

# A One Health Approach Examining the Potential Linkage between Agricultural Livestock and Human Antibiotic Resistance at the Watershed Level

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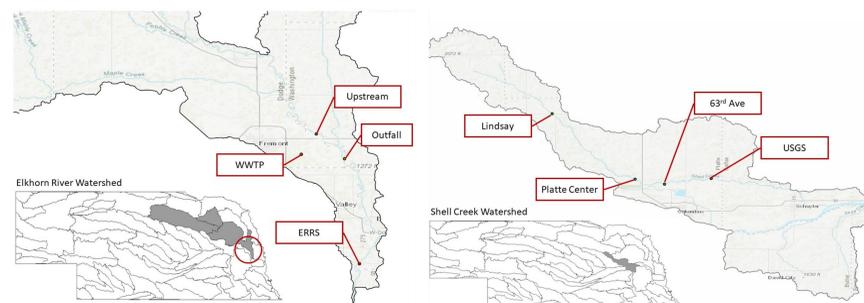
## BACKGROUND

Antibiotic resistance is considered one of the most significant public health threats and challenges of our time. Widespread use of antibiotics in agriculture is increasing the concern about agricultural contributions to the dissemination of antibiotic resistance.<sup>1</sup> Antibiotics and antibiotic resistance genes are excreted in animal manures and then the manure is applied as a fertilizer to soils that can lead to run-off of antibiotics and antibiotic resistance genes to surface water.<sup>2</sup> The use of antibiotics is linked to increasing bacterial resistance against these compounds. The aim of the project was to evaluate the level of exposure of humans and agricultures systems to antibiotic-resistant bacteria, develop a mechanistic understanding of fate and transport of beta-lactam and aminoglycoside antibiotics in watershed impacted by livestock wastes, and identify the significant antibiotic resistance genes (ARG) present in bacteria isolated from the watershed which are commonly associated with human infections. The project examined two agricultural regions in Nebraska with concentrated animal feed operations (CAFO) and proximate human populations. The project team was interdisciplinary, with expertise in biology, environmental engineering, environmental microbiology, and clinical medical microbiological sciences.

## METHODS

- Two watersheds that are intensively agricultural, with both animal and row crop production, were sampled from March through November in 2018.
- The Elkhorn River and Shell Creek consisted of four collection locations each (see Figure 1).
- At each location, samples were collected for each spring, summer, and fall for a total of 24 samples.
- Water quality parameters were measured monthly at each location including temperature, pH, dissolved oxygen, nitrate, phosphate, and total coliforms and *E. Coli* (data not shown).
- Passive organic chemical integrative samplers (POCIS) were deployed for 30-day periods from August – November 2018 to allow for the measurement of clinical and veterinary pharmaceuticals and antibiotics.
- The water samples were cultured using HardyCHROM™ ESBL Agar to screen for the presence of ESBL producing *Enterobacteriaceae* that are potentially non-susceptible to ceftazidime and cefpodoxime.
- The water samples were cultured to isolate and identify the different bacterial colonies. Organisms capable of causing human infections had whole genome sequencing performed. Antibiotic resistance genes (ARGs) present on mobile genetic elements (MGE) that had >95% similarity to mobile ARG present in isolates recorded in the NCBI GenBank database was identified using ResFinder.

Figure 1. Sampling locations within the two watersheds.



## RESULTS

- The study demonstrated significant antibiotic levels present widely throughout the studied watershed, with five of them being associated with human usage (Table 1).

Compound	Frequency of Detection (%)	Range (ng/POCIS)
Monensin	100.0	3.42 - 718.69
Lincomycin	95.8	1.13 - 163.61
Tylosin	79.2	0.10 - 134.76
Trimethoprim	62.5	12.13 - 345.02
Chlortetracycline	54.2	2.41 - 245.28
Erythromycin	54.2	0.15 - 145.60
Anhydro-	33.3	2.33 - 164.83
Sulfamethoxazole <sup>ER</sup>	29.2	6.46 - 694.69
Ractopamine <sup>ER</sup>	29.2	0.19 - 1.86
Tiamulin	25.0	2.39 - 65.34
Tetracycline	20.8	0.44 - 65.72
Sulfadimethoxine	12.5	41.12 - 88.07
Oxytetracycline <sup>ER</sup>	8.3	0.73 - 2.82
Sulfamethazine <sup>SC</sup>	8.3	0.13 - 0.71
Sulfadiazine <sup>ER</sup>		
Erythromycin <sup>SC</sup>		

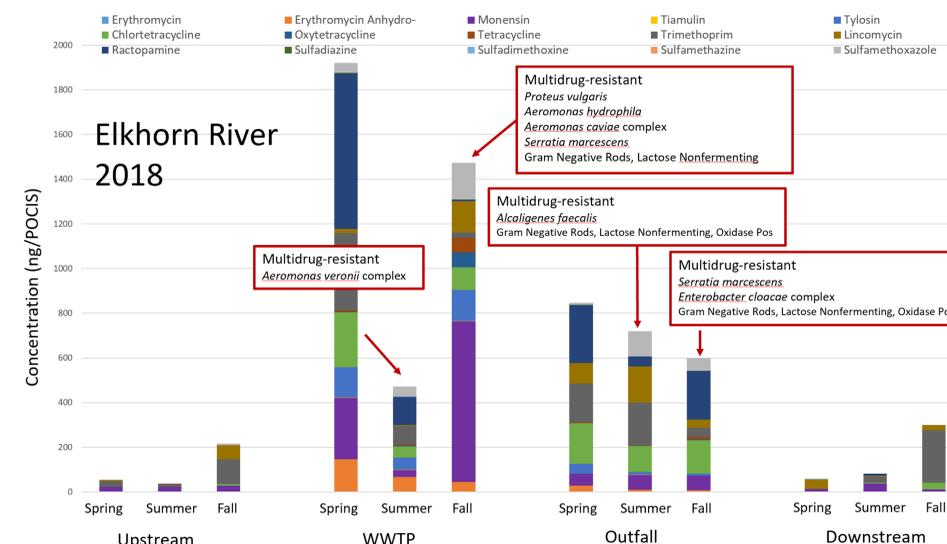
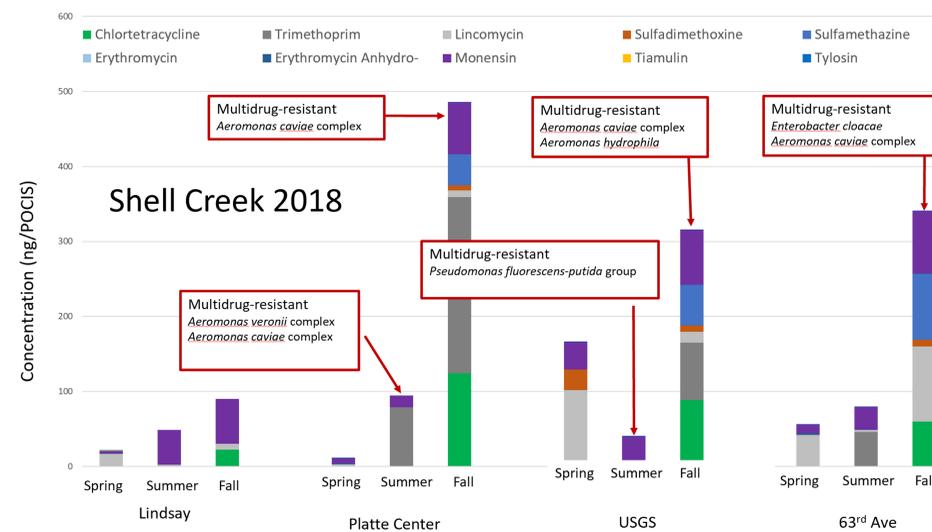
<sup>ER</sup> Analyte only detected in Elkhorn River watershed.  
<sup>SC</sup> Analyte only detected in Shell Creek watershed.

Table 1. Pharmaceutical levels in the watershed

## RESULTS

- Seasonally based drug-resistant bacterial species associated with the respective antibiotic levels is shown in Figure 2.
- A total of 146 bacterial colonies were identified using MALDI-TOF.
- Bacteria which commonly cause human infections had whole genome sequencing performed to identify ARGs that were present on MGE (Figure 3).
- Mobile ARGs were detected in 87.50% of isolates collected from the Elkhorn River, with beta-lactam resistance present in 14 of 16 samples and detected at all sampling sites except ERRS.
- ARG for coding for tetracycline resistance were the next most commonly detected (4 out of 16 isolates) but were only detected at the WWTP site in July and October. Phenicol and trimethoprim mobile ARGs were only detected in the WWTP site.
- Within the Shell Creek, mobile ARGs were detected within 80% of samples. Similar to the Elkhorn River, beta-lactam ARG were the most commonly detected (11 out of 15 isolates), followed by Fosfomycin ARG (6 out of 15 isolates), with both detected throughout the watershed.

Figure 2. Antibiotic levels and drug-resistant bacteria in the watershed



## RESULTS

Figure 3. Antibiotic resistance observed from each isolate at every sampling date and site. A colored bar denotes that resistance to that antibiotic was observed.

Antibiotic	July USGS	October USGS	April Platte Center	July Platte Center	October Platte Center	July 63rd Ave	October 63rd Ave								
Aminoglycoside															
Beta-Lactam															
Fosfomycin															
Quinolone															
Tetracycline															
Species	<i>Klebsiella variicola</i>	<i>Klebsiella variicola</i>	<i>Pseudomonas monteilii</i>	<i>Aeromonas veronii</i>	<i>Aeromonas hydrophila</i>	<i>Aeromonas salmonicida</i>	<i>Aeromonas hydrophila</i>	<i>Aeromonas hydrophila</i>	<i>Aeromonas media</i>	<i>Acinetobacter johnsonii</i>	<i>Aeromonas hydrophila</i>	<i>Aeromonas hydrophila</i>	<i>Aeromonas veronii</i>	<i>Enterobacter ludwigii/cloacae</i>	<i>Aeromonas veronii</i>
Month	July	October	April	July	October	July	October								
Site	USGS		Platte Center				63rd Ave								

Antibiotic	July Upstream	April WWTP	July WWTP	October WWTP	July Outfall	October Outfall	July ERRS									
Aminoglycoside																
Beta-Lactam																
Fosfomycin																
Macrolide																
Phenicol																
Quinolone																
Tetracycline																
Trimethoprim																
Species	<i>Klebsiella pneumoniae</i>	<i>Aeromonas hydrophila</i>	<i>Klebsiella oxytoca</i>	<i>Aeromonas caviae</i>	<i>Aeromonas hydrophila</i>	<i>Serratia marcescens</i>	<i>Proteus sp</i>	<i>Aeromonas hydrophila</i>	<i>Aeromonas hydrophila</i>	<i>Aeromonas veronii</i>	<i>Aeromonas veronii</i>	<i>Aeromonas veronii</i>	<i>Pseudomonas mendocina</i>	<i>Enterobacter cabrierus</i>	<i>Serratia marcescens</i>	<i>Alcaligenes faecalis</i>
Month	July	April	July	October	July	October	July	October	July							
Site	Upstream	WWTP			Outfall			ERRS								

## CONCLUSION

- The results indicated a pervasive acquisition of beta-lactam ARGs in both the Elkhorn River and Shell Creek, although all isolates also had intrinsic resistance to beta-lactams.
- Acquisition of novel ARG occurred frequently in both watersheds, suggesting potential transfer of ARGs between bacterial species.
- These results present evidence of transfer of highly mobile ARGs between environment, clinical, and animal-associated bacteria and highlight the need for a One Health perspective in assessing the spread of antibiotic resistance.
- The presence of significant levels of antibiotics persisting in this agricultural watershed points out the need for ongoing monitoring of compliance with the FDA recommendation of veterinarian oversight of the use of antibiotics in the use of veterinary feed directive applications.

## REFERENCES

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