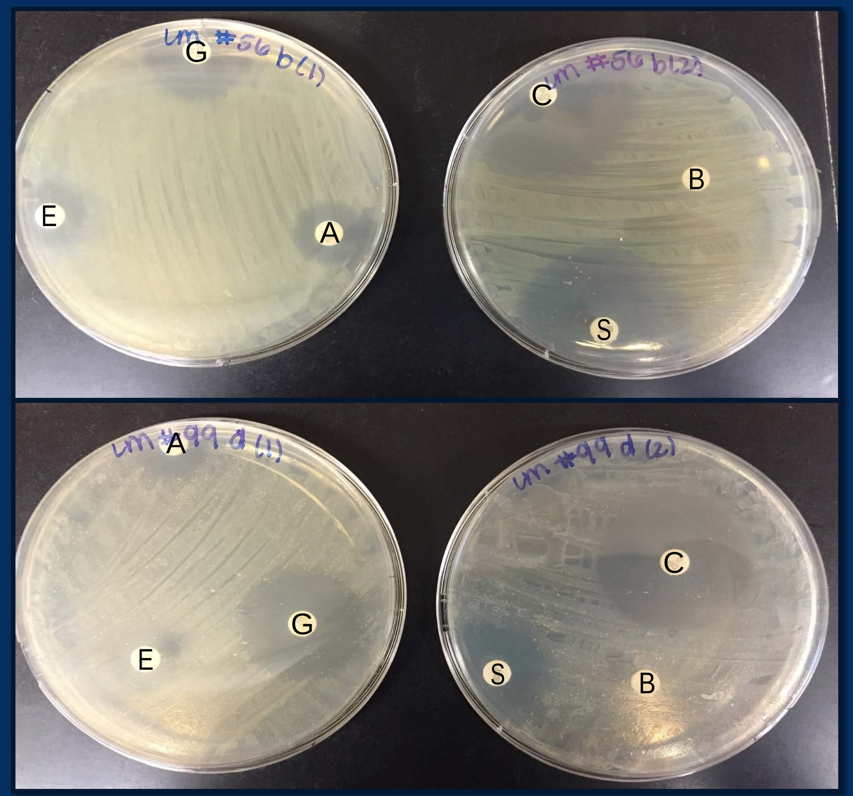

Figure 4. Efficacy of antibiotics against *Pantoea agglomerans* (top) and *Bacillus pumilus* (bottom). Antibiotics are denoted as: A - Ampicillin, B – blank, C - Ciprofloxacin, E - Erythromycin, G - Gentamycin, S - Sulfamethoxazol.

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