

Article

# *Escherichia coli* Antimicrobial Resistance Variability in Water Runoff and Soil from a Remnant Native Prairie, an Improved Pasture, and a Cultivated Agricultural Watershed

Maitreyee Mukherjee <sup>1,2,\*</sup>, Terry Gentry <sup>1</sup>, Heidi Mjelde <sup>1</sup>, John P. Brooks <sup>3</sup>, Daren Harmel <sup>4</sup>, Lucas Gregory <sup>5</sup> and Kevin Wagner <sup>6</sup>

## Supplementary Materials

Runoff				Soil			
Tetracycline	Prairie	Hay Pasture	Cropland	Tetracycline	Prairie	Hay Pasture	Cropland
Prairie		0.076795	0.585012	Prairie		-	-
Hay Pasture			1.092761	Hay Pasture			-
Cropland				Cropland			
Cephalothin	Prairie	Hay Pasture	Cropland	Cephalothin	Prairie	Hay Pasture	Cropland
Prairie		1.830642	0.024796	Prairie		8.610186	0.815304
Hay Pasture			1.515529	Hay Pasture			3.317308
Cropland				Cropland			
Ampicillin	Prairie	Hay Pasture	Cropland	Ampicillin	Prairie	Hay Pasture	Cropland
Prairie		12.82306	5.744888	Prairie		2.476985	1.355389
Hay Pasture			2.385037	Hay Pasture			3.895161
Cropland				Cropland			
Sulfamethoxazole	Prairie	Hay Pasture	Cropland	Sulfamethoxazole	Prairie	Hay Pasture	Cropland
Prairie		0.323839	1.721975	Prairie		0.929684	0.466389
Hay Pasture			3.1811	Hay Pasture			-
Cropland				Cropland			

**Figure S1.** Chi-square test values for rates of isolate resistance between runoff and soil in sampling sites by antibiotic. A significant difference ( $p < 0.05$ ) between sites existed for test values  $> 3.84$  (critical value for 1 degree of freedom). Orange shaded cells are tests that reported a significant difference in isolate resistance rates for that antibiotic. Blue shaded values were significant after the Chi-square but not after Bonferonni correction. Cells with no value (-) indicate that no isolate resistance was detected at one of the sites.

Prairie	Tetracycline	Cephalothin	Ampicillin	Sulfamethoxazole
	Soil	Soil	Soil	Soil
Runoff	16.56303602	4.319315895	10.8372656	0.286197484
Hay Pasture	Tetracycline	Cephalothin	Ampicillin	Sulfamethoxazole
	Soil	Soil	Soil	Soil
Runoff	16.91557223	0.007681856	3.78416611	0.893735131
Cropland	Tetracycline	Cephalothin	Ampicillin	Sulfamethoxazole
	Soil	Soil	Soil	Soil
Runoff	6.02259887	0.831596308	2.98350282	2.523639607

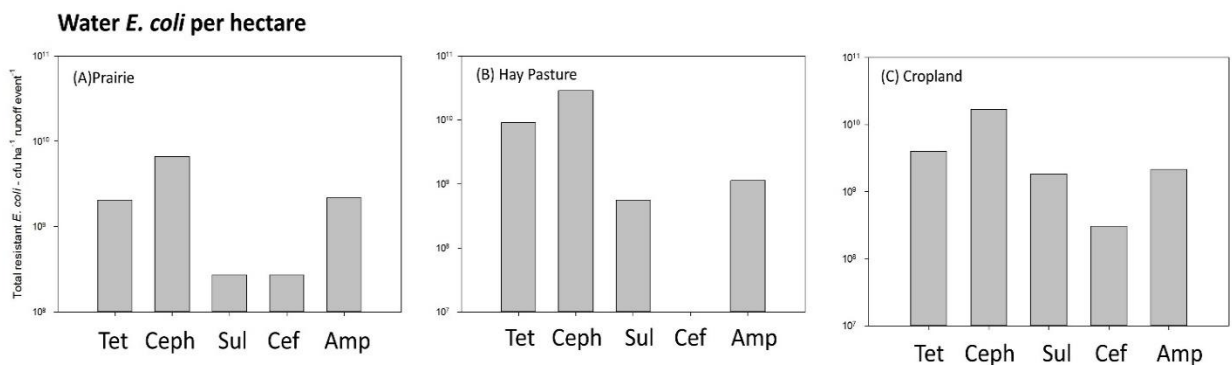
**Figure S2.** Chi-square test values for rates of isolate resistance runoff vs. soil in all sampling sites for each antibiotic. A significant difference ( $p < 0.05$ ) between sites existed for test values  $> 3.84$  (critical value for 1 degree of freedom). Orange shaded cells are tests that reported a significant difference in isolate resistance rates for that antibiotic. Cells with no value (-) indicate that no isolate resistance was detected at one of the sites.

Prairie	$\geq 1$ Agents	$\geq 2$ Agents	$\geq 3$ Agents	$\geq 4$ Agents
	Soil	Soil	Soil	Soil
Runoff	2.29741506	29.12454773	27.1800044	27.82703266
Hay Pasture	$\geq 1$ Agents	$\geq 2$ Agents	$\geq 3$ Agents	$\geq 4$ Agents
	Soil	Soil	Soil	Soil
Runoff	0.87803539	20.40076663	28.190339	-
Cropland	$\geq 1$ Agents	$\geq 2$ Agents	$\geq 3$ Agents	$\geq 4$ Agents
	Soil	Soil	Soil	Soil
Runoff	2.80099945	35.35774757	38.1291263	-

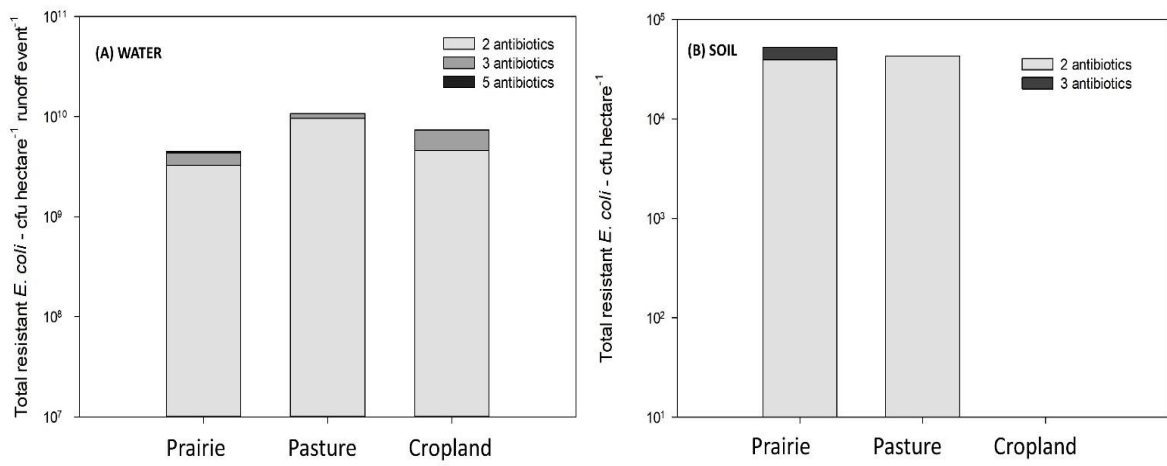
**Figure S3.** Chi-square test values for rates of isolate MDR runoff vs. soil in all sampling sites. A significant difference ( $p < 0.05$ ) between sites existed for test values  $> 3.84$  (critical value for 1 degree of freedom). Orange shaded cells are tests that reported a significant difference in isolate resistance rates for that antibiotic. Cells with no value (-) indicate that no isolate resistance was detected at one of the sites.

≥ 1 Agents		Sampling Site			≥ 1 Agents		Sampling Site			
		Prairie	Hay Pasture	Cropland			Prairie	Hay Pasture	Cropland	
Sampling Site	Prairie		1.000467926	0.06	Sampling Site	Prairie		8.61018634	0.82	
	Hay Pasture			1.52		Hay Pasture				3.32
	Cropland					Cropland				
≥ 2 Agents		Sampling Site			≥ 2 Agents		Sampling Site			
		Prairie	Hay Pasture	Cropland			Prairie	Hay Pasture	Cropland	
Sampling Site	Prairie		1.75	4.85	Sampling Site	Prairie		2.18	1.4391429	
	Hay Pasture			0.71		Hay Pasture				3.90
	Cropland					Cropland				
≥ 3 Agents		Sampling Site			≥ 3 Agents		Sampling Site			
		Prairie	Hay Pasture	Cropland			Prairie	Hay Pasture	Cropland	
Sampling Site	Prairie		3.853983653	0.00055	Sampling Site	Prairie		0.92968421	0.46639	
	Hay Pasture			4.03		Hay Pasture				-
	Cropland					Cropland				
≥ 4 Agents		Sampling Site			≥ 4 Agents		Sampling Site			
		Prairie	Hay Pasture	Cropland			Prairie	Hay Pasture	Cropland	
Sampling Site	Prairie		0.99	1.12	Sampling Site	Prairie		-	-	
	Hay Pasture			~		Hay Pasture				-
	Cropland					Cropland				

**Figure S4.** Chi-square test values for rates of isolate MDR between all sampling sites within runoff and soil isolates. A significant difference ( $p < 0.05$ ) between sites existed for test values  $> 3.84$  (critical value for 1 degree of freedom). Orange shaded cells are tests that reported a significant difference in isolate resistance rates for that antibiotic. Cells with no value (-) indicate that no isolate resistance was detected at one of the sites.



**Figure S5.** Total antibiotic resistant *E. coli* (cfu) observed within each of the three sites, (A) Prairie, (B) Pasture and (C) Cropland in water runoff per rainfall event per hectare. Tet – Tetracycline, Ceph – Cephalothin, Sul – Sulfamethoxazole, Cef – Cefoperazone, Amp – Ampicillin.



**Figure S6.** Total multidrug resistant (MDR) *E. coli* (cfu) observed, **(A)** per hectare per rainfall event (runoff) and **(B)** per hectare of soil within each of the three sites Prairie, Pasture and Cropland.