Supporting Information

TITLE: Validation of a Modified IDEXX Defined-Substrate Assay for Detection of Antimicrobial Resistant *E. coli* in Environmental Reservoirs

AUTHORS: Gracie M. Hornsby[1], Temitope D. Ibitoye[2], Shivaramu Keelara[3], Angela Harris[2]*

[1]Department of Civil and Environmental Engineering, Stanford University School of Engineering, Stanford, CA, USA

[2]Department of Civil, Construction and Environmental Engineering, North Carolina State University, College of Engineering, Raleigh, NC, USA

[3]College of Veterinary Medicine, North Carolina State University, Raleigh, NC, USA

SUPPORTING INFORMATION CONTENTS, 17 pages, 6 tables, 1 figure

TABLE OF CONTENTS:

Abbreviations

Antimicrobial resistant (AMR), World Health Organization (WHO), Tryptone Bile X-glucuronide (TBX), Most Probably Number (MPN), deionized (DI), colony forming unit (CFU), heterotrophic plate counts (HPC)

METHODS

Control Strain Details

EC NC11 is a commensal organism and cefotaxime resistant *E. coli* strain which harbors the CTX M type gene, confirming this isolate is also an ESBL (extended-spectrum beta-lactamase) producer. EC NC11 was isolated from a food animal fecal sample from a local farm in a low- or middle-income country in 2018. EC 25922 is an ATCC reference strain of non-pathogenic *E. coli* which is susceptible to all antibiotics. *Klebsiella pneumoniae* is an ATCC reference strain of (non-*E. coli*) total coliform which acted as a negative control for *E. coli* detection.

Control Strain Preparation

Stocks of the control strains were stored at -80°C. To prepare for an experiment, strains were plated on blood agar and stored at 2-8°C. Before use in controlled experiments, a single colony from each control strain plate was selected to inoculate 10mL of LB broth (Miller) and incubated overnight (12-16 hours) in a shaking incubator (140 rpm) at 37°C. Cultures were serially diluted to obtain a final concentration in the range of quantification (below 2420 MPN for IDEXX; below 500 CFU for plating methods) of the enumeration assays described below

(typically cultures were at a concentration of 10^{10} CFU/mL after overnight incubation and we targeted a final concentration of approximately 10^{1} (generally 20-50 MPN or CFU) in samples).

Environmental sample collection and preparation details

Surface water, surface soil, and waterfowl fecal samples were collected from various public locations around Raleigh, NC. Sample locations were chosen purposively to prioritize locations where we thought we could co-locate surface water, surface soil, and waterfowl fecal samples. Samples were taken between 8am and 12pm from shaded areas to avoid the effects of UV inactivation. All samples were stored in a cooler with ice packs and transported to the lab for processing within 1 hour of collection (fecal samples were placed in a sealed plastic bag to further protect other samples from cross-contamination). Sample collection occurred over a 7-month time period starting in June 2020. A five-day period (August 3rd to 7th, 2020) of daily sampling took place at 3 sample sites to capture an intense rainfall event and increased stream gauge measurements from Hurricane Isaias (August 3rd-4th, 2020).

Surface Water. Surface water samples came from 3 urban lakes and 2 sites along an urban creek. A 1-liter washed and autoclaved Nalgene bottle was used to collect a surface water sample. Sample containers were rinsed three times with surface water downstream prior to collecting each surface water sample. Bottles were submerged horizontally to fill with water.

Surface Soil. Soil samples were co-located with the surface water samples (within 5m). A 1-liter washed and autoclaved Nalgene bottle was used to collect a surface soil sample. Soil was collected along a 0.6m transect, 5-10cm deep using the lip of the Nalgene bottle. To prepare surface soil samples for processing, soil elutions were made with the proportion 6 grams of soil per 40mL of DI water. Depending on the number of replicates required for various experiments,

65 elutions would range from 40mL to 120mL in volume. Mixtures were shaken vigorously for 3
66 minutes and then left undisturbed for 5 minutes[1]. A sterile pipette was used to withdraw the
67 supernatant (half of the elution volume). In cases where multiple replicates were being
68 processed, the supernatant was re-homogenized in a sterile 1-liter Nalgene container before
69 being added to sample vessels (a total wet weight of 3 grams of soil is represented in each
70 100mL sample; see Table 1 for additional information on sample volumes).
71 *Waterfowl Feces*. Waterfowl feces were collected in the general vicinity of the water and
72 soil sample sites (within 20m). Duck (n=1) and geese (n=7) feces were targeted. We aimed to
73 collect fresh feces when possible so we could positively identify the species of bird it was
74 deposited by. In cases where we could not observe a bird depositing feces on the ground, we
75 aimed to collect feces that appeared fresh. Sterile 50mL disposable conical tubes were used to
76 collect waterfowl (i.e. goose, duck) fecal samples. To avoid contamination from the soil below
77 fecal deposits, only portions of fecal deposits which had not contacted the ground were collected
78 (the top half of the deposit) using sterile disposable scoops (v-scoop spatula, Corning, New
79 York). To prepare fecal samples for processing, fecal slurries were created by vortexing 2 grams
80 of feces with 15mL of DI water for one minute. Additional DI water was then added to bring the
81 slurry to a total volume of 20mL. The slurry was vortexed for another 5 minutes then allowed to
82 rest for another 5 minutes[2]. The slurry was diluted to create final dilutions of $1{:}10^2$, $1{:}10^4$, and
83 $1{:}10^6$ for samples treated with cefotaxime, and $1{:}10^4$, $1{:}10^6$, and $1{:}10^8$ for samples not treated with
84 cefotaxime. Since concentrations of antibiotic resistant and total *E. coli* varied greatly between
85 fecal samples, each dilution described above was first tested in duplicate only using the IDEXX
86 assay in order to determine the dilution level that would result in concentrations of *E. coli* within
87 the range of quantification of all three assays. The slurry was then stored at 2-8°C overnight and

processed within 24 hours at the appropriate dilution level for IDEXX, TBX, and MacConkey as part of the Matrix Control experiments described in Table S1.

Enumeration using membrane filtration and plating on MacConkey and TBX

MacConkey (BD, Franklin Lakes, NJ) and TBX (Sigma Aldrich, St. Louis, MO) agar were prepared per manufacturer instructions. To enumerate the total CFU of *E. coli* in a sample, no further additions were made past manufacturer instructions. To enumerate cefotaxime resistant *E. coli*, agar was treated with cefotaxime (400µL of 5mg/mL cefotaxime solution per 500mL of agar for a final concentration of 4µg/mL) after cooling in a 50°C water bath for 30 minutes, before being poured into sterile plates.

Samples were membrane filtered using sterile disposable Nalgene analytical filter funnels with 0.45µm pore size cellulose nitrate membrane (Fisher Scientific, Waltham, MA) and a vacuum filtration system. Filter papers were placed on agar plates with metal forceps that were submerged in 100% ethanol and flame sterilized. The plates were incubated for 24 hours at 35°C before colonies were counted.

E. coli on MacConkey plates presented as pink growths with a small halo of pink on the filter paper. In instances where filter papers collected heavy amounts of sediment (i.e. soil samples or highly turbid water samples), *E. coli* would sometimes appear as large clear growths on MacConkey. A subset (5-10 colonies) of these growths were selected and propagated overnight in LB broth then re-plated on cefotaxime-treated agar the next day to confirm antibiotic resistance. *E. coli* on TBX plates presented as bright blue growths of varying sizes. Plates were classified as above the detection limit if greater than 500 separate growths appeared on a single plate. Plates were classified as "uncountable" if inhibition made growths impossible

to distinguish. Plates were counted in terms of colony forming units (CFU), and each growth on the filter paper which was not touching or conjoined with other growths was regarded as one CFU.

Standard Methods for IDEXX Quanti-Tray/2000

The standard protocol is designed to detect generic *E. coli* in environmental samples and requires a total sample volume of 100mL to fill each Quanti-Tray/2000. Colilert-18 media is dissolved completely in a 100mL sample then the solution is poured into a Quanti-Tray/2000 and sealed in an IDEXX tray sealer. The trays are then incubated at 35°C for 18-22 hours. *E. coli* concentrations are estimated using a most probable number (MPN) technique, based on the number of small and large wells on each tray that appear yellow under normal light and fluoresce under UV light after incubation.

Confirmation Testing Methods

To withdraw culture from IDEXX tray wells for confirmation testing, the back of the IDEXX tray is first wiped with ethanol. Next, a flame sterilized box cutter is used to cut an 'X' shape into an identified well. The box cutter is then used to push back the paper to keep it from touching the culture and allow space for pipetting. The culture is pipetted from the well and dispensed into a labeled microcentrifuge tube for storage.

The IDEXX isolates were initially streaked onto cefotaxime-treated MacConkey plates and incubated at 37 C for 24 hours. An isolate was then picked and streaked on blood agar plates (BD, Franklin Lakes, NJ) and grown at 37°C for 12-16 hours. The concentrations of the culture were adjusted to 0.5 McFarland standard and streaked onto Mueller Hinton agar (MHA) plates.

The following antibiotic discs were placed on the plates and incubated overnight at 37°C: cefotaxime (30 µg), cefotaxime (30 µg) + clavulanic acid (10 µg), ceftazidime (30 µg), and ceftazidime (30 µg) + clavulanic acid (10 µg). A ≥5 mm increase in the inhibition zone for the cefotaxime and ceftazidime discs with and without β-lactamase inhibitor, clavulanic acid, confirmed ESBL production. Positive (*E. coli* NC11) and negative (*E. coli* ATCC25922) controls were also tested.

To archive the isolates from the IDEXX assay, a flame sterilized scalpel was used to open the IDEXX tray. The sample was then transferred to a 1.5 mL microcentrifuge tube. To archive the isolates from the MacConkey and TBX plates, a single cefotaxime-resistant colony was picked using a sterile inoculation loop and placed in 1 mL of LB broth. 1-5 isolates per each unique environmental sample were archived and stored at -80°C. We analyzed 47 isolates from water samples, 24 isolates from soil samples, and 26 isolates from fecal samples. Of the isolates collected, 4 unique water sample, 1 soil sample, and 1 fecal sample had isolates from IDEXX, TBX, and MacConkey and were evaluated for confirmation of ESBL production.

Waste Disposal Recommendations

Used IDEXX trays were treated as biowaste and sterilized in an autoclave (90-minute liquid cycle) before disposal in a lab waste bin. For resource-restricted settings, pressure cookers are an affordable alternative to an autoclave and many commercial models can achieve the required temperature and pressure (121 °C, 15 psi) for sterilization.

Data Analysis Details

Samples that had no *E. coli* detected using IDEXX, MacConkey, or TBX assays were assigned 0 MPN or CFU (colony forming unit), depending on method, per sample volume. Samples which met or exceeded the IDEXX assay's detection limit of 2420 MPN/sample volume were assigned this detection limit for data analysis. For both plating methods, an upper detection limit of 500 CFU/plate was used. Plates which exceeded this value were labeled too numerous to count (TNTC) and were assigned a value of 500 CFU for data analysis. Additionally, samples which resulted in indistinguishable results due to inhibition on plates and were omitted from data analysis.

Statistical Power

Post-hoc power calculations for our key comparisons indicate our study was adequately powered to detect statistical significance for relevant magnitudes of differences. Based on our observed sample variability (average within group variation=8.90) for Pure Culture Experiments where spiked samples are treated with cefotaxime and processed by each assay, we were powered to detect mean differences between groups (assays) of 9 MPN/100mL or higher with ANOVA (power = 0.80, alpha=0.05, n=3). Based on our observed sample variability (average within group variation=18.87) for matrix control samples where environmental samples are spiked with culture, treated with cefotaxime, and processed by each assay, we were powered to detect differences between group (assay) means of 6 MPN/100mL or higher with ANOVA (power = 0.80, alpha=0.05, n=9).

Further, Most Probable Number techniques and plating techniques are not perfectly reproducible and commonly show some variation in results between replicates. Despite our experimental results showing some variation between replicates (see spread of Figures 1 and 2),

we still had sufficient statistical power to detect significant differences. Standard deviations were comparable across methods (IDEXX, MacConkey, TBX) and standard deviations were not influenced by the addition of cefotaxime. Standard deviations among replicates in matrix control experiments ranged from 1.3-9.4 MPN for IDEXX, 2.1-9.8 MPN for MacConkey, and 0.6-7.5 MPN for TBX. Moreover, results of environmental samples processed for *E. coli* are often reported on a log scale, meaning replicates would be expected to be of the same power of 10. Given this context, replicate variability up to 20 MPN in this study is an adequately reproducible method.

RESULTS

Pure Culture Experiment Results

One-way ANOVA tests indicated statistically significant differences between assays within each of these treatment conditions, shown on the x-axis of Figure 1. Tukey multiple pairwise comparisons revealed TBX performed significantly differently than IDEXX ($p=0.013$) and MacConkey ($p=0.013$) when samples were spiked with EC NC11 and treated with cefotaxime and when samples were spiked with EC NC11 and not treated with cefotaxime ($p=0.020$; $p=0.006$). Both TBX and MacConkey are widely accepted methods for enumerating cefotaxime resistant *E.* coli in environmental samples. Thus, despite the significantly different performance of the TBX assay, these results suggest the modified IDEXX protocol is a valid method for enumerating cefotaxime-resistant *E. coli* based on its highly similar performance to the MacConkey assay.

Confirmation Testing and QA/QC

For samples processed with IDEXX, a total of 42, 60% of water isolates (9/15), 43% of soil isolates (3/7), and 0% of fecal isolates (0/20) were confirmed as ESBL producing. For samples processed with MacConkey, a total of 33, 5% of water isolates (2/20), 30% of soil isolates (3/10), and 33% of fecal isolates (1/3) were confirmed as ESBL producing. For samples processed with TBX, a total of 22, 0% of water isolates (0/12), 14% of soil isolates (1/7), and 0% of fecal isolates (0/3) were confirmed as ESBL producing. In total, 19% (19/97) of isolates tested were identified as ESBL-producing *E. coli* according to the zone diameter specified by CLSI. The modified IDEXX assay detected a significantly larger fraction of ESBL producing *E. coli*

than the MacConkey ($p=0.002$) and TBX ($p=0.001$) assays in surface water (difference of proportions z-test). No significant differences were observed for soil and fecal isolates from different assays.

Roughly 5% of samples processed with the IDEXX assay exceeded the enumeration limit of 2420 MPN/100mL and had to be assigned the value 2420 MPN for data analysis. Less than 10% of samples processed with either MacConkey or TBX exceeded the enumeration limit of 500 CFU/100mL and were assigned the value 500 CFU for data analysis. Lastly, less than 3% of samples processed were omitted from data analysis due to indistinguishable results.

222 **Table S1.** Experimental Design

Experiment	Matrices	Control Strains	Antibiotic Treatment	Replicates
Pure culture	99mL sterile H20	EC NC11 (resistant)	80µL cefotaxime	3
			80µL of sterile H20	3
			No addition	3
		EC 25922 (susceptible)	80µL cefotaxime	3
			80µL of sterile H20	3
			No addition	3
		K. pneumoniae	80µL cefotaxime	3
			80µL of sterile H20	3
			No addition	3
Matrix control	99mL surface water	EC NC11 (resistant)	80µL cefotaxime	3
			No addition	3
		EC 25922 (susceptible)	80µL cefotaxime	3
			No addition	3
		None	80µL cefotaxime	3
			No addition	3
	20mL soil elution and 79mL sterile water	EC NC11 (resistant)	80µL cefotaxime	3
			No addition	3
		EC 25922 (susceptible)	80µL cefotaxime	3
			No addition	3
		None	80µL cefotaxime	3
			No addition	3
	1mL diluted fecal slurry and 98mL sterile water	EC NC11 (resistant)	80µL cefotaxime	3
			No addition	3
		EC 25922 (susceptible)	80µL cefotaxime	3
			No addition	3
		None	80µL cefotaxime	3
			No addition	3
Environmental	100mL surface water	None	80µL cefotaxime	1
			No addition	1
	20mL soil elution and 80mL sterile water	None	80µL cefotaxime	1
			No addition	1
	1mL diluted fecal slurry and 99mL sterile water	None	80µL cefotaxime	1
			No addition	1

For the Pure culture experiments only, we tested a third treatment condition, "DI Water," consisting of an 80µL addition of sterile DI water (Table S1) to mimic the change in total sample volume due to addition of antibiotic. The Quanti-Tray/2000s are able to handle this small extra volume due to the large 'overflow' well at the top of the tray.

Table S2. Full Results of the Pure Culture Validation Experiment

	Pure Cultures Experiment - Mean (StDev)								
	EC NC 11			EC25922			K. Pneumoniae		
	Antibiotic	DI Water	None	Antibiotic	DI Water	None	Antibiotic	DI Water	None
IDEXX	25.4 (3.4)	31.8 (3.6)	33.2 (7.9)	0 (0)	28.2 (5.5)	27.9 (5.5)	0 (0)	0 (0)	0 (0)
TBX	15.0 (1.7)	NA	11.0 (4.4)	0 (0)	NA	41.0 (4.0)	0 (0)	NA	0 (0)
MacConkey	25.3 (3.5)	NA	28.3 (3.2)	0 (0)	NA	30.7 (5.9)	0 (0)	NA	0 (0)

Table S2. Full results of the pure culture validation experiment. Mean and standard deviation (in MPN or CFU) of all experimental conditions tested during the pure culture validation experiment. All conditions were tested in triplicate and the mean and standard deviation of these triplicate measurements are reported above. Columns with "NA" represent experimental conditions not tested.

Table S3. Justifications for Sample Sizes Reported in GEE Analysis of Environmental Sample Validation Experiments

Matrix	n	Justification
Surface water	60	3 assays run per location (3 sites) per day for 7 days of sampling, except only 2 assays were run on the last day of sampling due to material shortages (3x3x7-3)
Surface soil	58	3 assays run per location (3 sites) per day for 7 days of sampling, except only 2 assays were run on the last day of sampling due to material shortages and one site was unavailable on the last day of sampling (3x3x7-5)
Waterfowl feces	10	3 assays run per location (1 site) for 4 days where fecal samples were able to be collected except only the IDEXX assay was performed on the last day of sampling due to material shortages (3x1x4-2)

Table S4. Surface water, surface soil, and waterfowl feces contingency table for McNemar's exact tests between IDEXX-MacConkey and IDEXX-TBX*

	Surface Water				Surface Soil				Waterfowl Feces			
	MAC		TBX		MAC		TBX		MAC		TBX	
	(+)	(-)	(+)	(-)	(+)	(-)	(+)	(-)	(+)	(-)	(+)	(-)
IDEXX (+)	6	7	5	8	6	5	8	3	1	3	1	3
IDEXX (-)	4	11	6	9	9	8	5	12	0	3	0	3

*n=28 for surface water-MacConkey, surface water-TBX, surface soil-MacConkey, and surface soil-TBX and do not sum to n=60 and n=58 as mentioned above. Supply issues during COVID-19 prevented us from processing one day of samples with MacConkey and TBX, hence they could not be matched with IDEXX results in the 2x2 table.

Table S5. Summary of bivariate GEE model outputs for concentrations of cefotaxime resistant *E. coli* in surface water and surface soil

Concentrations of AMR E. coli

		Surface Water				*Surface Soil*			
	Variable	β	p-value	Lower 95% CI	Upper 95% CI	β	p-value	Lower 95% CI	Upper 95% CI
Model 1	intercept	0.932	0.013*	0.195	1.669	-0.369	0.616	-1.813	1.075
	Rainfall in last 24 hours	-0.965	0.069	-2.008	0.078	-0.968	0.416	-3.302	1.366
Model 2	intercept	-0.437	0.228	-1.147	0.273	-4.571	0.004*	-7.639	-1.503
	Concentration of total *E. coli*	0.001	<0.001*	0.0005	0.0015	0.006	0.013*	0.001	0.011

The outcome of interest in Model 1 and Model 2 is the concentration (MPN/100mL) of cefotaxime resistant *E. coli* detected. Both models are clustered by sample location. Model 1 uses a binary predictor coded as 1 if there was rainfall during the prior 24 hours from when the sample was collected and coded as 0 if there was no rainfall in the prior 24 hours. Model 2 uses the concentration of total *E. coli* to predict resistant *E. coli* concentration with a unit of 1 MPN/100mL. Concentration of total *E. coli* was found to be a significant predictor of the concentration of cefotaxime resistant *E. coli* in both the surface water ($p<0.001$) and surface soil ($p=0.013$) models, indicating concentrations of total *E. coli* and resistant *E. coli* co-vary (see Table 2, Model 2).

Table S6. Summary of Matrix Control experiment results with pure culture controls reported

Surface Water

	Sample 1				Sample 2				Sample 3			
	Spiked Env Sample	No Spike Env	Pure Culture	% Recovery	Spiked Env Sample	No Spike Env	Pure Culture	% Recovery	Spiked Env Sample	No Spike Env	Pure Culture	% Recovery
IDEXX	18.8 (1.3)	1.7 (0.6)	27.5	62.2%	18.6 (2.1)	1.0 (1.0)	27.5	64.0%	23.7 (4.5)	0.7 (1.2)	38.5	59.7%
TBX	20.7 (0.6)	0.3 (0.6)	16.0	127.5%	22.0 (5.3)	0 (0)	16.0	137.5%	27.3 (4.2)	0.3 (0.6)	28.3	95.4%
MacConkey	22.3 (2.1)	0.7 (1.2)	22.0	98.2%	14.3 (2.3)	0 (0)	22.0	65.0%	32.7 (2.5)	1.0 (1.0)	34.7	91.4%

Surface Soil

	Sample 1				Sample 2			
	Spiked Env Sample	No Spike Env	Pure Culture	% Recovery	Spiked Env Sample	No Spike Env	Pure Culture	% Recovery
IDEXX	36.4 (6.5)	0 (0)	34.1	106.7%	42.6 (5.1)	0 (0)	48.1	88.6%
TBX	32.7 (4.7)	0 (0)	29.0	112.8%	36.3 (4.6)	1.3 (1.5)	24.0	145.8%
MacConkey	17.3 (3.5)	0 (0)	32.0	54.1%	35.7 (4.5)	0 (0)	39.0	91.5%

Waterfowl Feces

	Sample 1				Sample 2				Sample 3			
	Spiked Env Sample	No Spike Env	Pure Culture	% Recovery	Spiked Env Sample	No Spike Env	Pure Culture	% Recovery	Spiked Env Sample	No Spike Env	Pure Culture	% Recovery
IDEXX	46.4 (2.1)	0.5 (0)	35.9	127.9%	21.0 (9.4)	0.5 (0)	34.2	59.9%	28.5 (6.2)	0 (0)	34.9	81.7%
TBX	31.3 (2.5)	0 (0)	27.7	113.0%	26.7 (6.7)	0 (0)	25.0	106.8%	30.0 (7.5)	0 (0)	22.7	132.2%
MacConkey	18.7 (2.1)	0 (0)	18.3	102.2%	22.3 (4.2)	0 (0)	17.7	126.0%	14.7 (9.8)	0 (0)	24.0	61.3%

*All samples were spiked with ECNC 11, treated with cefotaxime, and reported as Mean (St Dev) of triplicate samples processed

*Only two soil samples are presented since one sample yielded almost entirely TNTC or >2420 MPN results

Percent recovery ranged from 59.7%-127.9% for IDEXX, 95.4%-145.8% for TBX, and 54.1%-126% for MacConkey. Some Pure Culture samples resulted in lower enumeration of organisms than spiked environmental samples, even after naturally occurring resistant *E. coli* ("No Spike Env") was subtracted out. Thus, recovery rates over 100% are likely due natural variability in replicate measurements. All 3 assays showed sufficient recovery and point to little evidence of inhibition from environmental matrices.

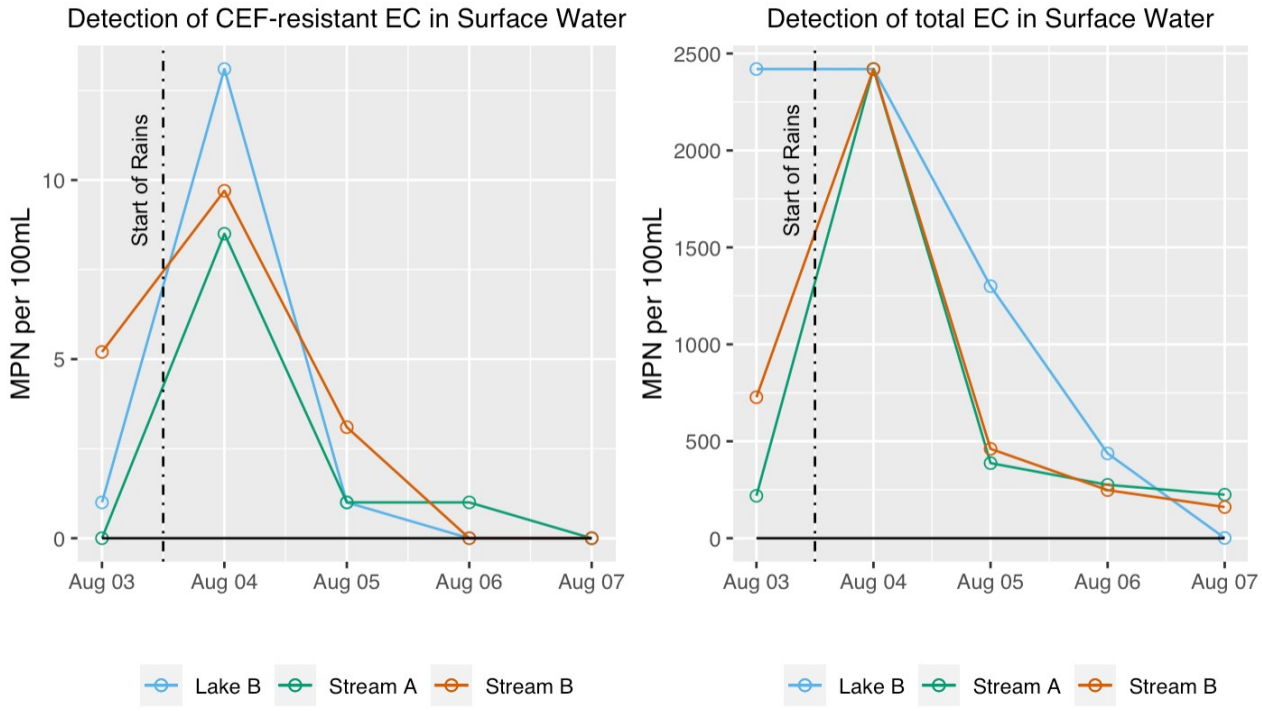

Figure S1. Trends in concentration of cefotaxime resistant and total *E. coli* in surface water after heavy rains. Data represent concentrations of cefotaxime-resistant and total *E coli* in surface water samples collected on five consecutive mornings from August 3rd, 2020 to August 7th, 2020 at one lake and two urban streams near Raleigh, NC. Heavy rains from Hurricane Isaias began on the evening of August 3rd and continued through the early morning of August 4th but stopped before sample collection occurred. Cefotaxime resistant *E. coli* is shown in the left plot and total *E. coli* is shown on the right. Colored lines correspond to sample site listed in the legend and hollow dots represent the daily measurements. Note the x-axis is identical between plots but y-axes differ substantially. Axes have been adjusted to prioritize showing increase and decrease of concentrations rather than absolute levels. Readers can also note, in the total *E. coli* plot, on August 4th the sample from Stream A was over the maximum detection range (>2,419.6 MPN/100mL) and is represented in our dataset as 2420 MPN/100mL, whereas the samples from Lake B and Stream B were exactly 2,419.6 MPN/100mL. Readers can also note, in the total *E.*

coli plot, on August 4th the sample from Stream A was over the maximum detection range (>2,419.6 MPN/100mL) and is represented in our dataset as 2420 MPN/100mL, whereas the samples from Lake B and Stream B were exactly 2,419.6 MPN/100mL.

References

1. A. J. Pickering, T. R. Julian, S. J. Marks, M. C. Mattioli, A. B. Boehm, K. J. Schwab and J. Davis, Fecal contamination and diarrheal pathogens on surfaces and in soils among Tanzanian households with and without improved sanitation, *Environmental Science & Technology*, 2012, **46**, 5736–5743.
2. A. R. Harris, A. J. Pickering, M. Harris, S. Doza, M. S. Islam, L. Unicomb, S. Luby, J. Davis and A. B. Boehm, Ruminants contribute fecal contamination to the urban household environment in Dhaka, Bangladesh, *Environmental science & technology*, 2016, **50**, 4642–4649.