Methods for Surveillance of Antimicrobial Resistant Bacteria in Environmental Water and Wastewater

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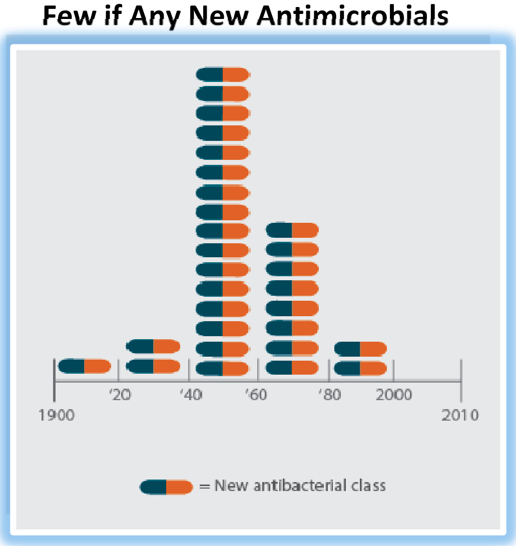
March 16th, 2017

Presented Graciously by Dr. Emanuele Sozzi

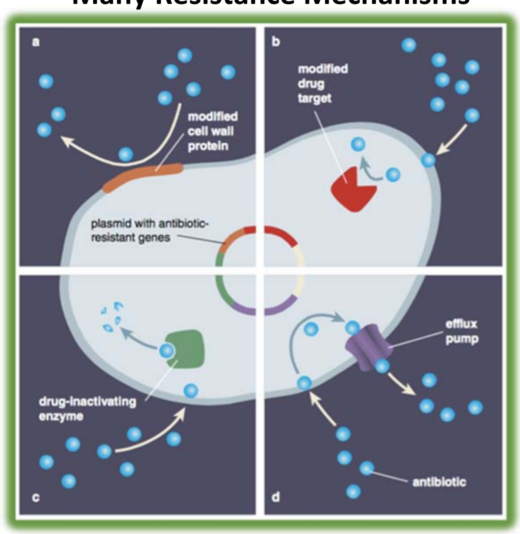
Antibiotic Resistance is an Increasing Threat to Health

Few if Any New Antimicrobials



https://i1.wp.com/www.me-med.com/html5/Web/2466/antibiotic_resistance-a.png

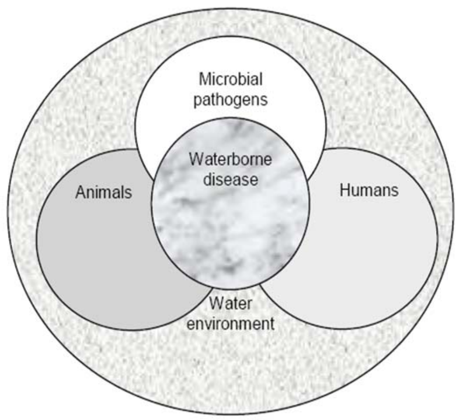
Many Resistance Mechanisms

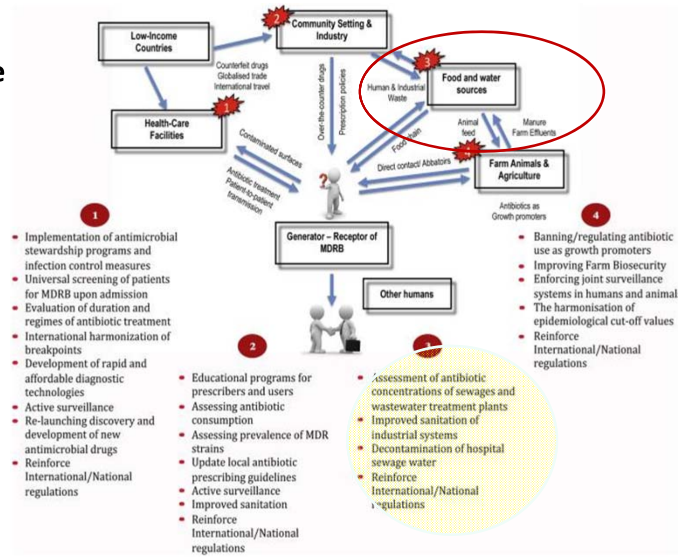


https://i1.wp.com/www.me-med.com/html5/Web/2466/antibiotic_resistance-a.png

AMR Hotspots as Targets for Interventions to Reduce Risks

Waterborne Disease Interactions in the Water Environment: A One Health Issue





Waterborne zoonoses : identification, causes, and control.
Cotruvo, J.A. et al. (eds), World Health Organization and Intl. Water Assoc., London

Roca et al. The global threat of antimicrobial resistance: science for intervention. New Microbes and New Infections, Volume 6, July 2015, Pages 22–29

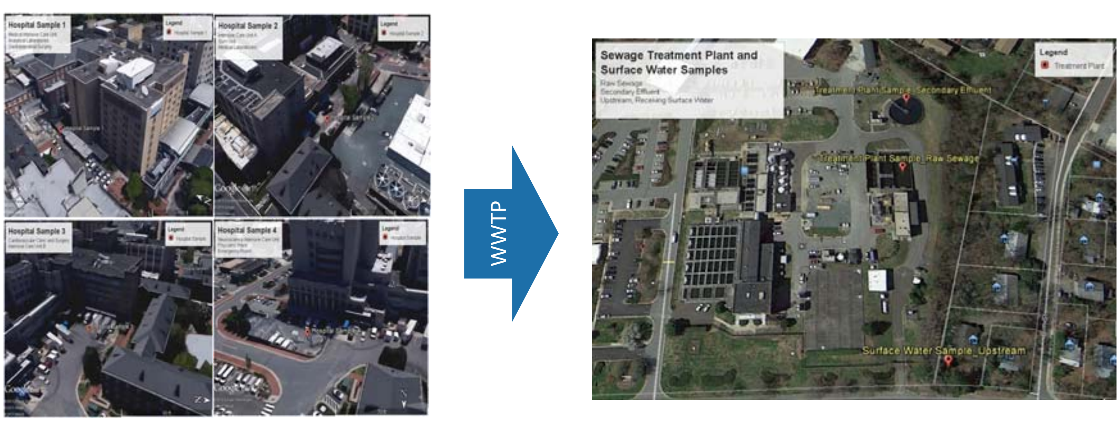
Surveillance System for Hotspot Sources of AMR

- **<u>There are currently no globally coordinated efforts for AMR surveillance, response, and prevention</u>.** (We're getting there…)
- Little is actually known about the magnitude, global landscape, and trends of ARB due to lack of harmonized, coordinated data and method for data collection.
- A **simple but robust** monitoring method is needed for the direct detection and quantification of target or indicator ARB in **exposure relevant hotspots**.
- System should be **accessible** for both high and lower income countries and **applicable** to clinical, agriculture, community, and environmental settings.

Project Objectives:

- Address the need for a simple, culture-based microbial method **to detect and quantify target ARBs of concern in environmental samples**.
- Implement **indicator system** proposed by World Health Organization as a proof of concept.
- Enumerate target AMR in environment, including ***E. coli*** **and other coliforms with reduced susceptibility to Extended-spectrum-β-lactams**
- **Performance evaluation of AMR culture media** (CHROMagar ESBL bacteriologic culture medium)

Sample Sites



2015 Sample Collection Dates

	9-Feb	16-Feb	2-Mar	17-Mar	23-Mar	31-Mar	9-Apr	6-May	11-May	18-May	26-May	1-Jun	1–Jul
Sample Site	**W1**	**W2**	**W3**	**W4**	**W5**	**W6**	**W7**	**W8**	**W9**	**W10**	**W11**	**W12**	**W13**
UNC Hospital	–	–	–	–	–	–	–	+	+	+	+	+	+
OWASA Raw Sewage	+	+	+	+	+	+	+	–	–	–	+	+	+
OWASA Secondary Effluent	+	+	+	+	+	+	+	–	–	–	+	+	+

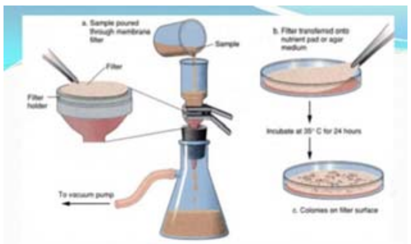
Original Protocol

Day 1

- Preparation of Bio Rad Rapid' E. coli 2 agar medium, CHROMagar™ ESBL, Tryptic Soy Agar (Difco™), Phosphate-buffered Saline, 1X Trypticase Soy Broth, and 40% Glycerol aqueous Solution

Day 2

- Sample Collection, Transport, and Storage
- Direct, One-Step Membrane Filter Method for analysis of samples on *Bio Rad Rapid' E. coli 2* and CHROMagar™ ESBL using procedures form EPA Method 1604



- 20 mL filter volume (Filtered 3 dilutions in triplicate)
- 0.45µm pore size, 47 mm diameter, gridded membrane filter (Millipore HA filter)
- 60 x 15 mm agar plates (Bio-Rad, ESBL and KPC)
- Incubation for 24 hours at 37°C.

Day 3

CFUs were counted for presumptive E. coli and other coliforms via colony color interpretation based on manufacturers' descriptions

CFU – LDL = 1, UDL = 250, TNTC >250

60x15mm filter plates of all 4 media after incubation

Original Protocol

Day 4

Day 5

Streak on 100mmx15mm ESBL plates of the same medium and incubate for about 24 hours at 37°C.

Day 6

Streak on 100mmx15mm TSA plates, incubate for 24 hours at 37°C

Day 7

One colony from each TSA plate was then inoculated into 1X trypic soy broth into a separate, 8mL sterile vial and incubated for 24 hrs at 37°C.

Day 8

Enrichment vials with discernable regrowth were stored in 1mL aliquots (0.5mL TSB culture and 0.5mL 20% glycerol stock solution) in 1.5mL sterile freezer tubes and stored at -80F for future characterization.

Later

Revival, purification, re-isolation

MALDI-TOF MS

VITEK 2 Analysis

Determine presence, concentration, and relative proportion of presumptive ESBL producing *E. coli* and other coliforms at sampled hotspots

Timeline and Summary of Events

13 sampling events

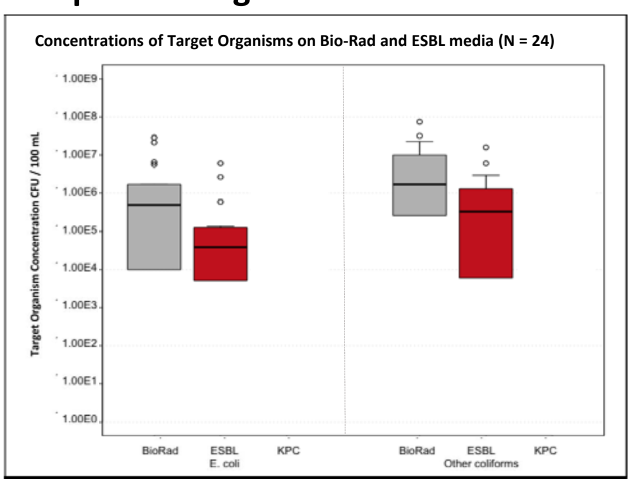
67 Assays

Calculation of Concentrations for all sites, assays, and media) using colony counts of presumptive *E. coli* and other coliforms

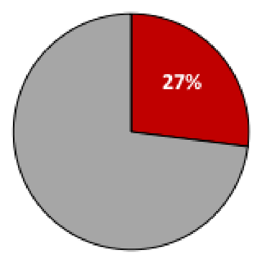
Calculations of the relative proportion of presumptive ESBL and KPC producing target Organisms

Results

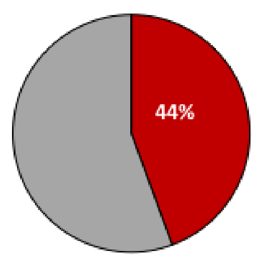
Hospital Sewage



Average concentration	*E. coli*		Other coliforms	
	CFU / 100 mL	95% CL (+/-)	CFU / 100 mL	95% CL (+/-)
Ave BR	4.30E+06	1.02E+03	7.74E+07	3.86E+03
Ave ESBL	5.00E+05	3.10E+02	1.62E+06	5.09E+02



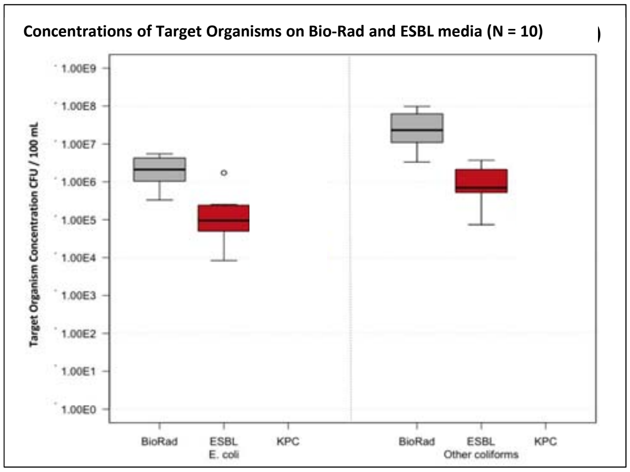
% ESBL *E. coli*



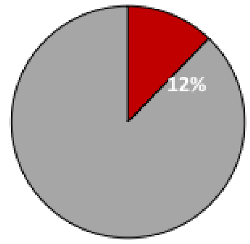
% ESBL Other coliforms

Results

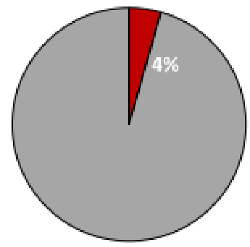
Raw Sewage



Average concentration	*E. coli*		Other coliforms	
	CFU / 100 mL	95% CL (+/-)	CFU / 100 mL	95% CL (+/-)
Ave BR	2.58E+06	1.11E+03	3.34E+07	3.58E+03
Ave ESBL	2.70E+05	3.22E+02	1.17E+06	6.71E+02



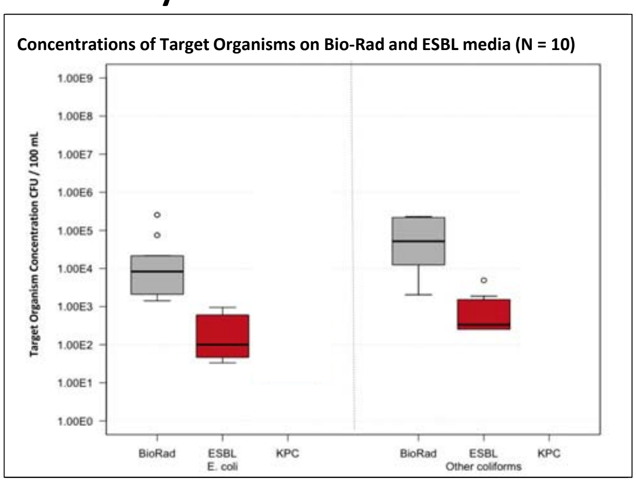
% ESBL *E. coli*



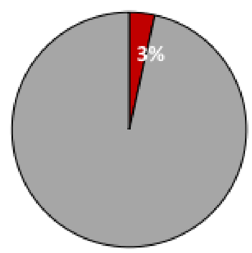
% ESBL Other coliforms

Results

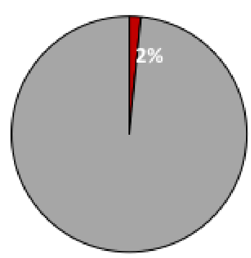
Secondary Effluent



Average concentration	*E. coli*		Other coliforms	
	CFU / 100 mL	95% CL (+/-)	CFU / 100 mL	95% CL (+/-)
Ave BR	3.93E+04	1.23E+02	9.58E+04	1.92E+02
Ave ESBL	2.89E+02	1.05E+01	1.11E+03	2.06E+01



% ESBL *E. coli*



% ESBL Other coliforms

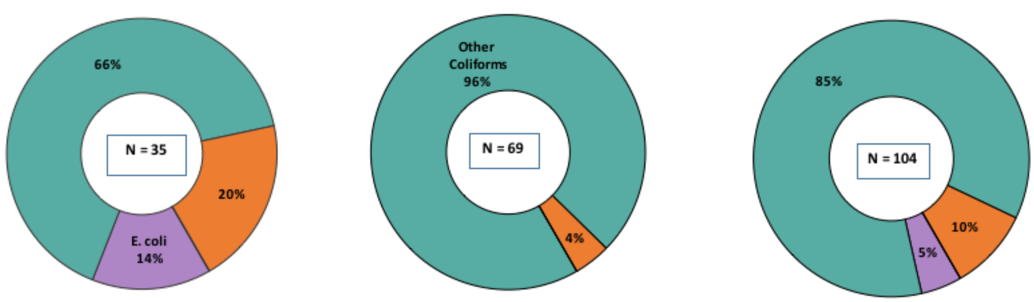
Performance Evaluation of CHROMagar ESBL media

Timeline and Summary of Events

- Select Isolate revival, purification, and re-isolation
- Identity confirmation via MALDI-TOF MS analysis
- Antimicrobial susceptibility testing via VITEK 2

Results

Hospital Sewage – ESBL MALDI-TOF Confirmation (N = 104)



Presumptive *E. coli*

14% Correctly Confirmed

Presumptive Other coliforms

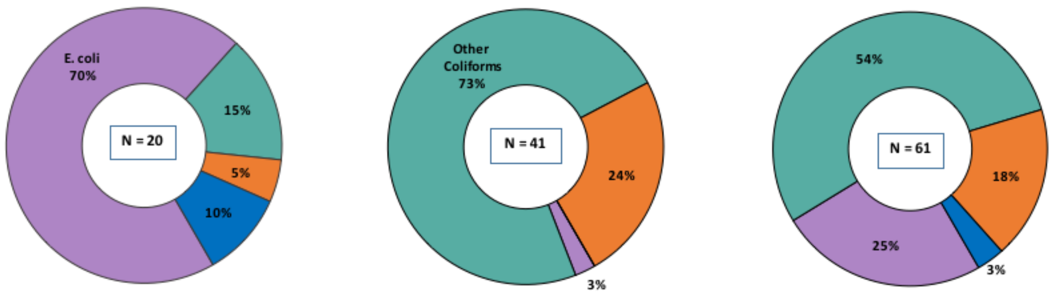
96% Correctly Confirmed

Total Isolates, post-MALDI

- *E. coli*
- Other coliforms
- Other Gram-Negative
- Gram-Positive

Results

Raw Sewage – ESBL MALDI-TOF Confirmation (N = 61)



Presumptive *E. coli*

70% Correctly Confirmed

Presumptive Other coliforms

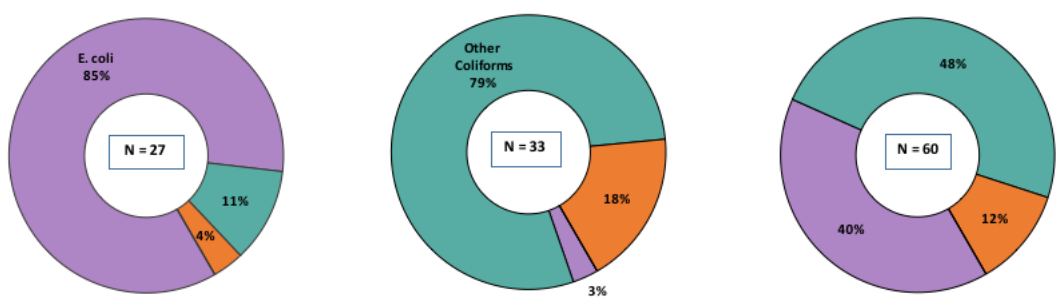
73% Correctly Confirmed

Total Isolates, post-MALDI

- *E. coli*
- Other coliforms
- Other Gram-Negative
- Gram-Positive

Results

Secondary Effluent – ESBL MALDI-TOF Confirmation (N = 60)



Presumptive *E. coli*

85% Correctly Confirmed

Presumptive Other coliforms

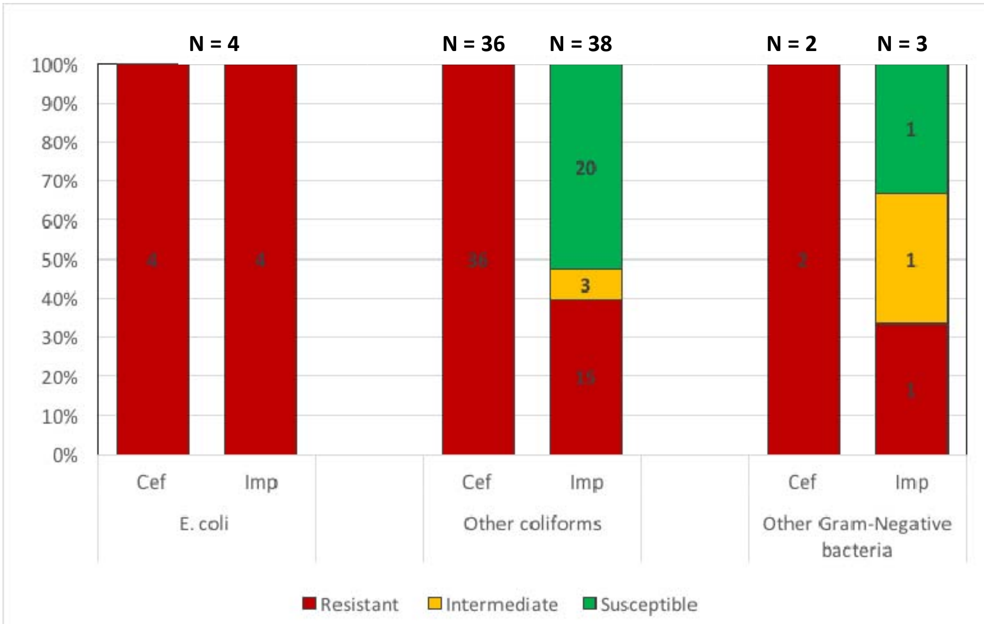
79% Correctly Confirmed

Total Isolates, post-MALDI

- *E. coli*
- Other coliforms
- Other Gram-Negative
- Gram-Positive

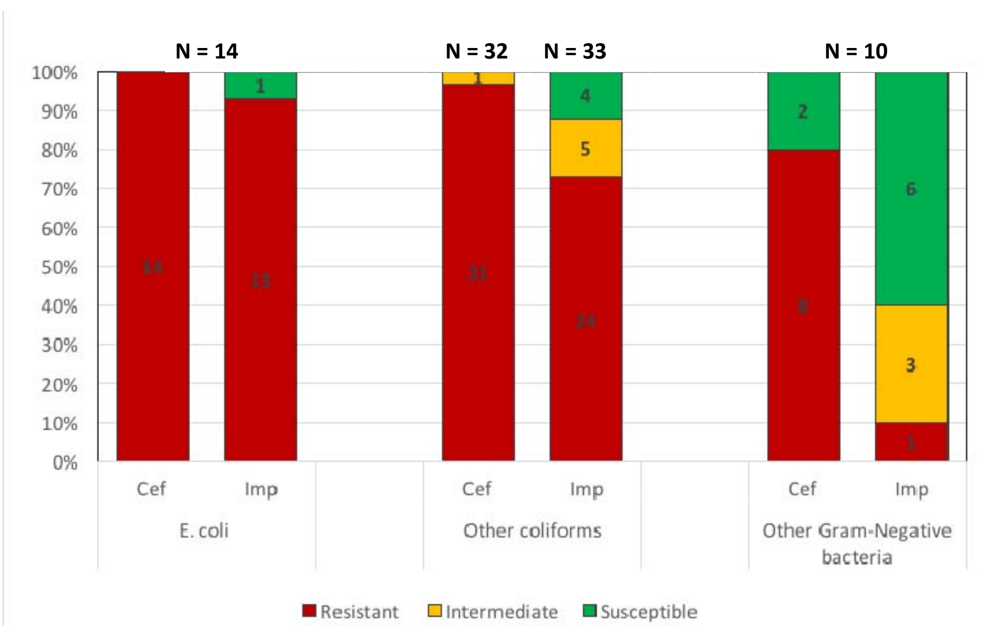
Results

Hospital Sewage – ESBL VITEK 2 Confirmation (N = 45)



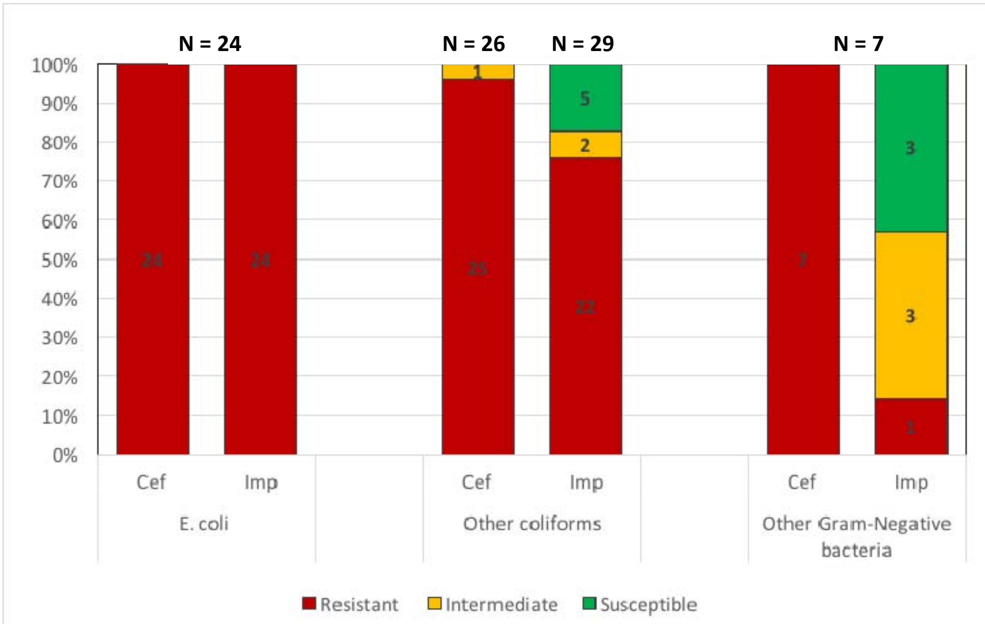
Results

Raw Sewage – ESBL VITEK 2 Confirmation (N = 57)



Results

Secondary Effluent – ESBL VITEK 2 Confirmation (N = 44)



Discussion:

- Presumptive ESBL and KPC producing bacteria were found and confirmed at all sites
- The highest concentrations and relative proportions of presumptive ESBL and KPC production in *E. coli* and other coliforms were detected in hospital sewage.
- Lower, but still detectable concentrations and proportions of presumptive ESBL and KPC producing bacteria found in raw sewage and secondary effluent.
- CHROMagar ESBL medium performed the best in secondary effluent samples, indicating the potential influence of selection pressure during treatment

Conclusions:

- Elevated concentrations of highly AMR bacteria in hospital and municipal sewage indicates the widespread presence in the population and their possible spread to other from exposure via environmental, food and person-to-person transmission routes.
- Global spread of ARB merits evaluation across other geographic regions in US and abroad using similar methods to identify ARB threats and detect outbreaks.
- These media and methods have promise as a candidate indicator system to detect and quantify ARB of health concern in environmental media as a monitoring system to support environmental surveillance as an element of a global action plan to combat AMR

Acknowledgements:

Mentorship:

- Dr. Mark Sobsey
- Dr. Jill Stewart
- Dr. Samuel Vilchez

University of North Carolina, Chapel Hill, specifically:

- AJ Karon, Knox Coleman, Yvonne Yuen, Emily Bailey, Joe Strasser, Elizabeth Christenson, and Sarah Rhodes Dept. of Environmental Sciences and Engineering, Gillings School of Global Public Health
- Mabry Griffin, Amy Guo, Andy Kolton, Sarah Miller, Katherine Mulligan, and Caroline Reed - undergraduate interns, UNC
- Lydia Abebe, PhD and Emmanuel Sotzi, PhD. Post-docs in Sobsey Lab,
- Sylvia Becker-Drepps MD, MPH, Director of the UNC Program in Nicaragua, Dept. of Family Medicine, UNC School of Medicine.
- Melissa B. Miller, PhD, D(ABMM), Director, and Rongpong Plongla, MD, MSc, Clinical Molecular Microbiology Laboratory, Department of Pathology and Laboratory Medicine, UNC School of Medicine.

My Non-Lab Friends and Family!

(Jordan, you saved me too many times to count)

North Carolina State University School, specifically:

- Megan Jacobs, MS, PhD, Director, Clinical Microbiology Lab, Dept. of Population Health and Pathobiology.
- Anna Rogers, Medical Lab Technologist, College of Veterinary Medicine.

UNAN-León, Department of Microbiology and Medical Sciences, specifically:

- Erick Amaya, PhD, Daniel Reyes, MD, PhD, Claudia Perez, MS, Johan Perez, MS, Lester Gutierrez and Evelin Martinez.

OWASA and UNC Hospital Staff – Thank you for letting me use some of your sewage

This work was supported by a grant from the UNC Institute for Global Health and Infectious Diseases and UNC Program in Nicaragua.

THE UNIVERSITY *of* NORTH CAROLINA *at* CHAPEL HILL

Thanks!

Questions?

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Materials and Methods:

Bacteriologic Culture Media	Purpose
Bio-Rad Rapid'*E. coli* 2	Chromogenic environmental medium validated for the detection and enumeration of *E. coli* and other coliforms bacteria in food and waste waters.
CHROMagar™ ESBL	Chromogenic medium for the detection of Gram-negative bacteria producing ESBL / resistant to extended beta lactams in stools and urine.
CHROMagar™ KPC	Chromogenic medium for the detection of Gram-negative bacteria with reduced susceptibility to most carbapenem agents in stools and urine

Target Organisms
E. coli and Other Non-*E. coli* coliforms (*Klebsiella, Enterobacter, Citrobacter,* and *Serratia*)

Materials and Methods:

Enumeration	Purpose
Concentrations and Proportions of target organisms	Colony forming units (CFUs) for presumptive *E. coli* and other coliforms were totaled for each plate and recorded as discrete counts according to colony color guides provided by the manufacturer. Proportions were calculated by dividing target organism CFU/100 mL, plated on ESBL or KPC by the CFU/100 mL in parallel assay, plated on Bio-Rad Rapid'*E. coli* 2, for the same sample
Bacteria Speciation via MALDI-TOF MS	Matrix-assisted laser desorption, time of flight mass spectrometry – soft ionization process that analyzes biomolecules and large organic molecule and compares them to a digital library of well characterized organisms
Susceptibility analysis via VITEK 2	For isolate originally detected on CHROMagar ESBL, reduced susceptibility to Extended-β-lactams (Cefpodoxime) and carbapenems (Imipenem) was evaluated via Vitek2 (Objective 3).

Recommendations:

- Initial and iterative performance evaluation
- Bacteria colony color and morphology referencing catalog
- Cost and Availability of Indicator System (specifically the for lower-income labs)
- Animal-free products
- Spread plate vs. membrane plate
- Partners, personnel, and planning
- Incorporate the One Health Philosophy by expanding AMR environmental surveillance using approaches and tools consistent with current medical and clinical methods

Limitations:

- VERY limited funding
- Retro-active confirmation of isolates during preliminary stage
- Mixed isolate cultures, repeated revivals, and freeze / thaw
- Sample comparison are hindered by disparate sample sizes and limited overlap in temporality
- Reliance on Beta-D-Galactosidase (GAL) and Beta-D-Glucuronidase (GLUC) to differentiate and positively ID coliform and *E. coli*, respectively.