Sinkholes, Springs Springs & Multi-Drug Resistant Bacteria

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sinkhole to springs connections in northeast iowa

SHERI SCHWERT WITH Dr. JACK HEDSTROM AND Dr. ERIC BAACK

LUTHER COLLEGE

OUTLINE

Background, Purpose, and Methods

Twin Springs Results

Middle Hesper Results

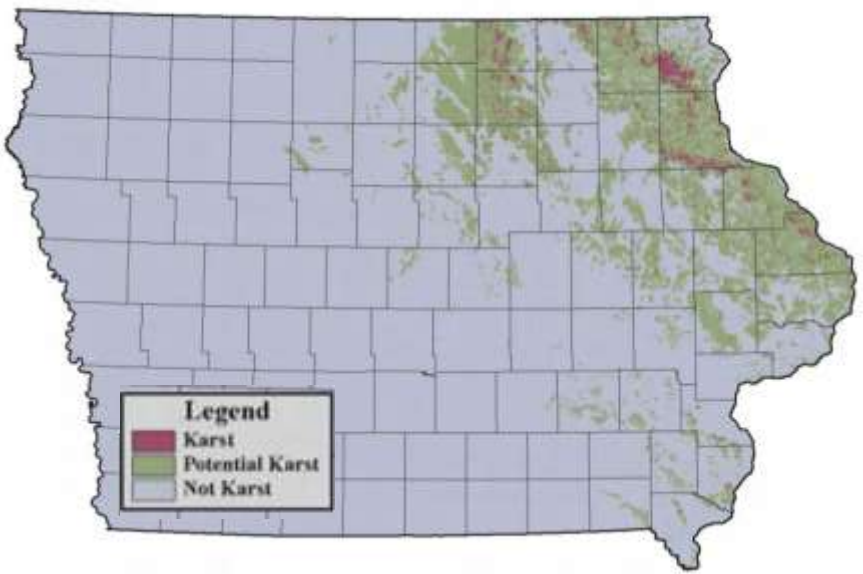
Conclusions and Discussion

BACKGROUND

Karst is limestone bedrock

Karst features

Karst is sensitive to water issues in ways other systems are not.



Ed. By Libra, RD. (2005). Living in karst, Iowa field conference for public policy makers, Northeast Iowa, Oct. 11-12, 2005. Iowa Dept. of Natural Resources.

PURPOSE

Dye tracing techniques

Sinkholes

Relationships with landowners

PURPOSE

METHODS

Around a pound of dye is mixed into water and introduced to sinkhole either A) before predicted rain) or B) during ice and snow melt

Activated charcoal receptors are placed at spring head and switched out every 24 h for first four days.

Water grab samples also taken at spring head as often as possible.

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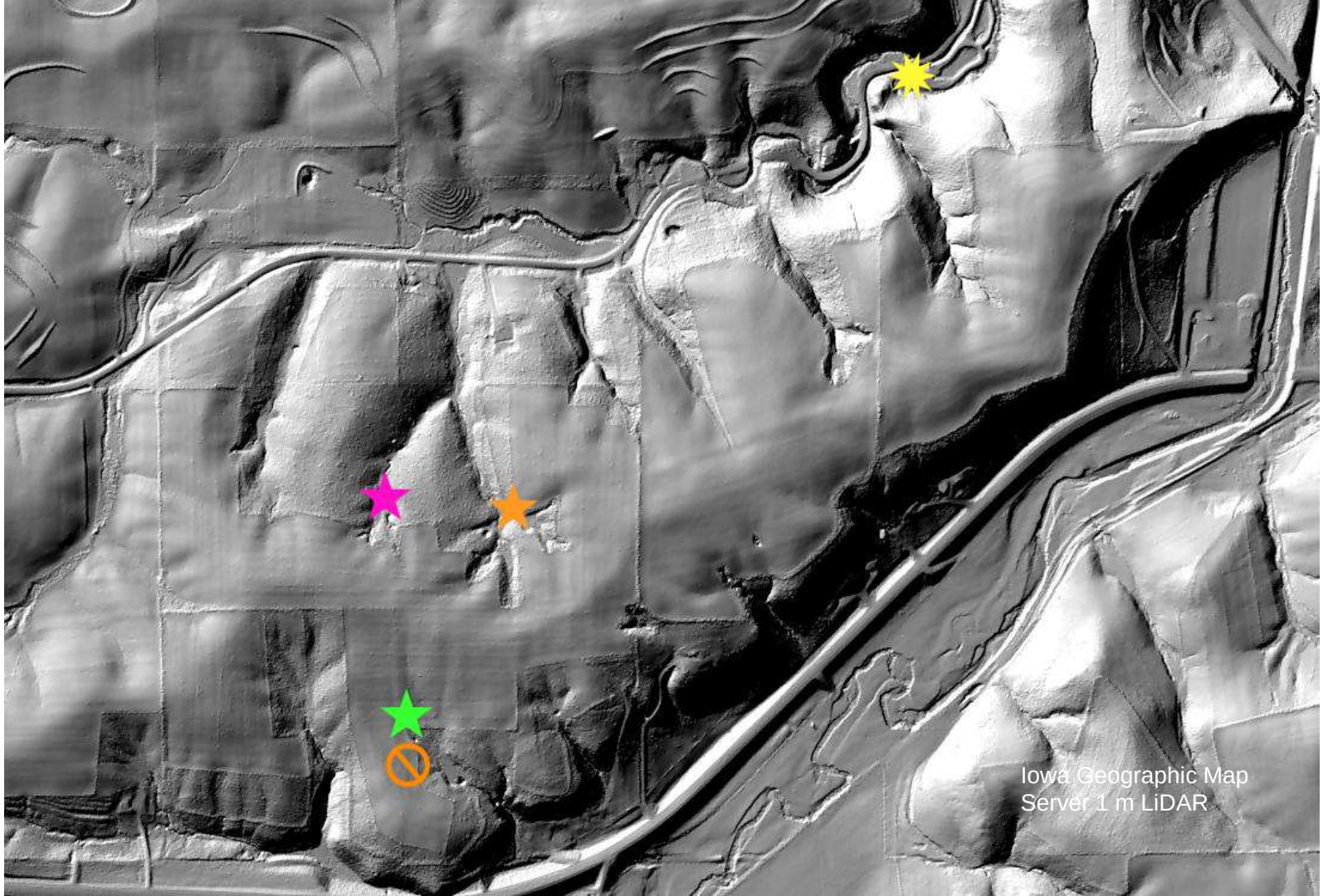
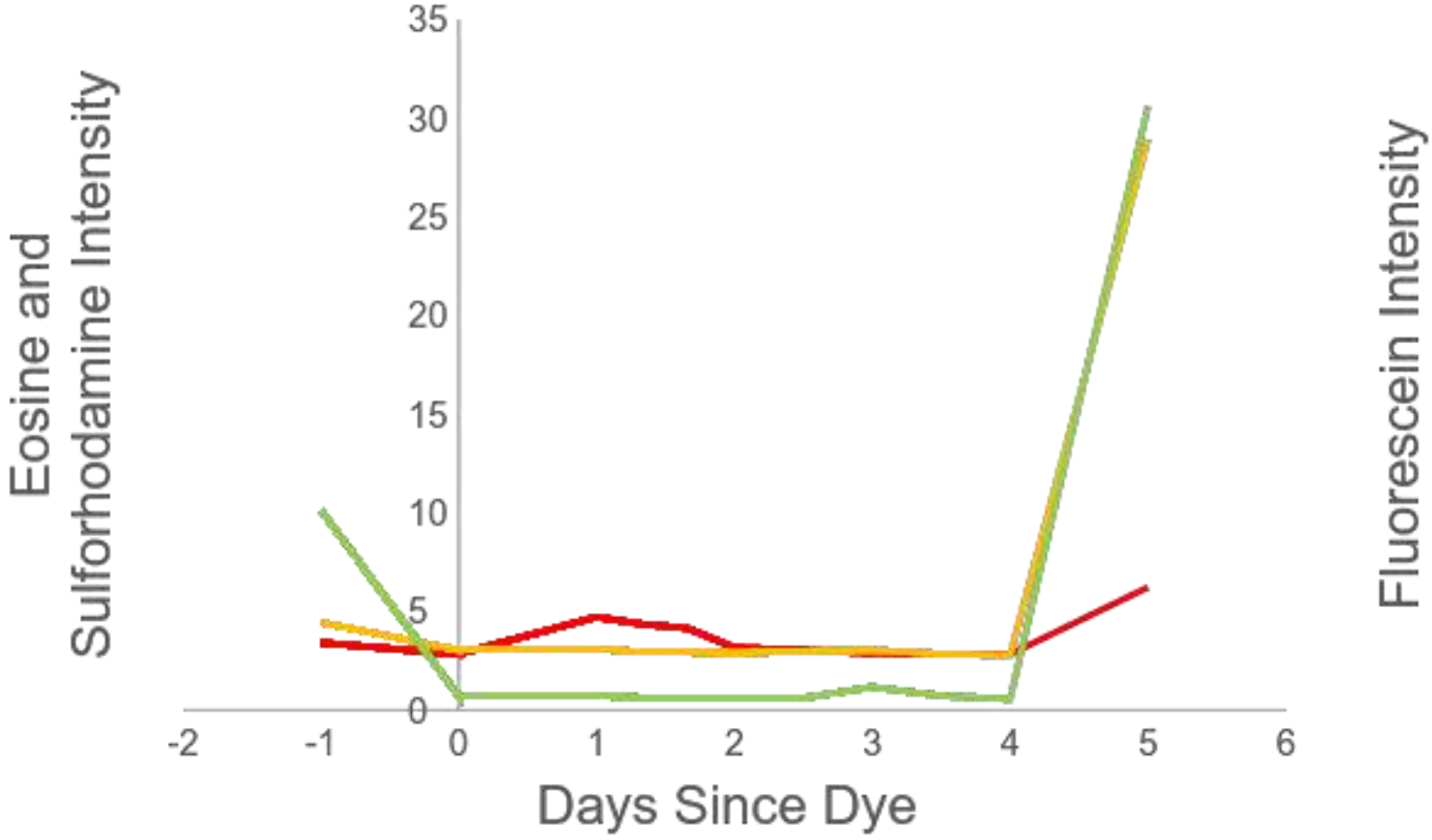

Iowa Geographic Map
Server 1 m LiDAR


Eosine and Sulforhodamine Intensity
35
30
25
20
15
10
5
0
-2
-1
0
1
2
3
4
5
6
Days Since Dye
Fluorescein Intensity

OUTLINE

Purpose, Background, and Methods

Twin Springs Results

Middle Hesper Results

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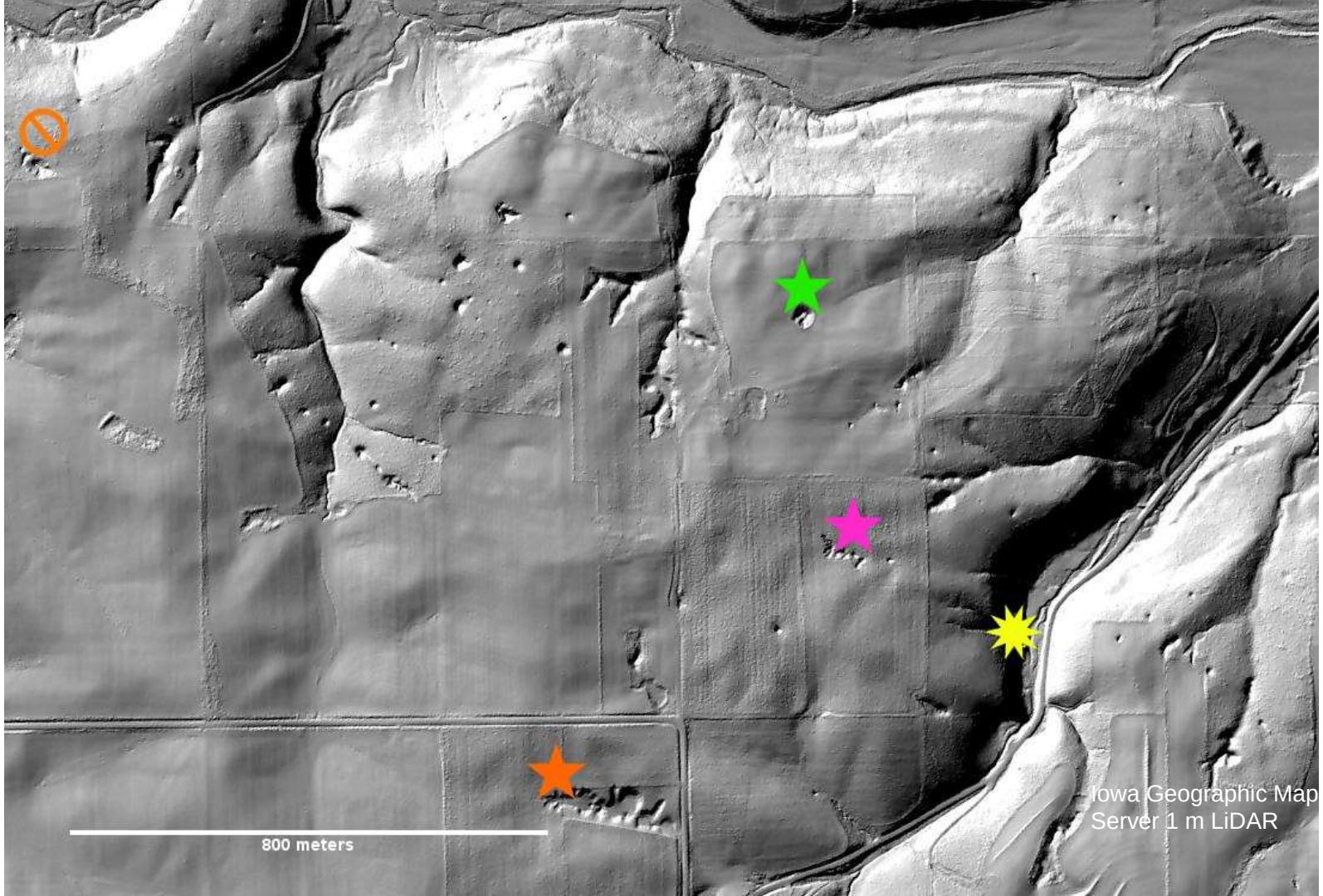
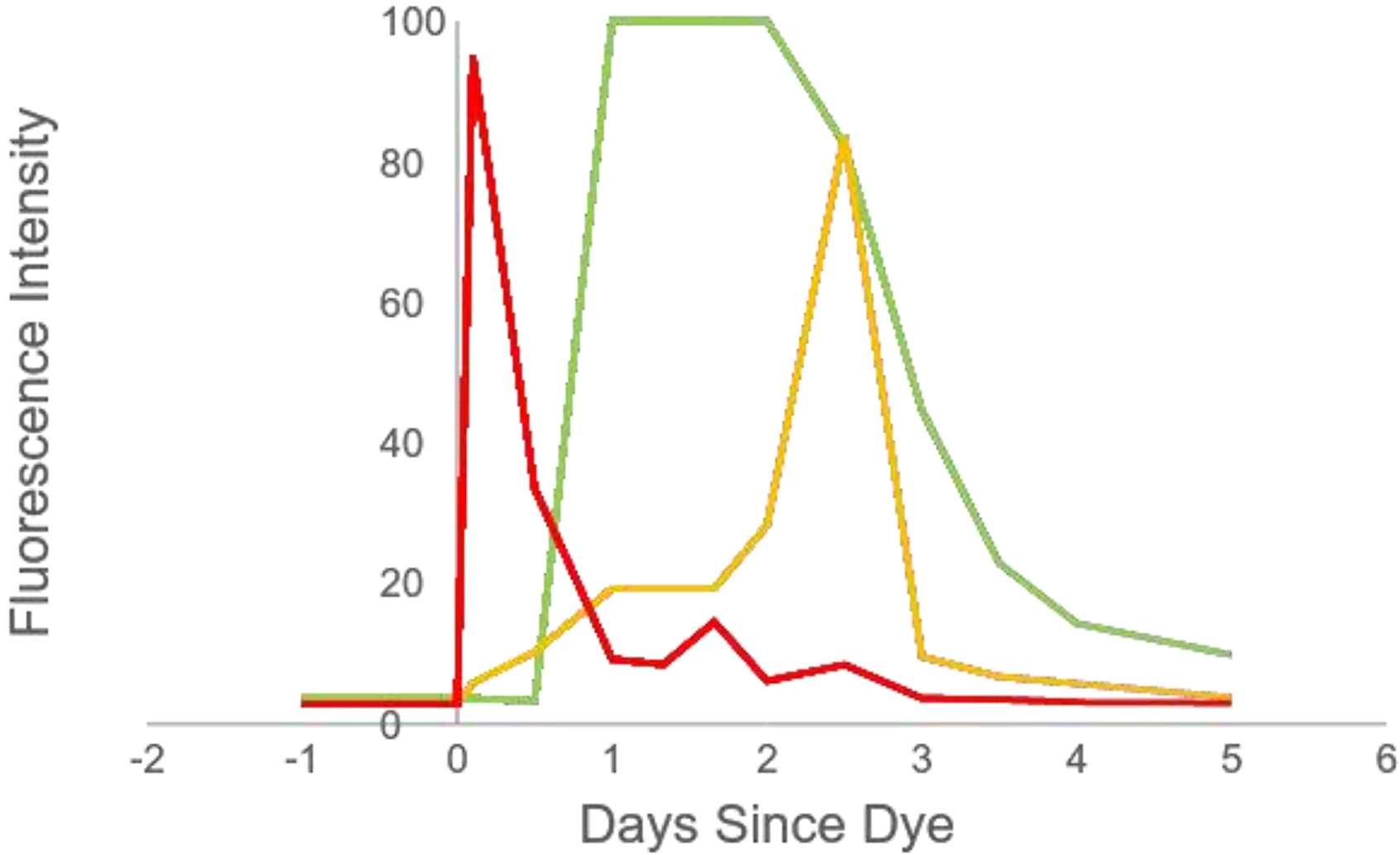

800 meters
Iowa Geographic Map Server 1 m LiDAR


Fluorescence Intensity
100
80
60
40
20
0
-2
-1
0
1
2
3
4
5
6
Days Since Dye

MIDDLE HESPER SPRING

CONCLUSIONS

The two closest sinkholes to Middle Hesper Spring have very strong connections

Sinkholes that are farther away at the Twin Springs site had longer travel times to the spring head, and Middle Hesper showed faster speed as well.

Using snow melt as carrier for dye is a much better method for tracing, but lower total flow.

FURTHER DISCUSSION

Method for water grab samples required solo sample collection. Could an automatic sampler be used, with consideration that Twin Springs is often visited by the public.

More dye possibilities, eosine dye peak lost under fluorescein peak at Middle Hesper Spring.

After Middle Hesper creek turned bright green, landowners were very curious about research, opportunity for sinkhole and runoff education?

ACKNOWLEDGEMENTS

▪IGWA for supporting this research; Claire Hruby and Cindy Quast

▪The Thompsons, Boedings, and the community at Seed Saver's Exchange.

▪Jeff Green for advice and resources.

▪The McElroy Foundation, who also supported this research.

- Dr. Baack and Dr. Hedstrom for attempting to answer all of my questions, joining me in the field, and letting me try just about anything.

Potential effects of livestock manure on water quality

-Pathogens: *Salmonella, E. coli, Campylobacter;* norovirus, rotavirus, hepatitis E

-Nutrients: nitrogen (organic, ammonia: 210 lbs/ ac/ yr), phosphorous (165 lbs/ ac/ yr P_2O_5). (Nitrate in Decorah springs: 5-14 mg / L, depending on site).

-Heavy metals: zinc (1.7 lbs /ac / yr), copper (.2 lbs / ac / yr)

-Antibiotics: especially tetracyclines (swine), monensin (cattle)

-Antibiotic resistance genes: for tetracycline, methicillin, vancomycin, etc.

US EPA 2013. Literature review of contaminants in livestock and poultry manure and implications for water quality.

Comas et al 2014. Input and leaching potential of copper, zinc and selenium in agricultural soil from swine slurry. *Archives Environmental Contamination Toxicology.* **66**: 277-286.

Koehler et al 2013. Effects of swine manure applications on soil nutrient levels. Minnesota Ag Extension.

Research on pathogens associated with livestock

E. coli and Salmonella can persist in soil after manure application for 30+ days, depending on soils (Fongaro et al 2017)

E. coli and Salmonella can reach tile drains groundwater when manure is applied to the soil (Fongaro *et al.* 2017; Hruby *et al.* 2016)

Workers at conventional hog confinements have higher levels of multidrug resistant *Staph aureus* than do others in the community (Wardyn et al 2014; Neyra et al 2014).

Fongaro *et al.* 2017. Different behavior of enteric bacteria and viruses in clay and sandy soils after biofertilization with swine digestate. *Frontiers Microbiology* **8**:74.

Hruby *et al.* 2016. Effects of tillage and poultry manure application rates on *Salmonella* and fecal indicator bacteria in tiles draining Des Moines lobe soils. *Journal of Environmental Management* **171:**60-69..

Wardyn *et al*. 2015. Swine farming is a risk factor for infection with and high prevalence of carriage of multidrug-resistant *Staphylococcus aureus*. *Clinical infectious disease* 61:59-66.

Research on heavy metals and antibiotics

Some antibiotics are excreted intact (*e.g*. tetracyclines; ~80%; Kumar *et al*. 2005)

Some antibiotics can persist in soil (*e.g*. tetracyclines) while others quickly break down (Kumar *et al.* 2005)

Some antibiotics are tightly bound to soil (*e.g*. tetracyclines) while others are more mobile in the environment (Kumar *et al.* 2005)

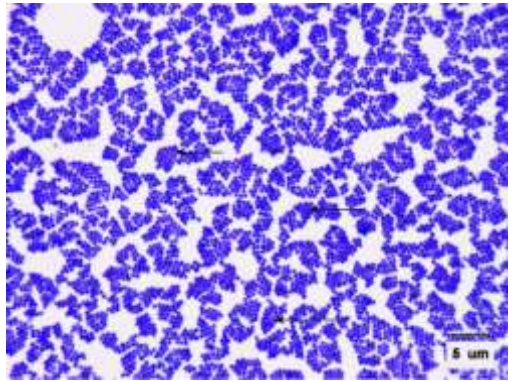
Copper remains tightly bound to soil and can accumulate (Jensen et al 2016)

Zinc is more mobile in the environment, but also accumulates (Jensen et al 2016)

Kumar *et al*. 2005. Antibiotic use in agriculture and its impact on the terrestrial environment. *Advances in Agronomy* **87**: 1-53.

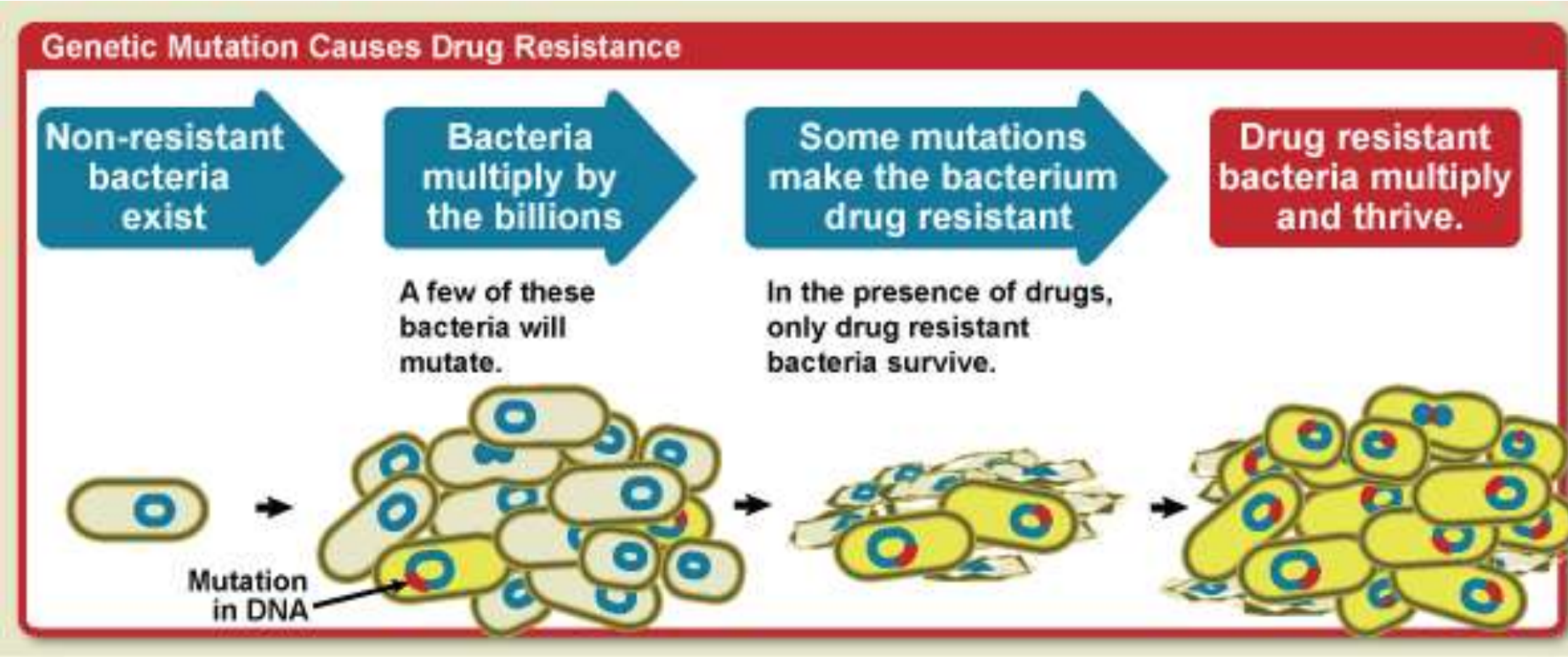
Jensen *et al.* 2016. National monitoring study in Denmark finds increased and critical levels of copper and zinc in arable soils fertilized with pig slurry. *Environmental Pollution* **214**: 334-350.

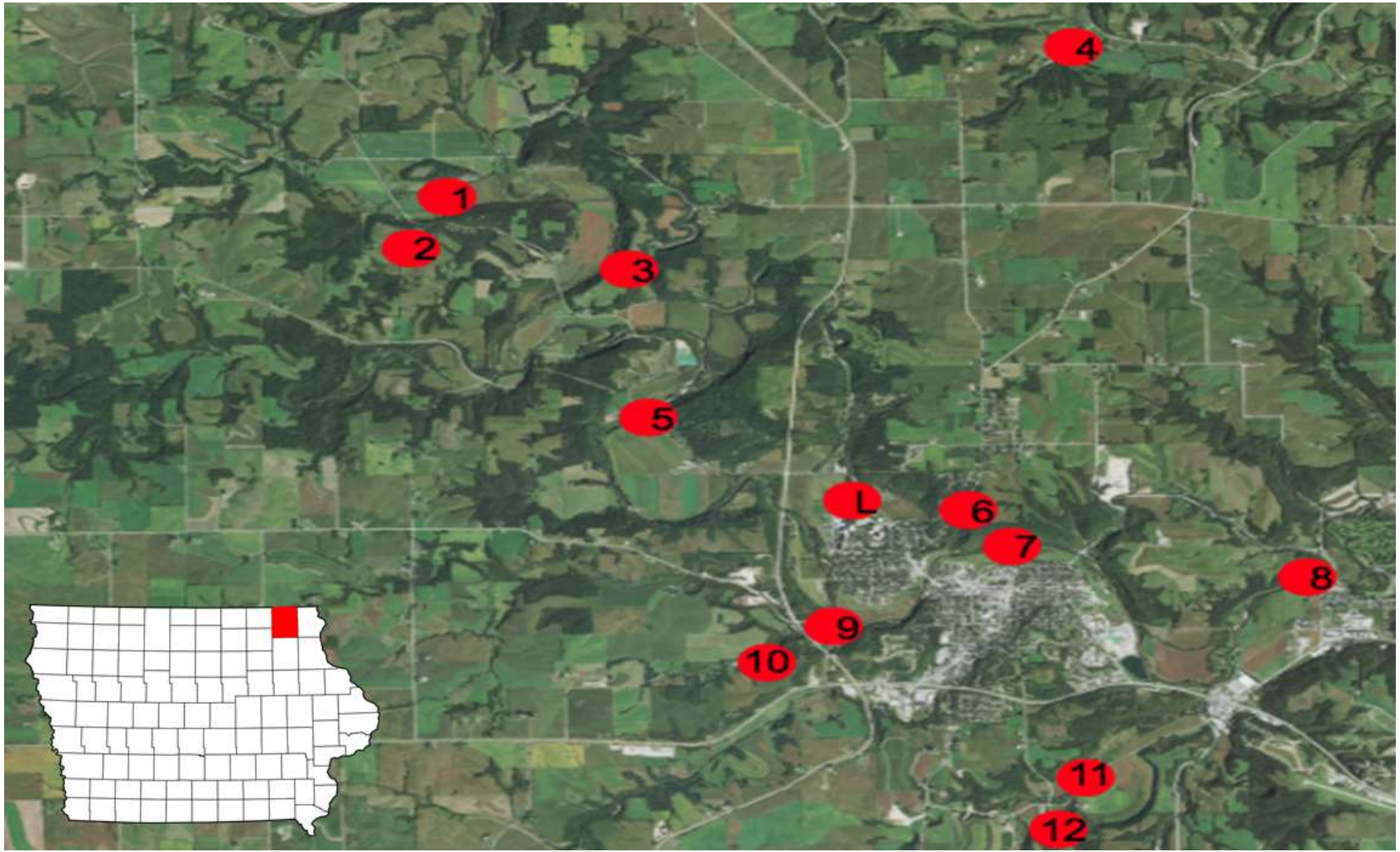
Bacteria tested & antibiotic resistance



- *E. coli*: normal part of gut bacteria for many species; indicates presence of feces
- *Staphylococcus aureus*: part of normal human microbiota (skin, nose)
- MRSA: Methicillin-resistant *Staphylococcus aureus*
- Methicillin no longer used in USA: oxacillin used instead.

Evolution of antibiotic resistance




4
1
2
3
5
L
6
7
8
9
10
11
12

Methods

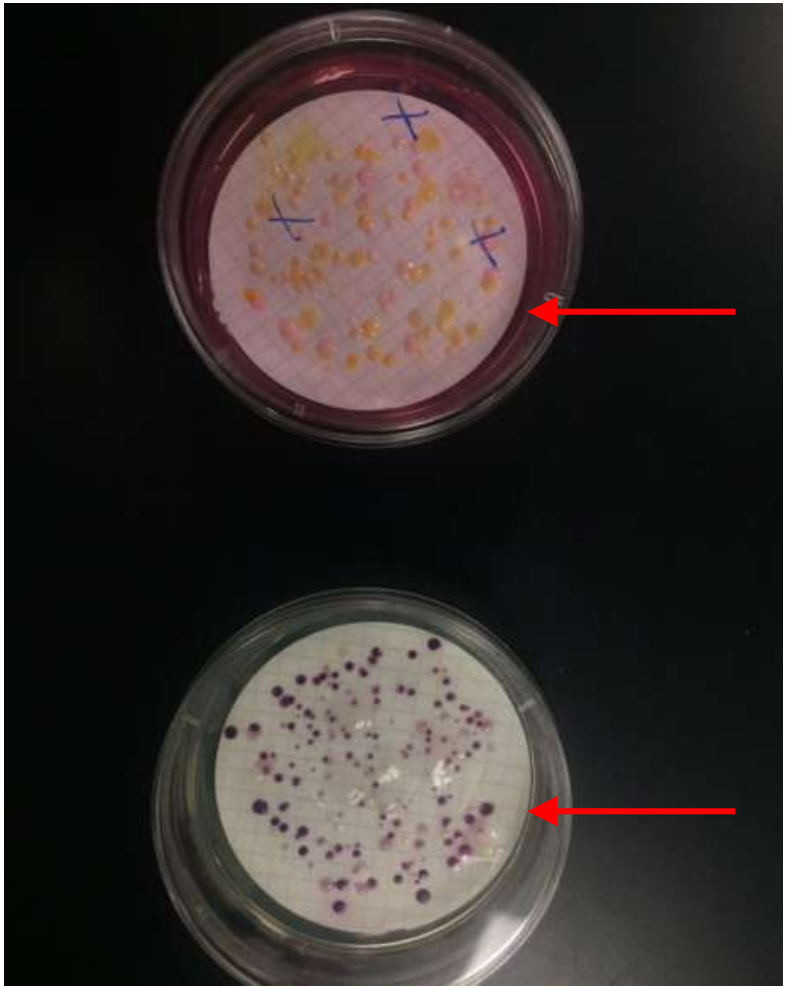
Collect Samples

↓

Filter Samples

↓

Isolate Bacteria on growth mediums



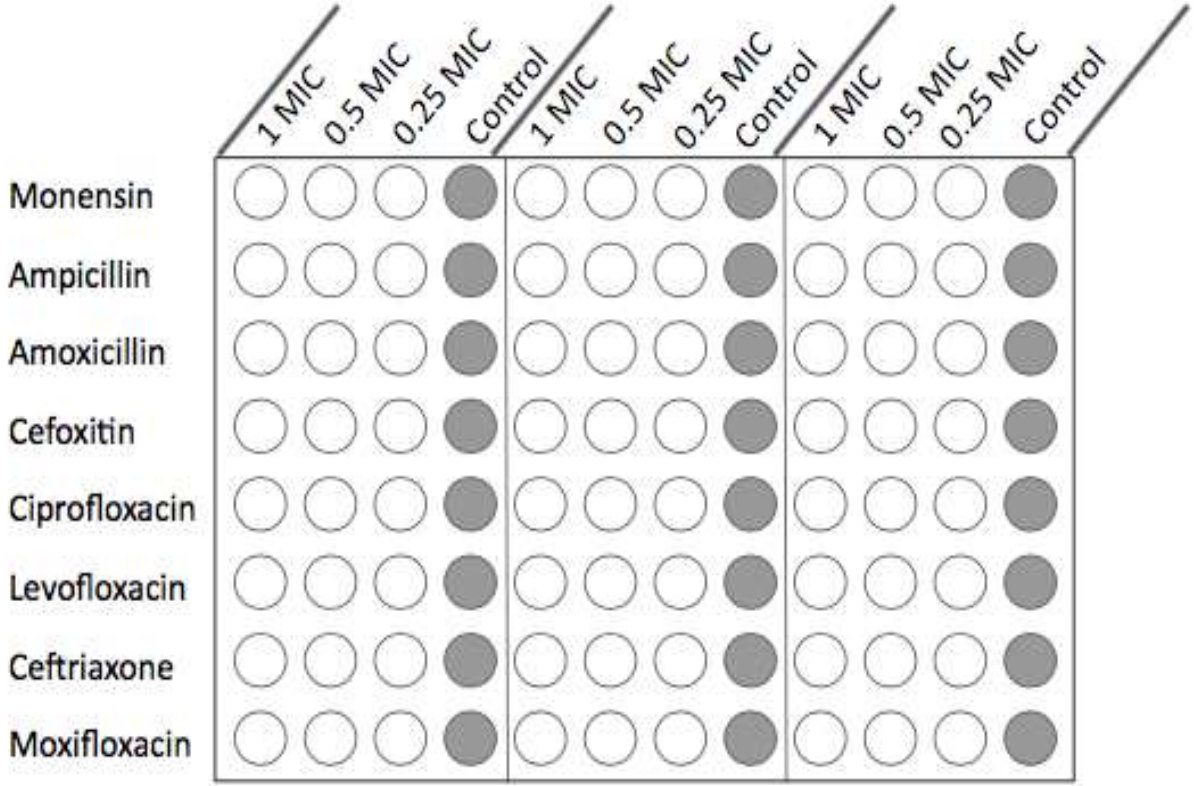
Staphylococcus plate

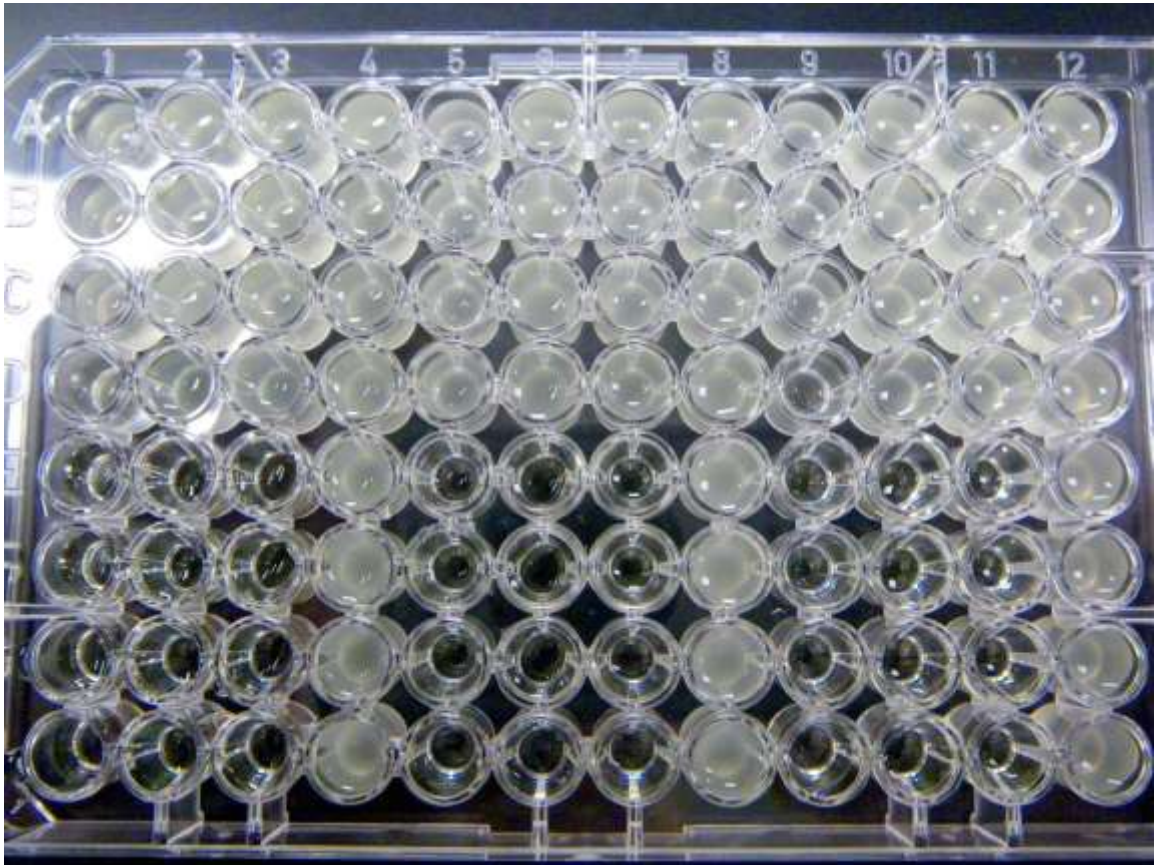
E. coli plate

Methods – testing for antibiotic resistance

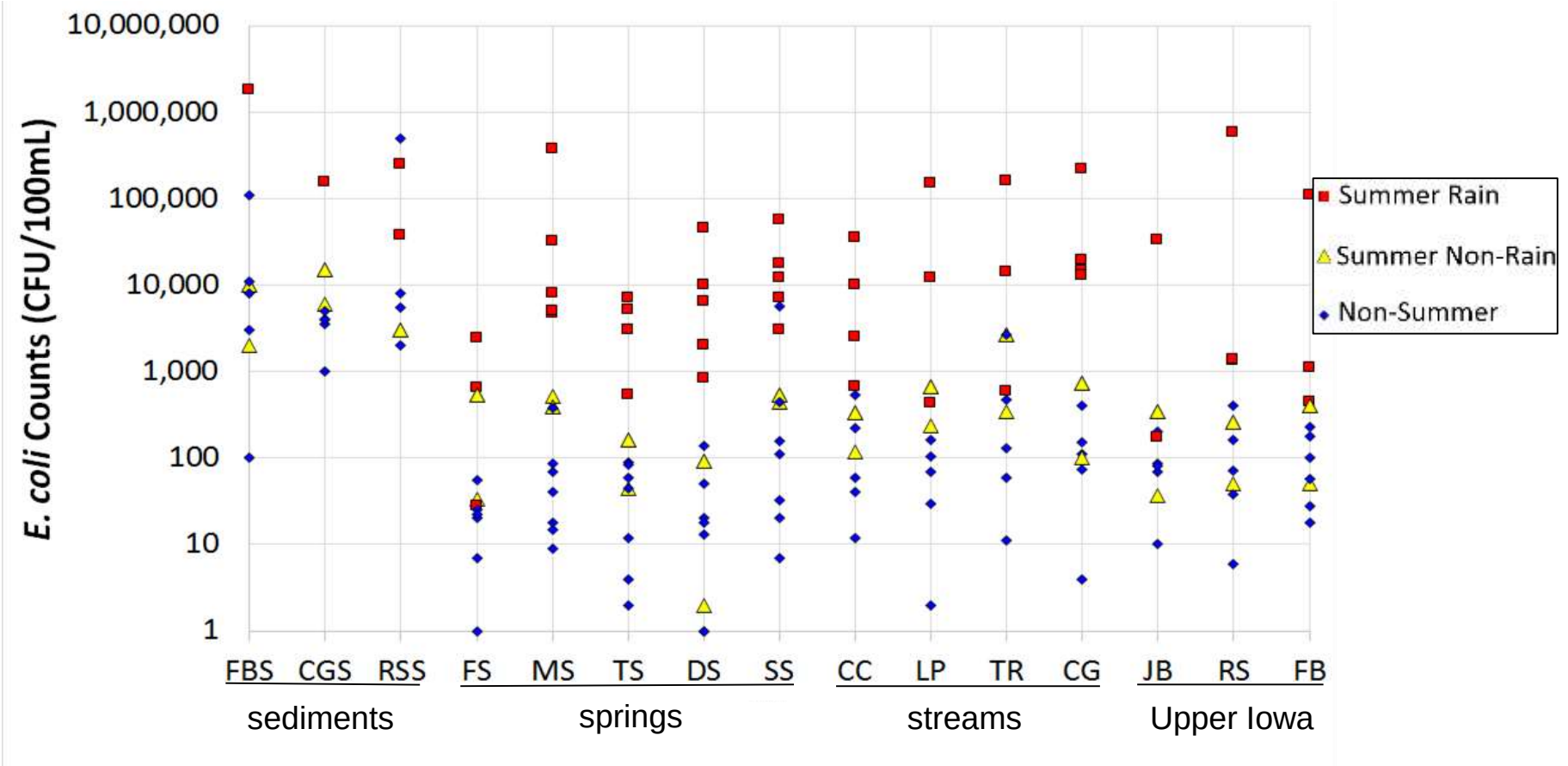
E. coli – test against 8 antibiotics

Staph: test for methicillin, then 12 antibiotics

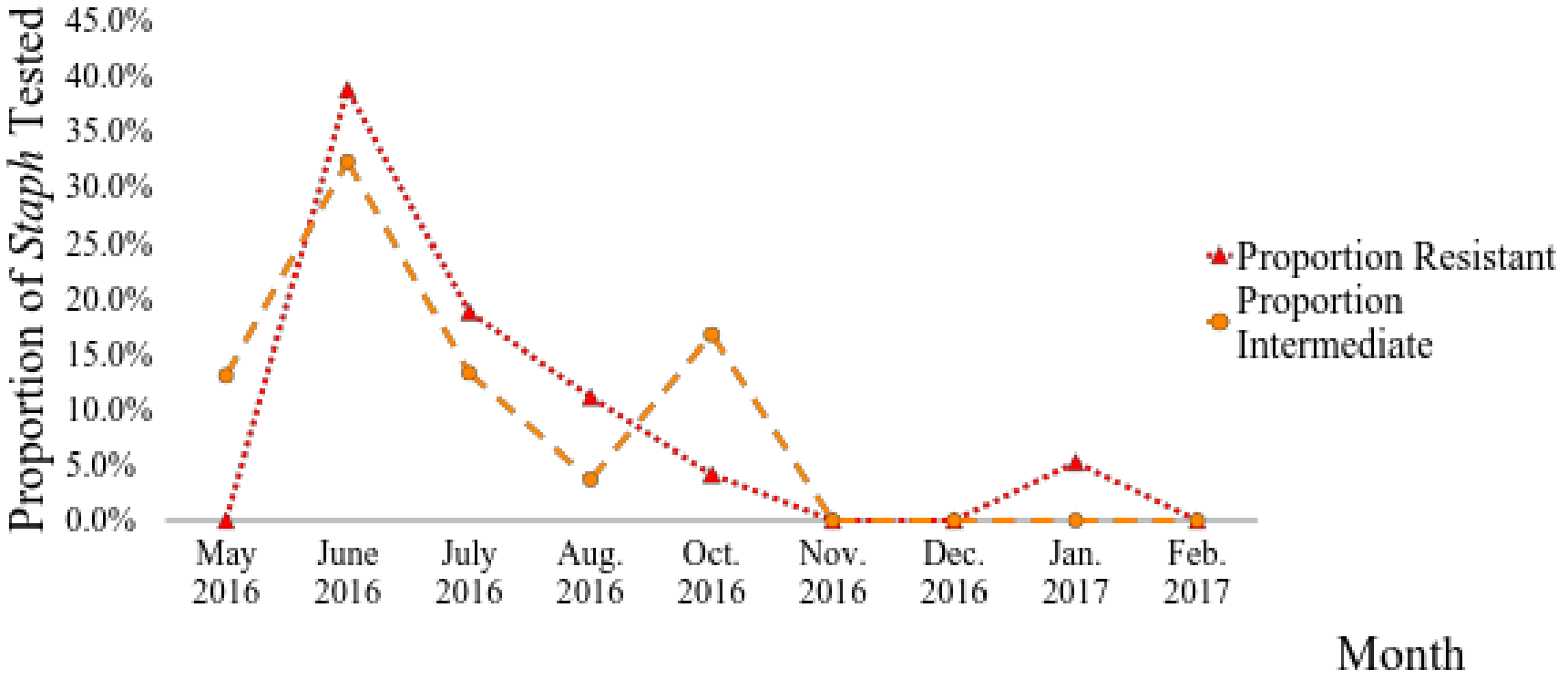



1 2 3 4 5 6 7 8 9 10 11 12
A
B
C

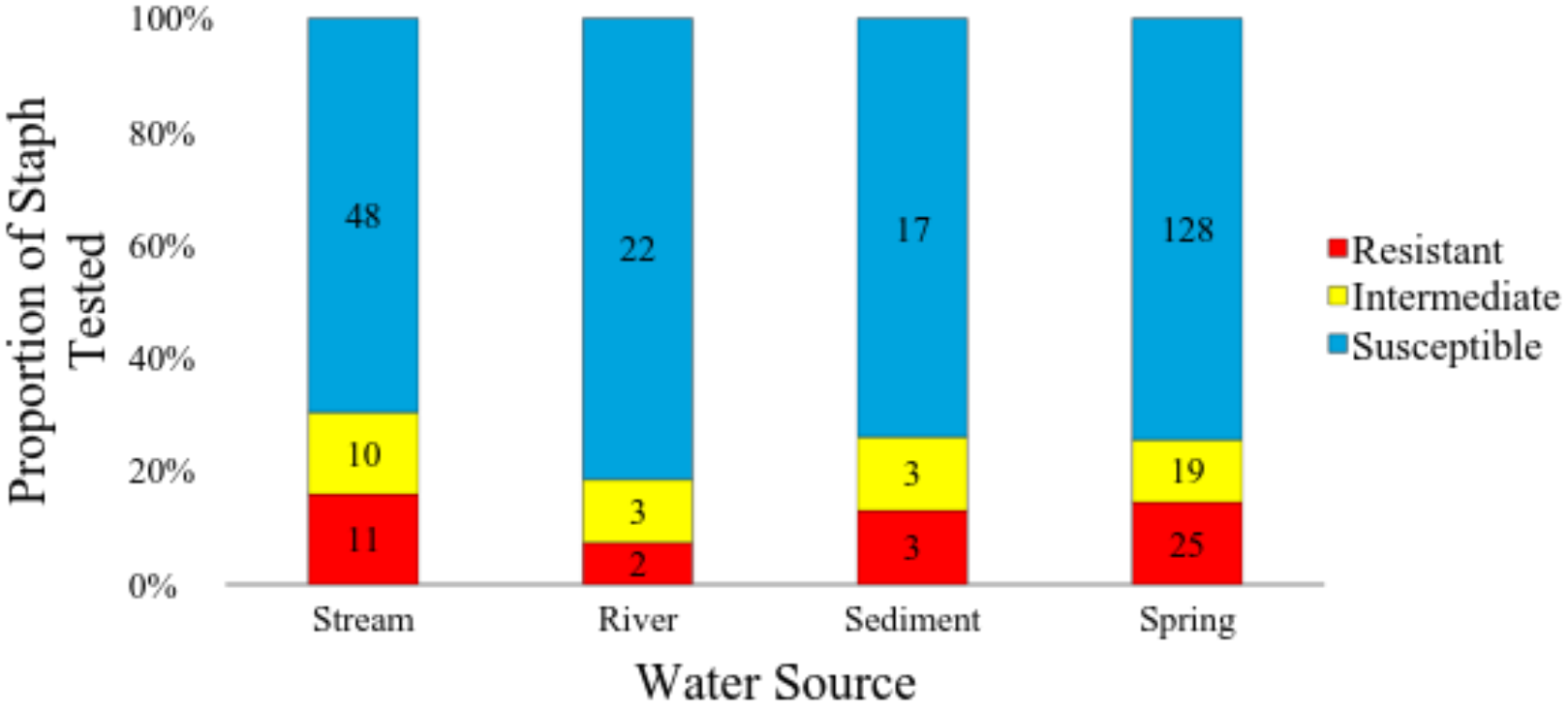
E. coli Colony Counts May 2016 – March 2017



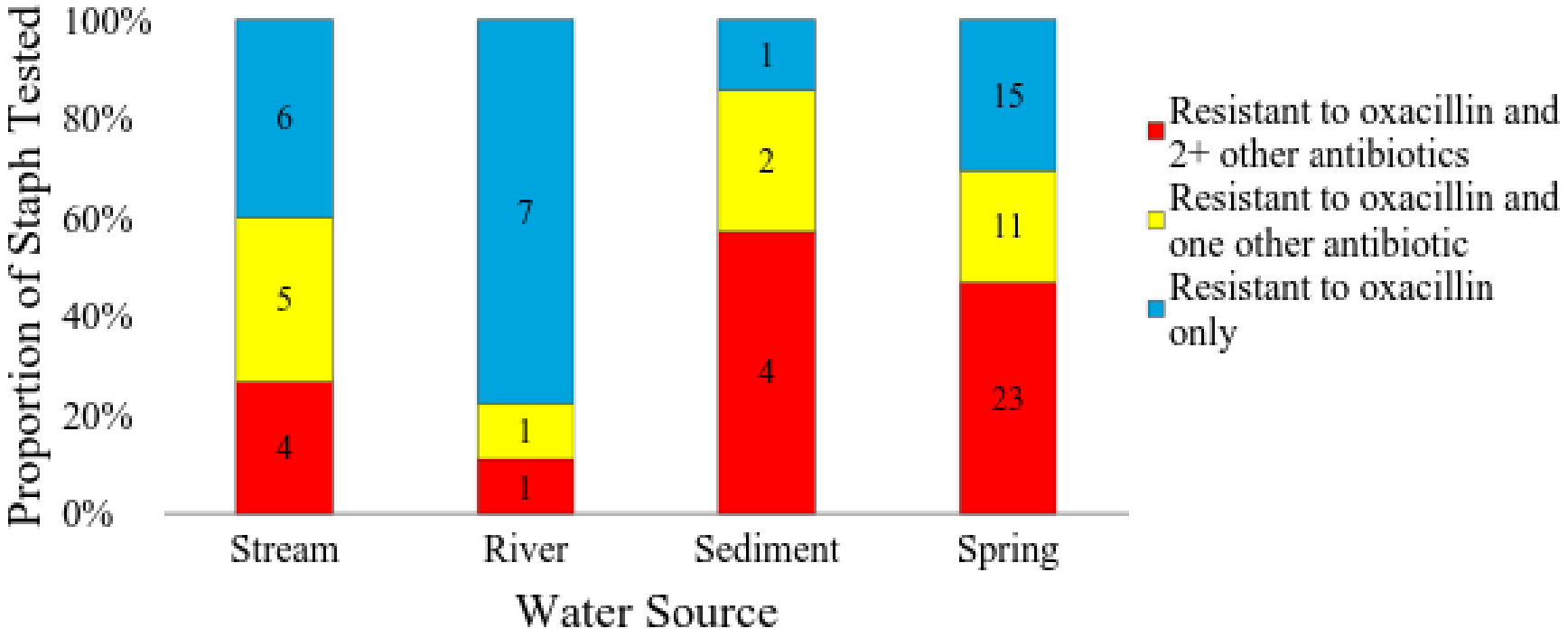
Resistance of *Staph* to Oxacillin



Resistance of *Staph* to Oxacillin

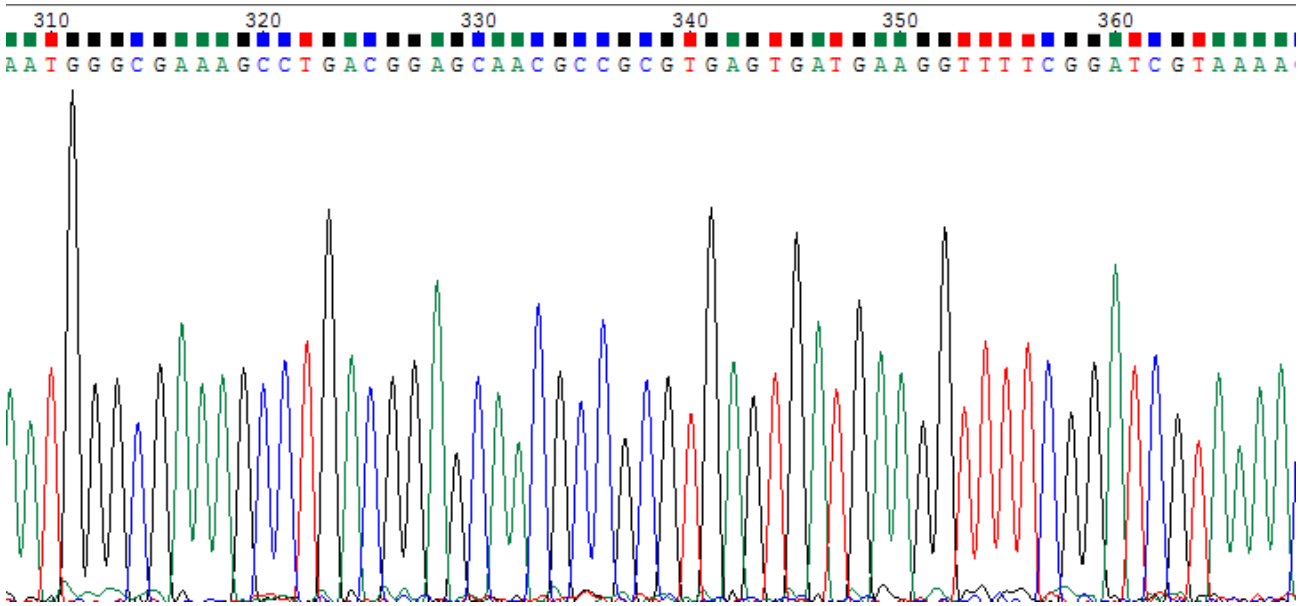


Resistance of Oxacillin-Resistant *Staph* to Other Drugs



Methods: Identification of *Staph* species using DNA

DNA Extraction

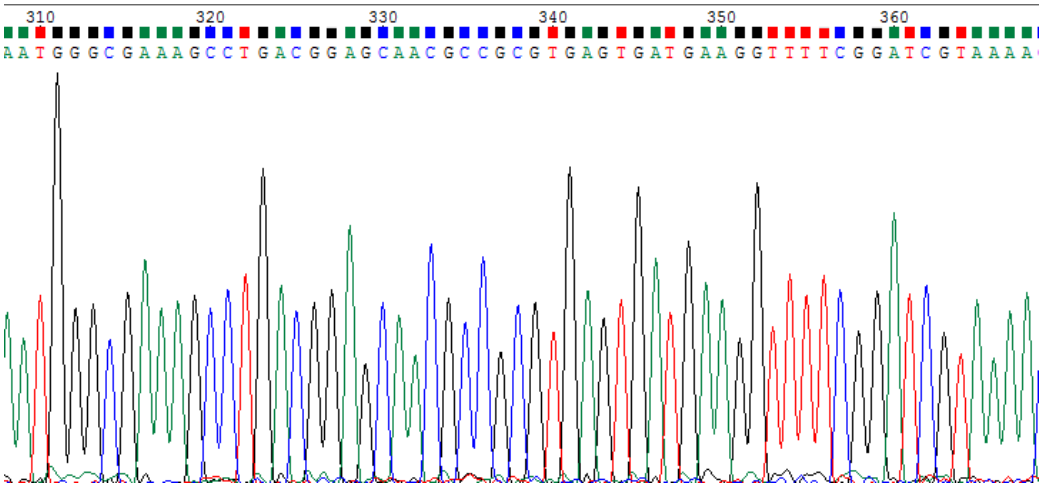


Amplify 16s gene

Analyze Samples via sequencing

PCR Results

- Drug resistant *Staph*:
  - *S. succinus*,
  - *S. xylosus*,
  - *S. sciuri*,
  - *S. lentus*,
  - *S. aureus*

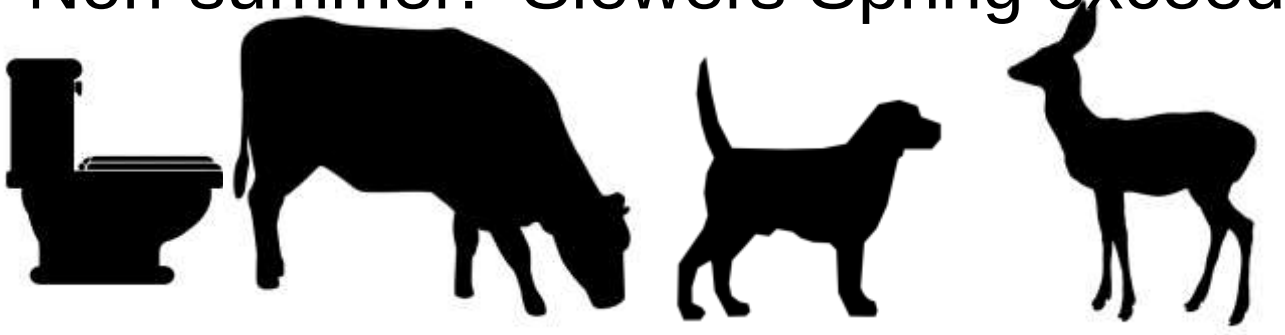


E. coli counts:

Summer Non-rain – Siewers Spring & Malanaphy Spring above recreational limit

Summer Rain: all springs exceed recreational limit.

Non-summer: Siewers Spring exceeds.



Antibiotic resistant *Staph*

- Up to 2/3 of samples showed some resistance to methicillin in June; lower in other months

- Resistance to multiple other drugs documented.

- Explanation for resistance?

- Which *Staph?*

Moodley *et al.* 2011. Effects of tetracycline and zinc on selection of methicillin-resistant *Staphylococcus aureus* (MRSA) sequence type 398 in pigs. *Veterinary Microbiology* 152: 420-423.

Peltier *et al.* 2010. Zinc-induced antibiotic resistance in activated sludge bioreactors. *Water research* **44**:3829-3836.

Human health implications

high fecal bacteria after summer rain

antibiotic resistance in *Staph* – infectious?

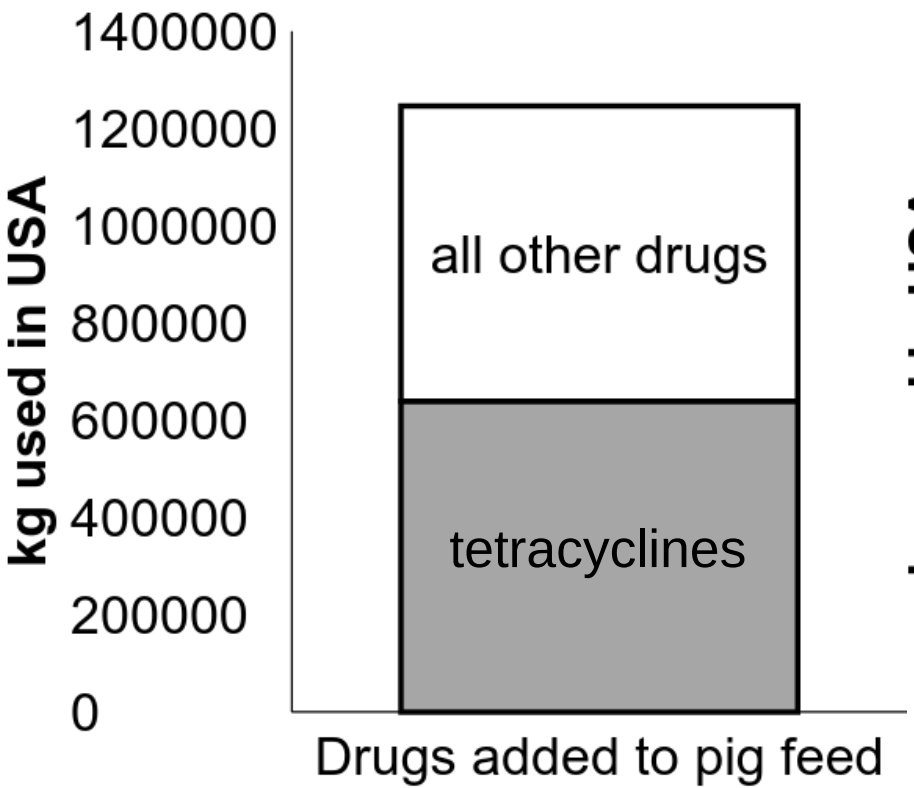
Environmental implications

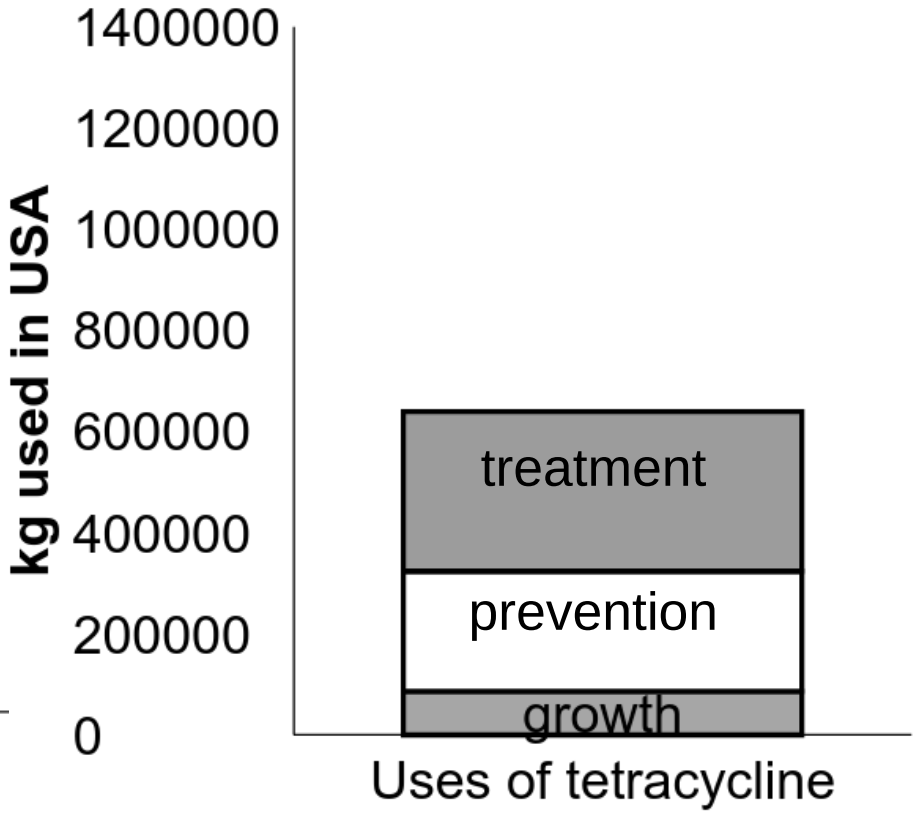
possible presence of antibiotics, heavy metals (Zinc)

Acknowledgements

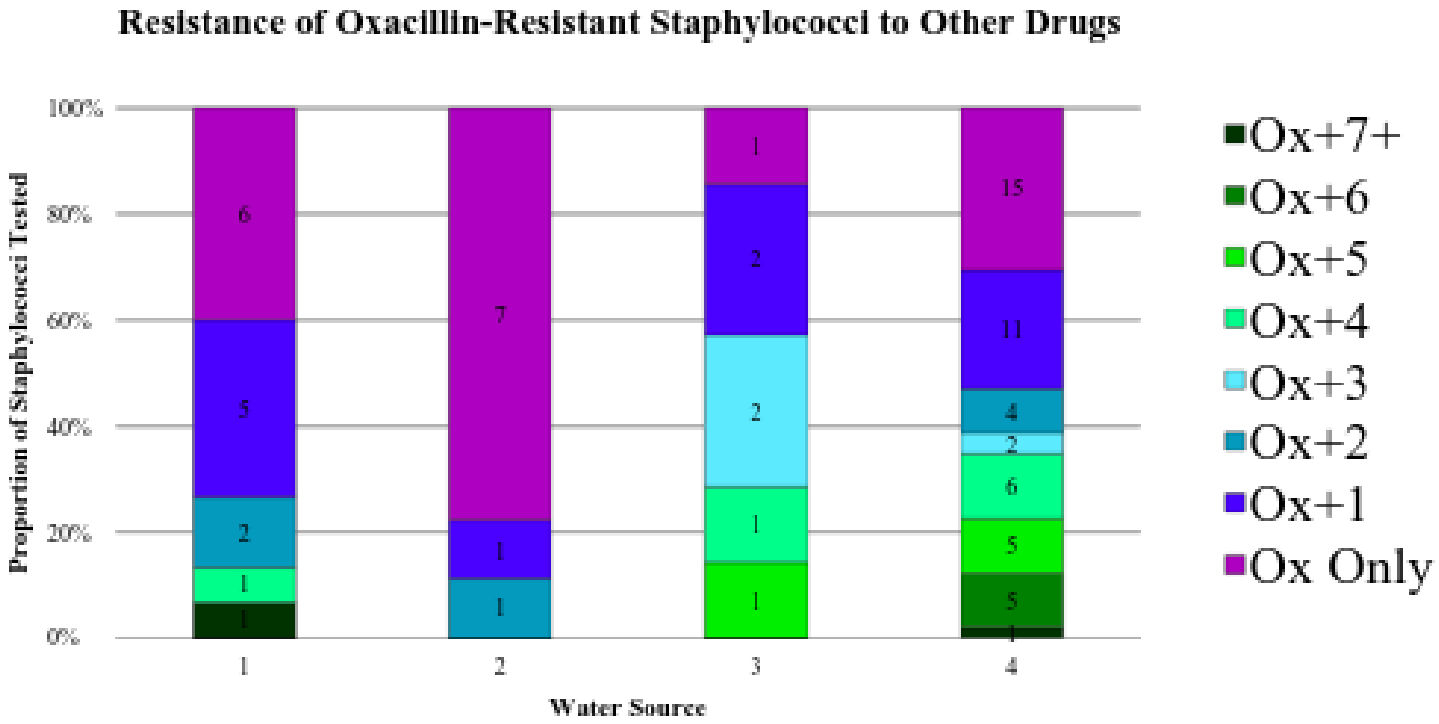
- Howard Hughes Medical Institute
- Luther College Rulon Chair Endowment
- Abby Mark, Isabel de Ayala, Natalie Wade, Lauren Knuckey, and Tiwonge Chirwa helped with sample collection and processing.

Estimated antibiotic use in pigs

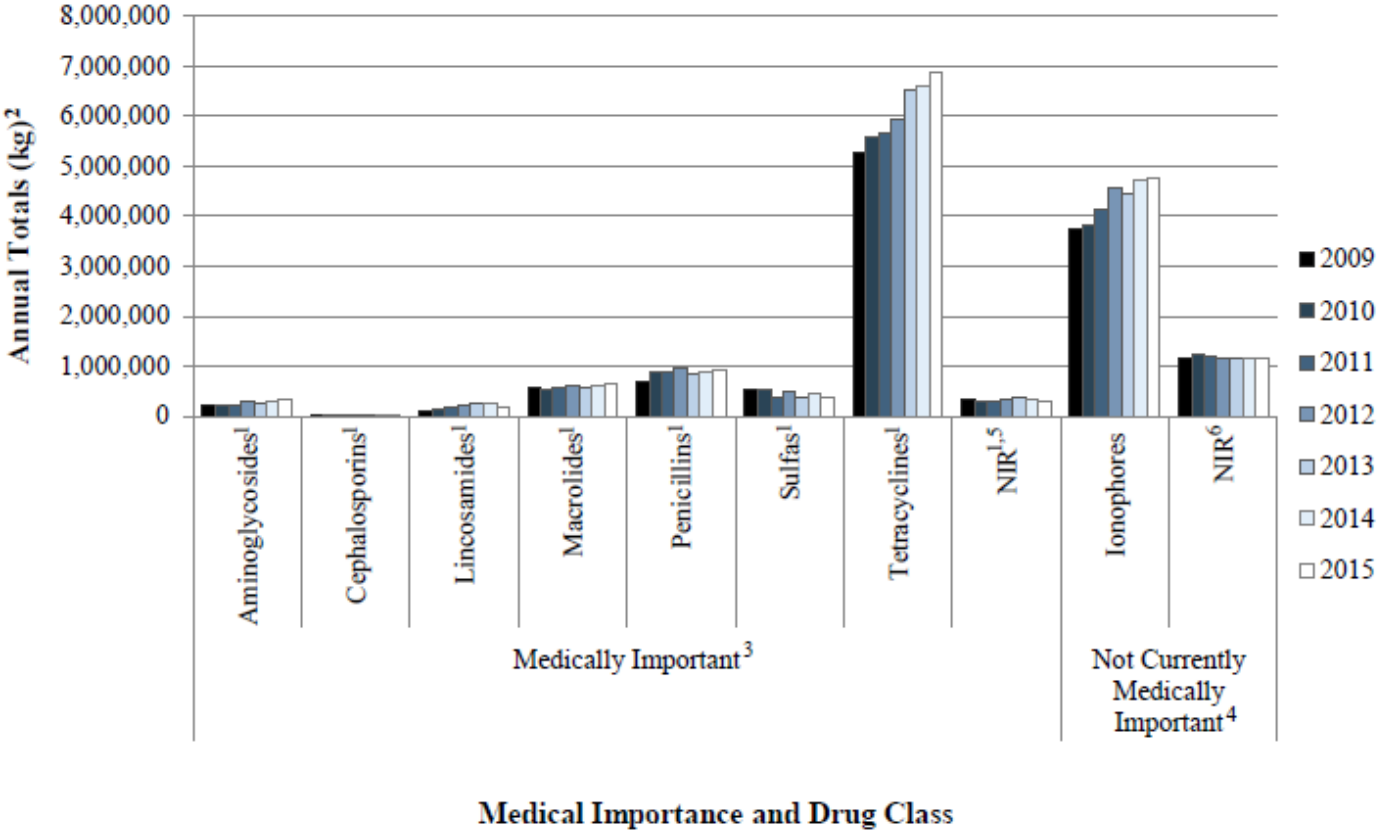


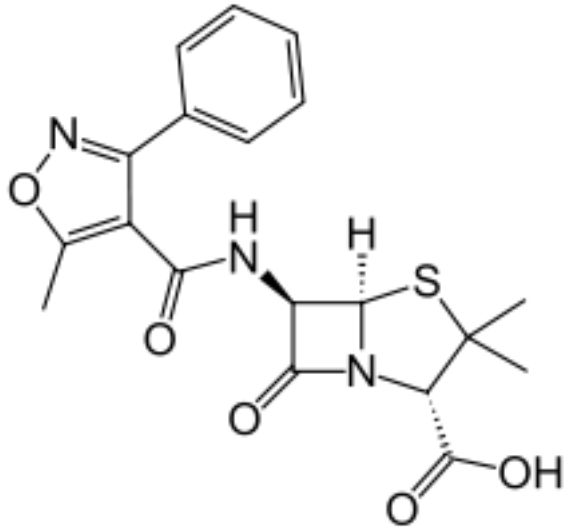


Estimates from Apley et al. 2012. Use estimates of in-feed antimicrobials in swine production in the United States. *Food borne pathogens & disease* **9**:1-8.

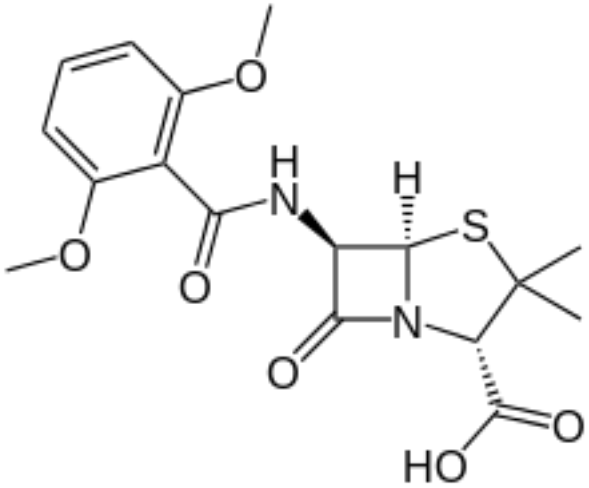

Resistance of Oxacillin-Resistant Staphylococci to Other Drugs
Proportion of Staphylococci Tested
100%
80%
60%
40%
20%
0%
1
2
3
4
Water Source
6
5
2
1
1
7
1
1
1
2
2
1
1
15
11
4
2
6
5
5
Ox+7+
Ox+6
Ox+5
Ox+4
Ox+3
Ox+2
Ox+1
Ox Only

ANTIMICROBIAL DRUGS APPROVED FOR USE IN FOOD-PRODUCING ANIMALS[1]
ACTIVELY MARKETED 2009-2015
DOMESTIC SALES AND DISTRIBUTION DATA
REPORTED BY MEDICAL IMPORTANCE AND DRUG CLASS





Oxacillin



Methicillin