SUPPLEMENT 1: Study Detailed Procedure

Study Data Collection

Upon arrival at a location, indicate whether it's private (R-access for limited individuals) or public (U-access for all individuals). We recorded either U or R at the end of the three-letter code describing the location; the first two letters were from the location's name. For DNA extraction of one sample, we massed 25 mg soil with a sensitive balance and used a metal spatula to add the soil to a 2 mL lysis tube with silicon lysis beads. We then added 750 µl lysis solution and bashed the lysis tube in a vortexes with the proper attachment for 40 minutes. Afterwards, we decanted the homogenate into a microcentrifuge tube (all tubes used were 1.5 mL) and centrifuged this tube for 3 minutes (open rotor centrifuge at maximum speed) to separate homogenate into pellet and supernatant. We pipetted 400 µl supernatant into a III-F spin filter with a collection tube, and centrifuged for 1 minute. After, we added 800 µl DNA binding buffer and 400 µl 95% ethanol to the collection tube. We then pipetted up and down 5-10 times with a 1000 µl pipette to mix the liquid. Afterward, we transferred 800 µl of this liquid to an II-CR spin column with a collection tube and centrifuge for 1 minute, then discarded the liquid in the collection tube and repeat this process for the remaining mixture. This isolated the DNA on the spin column.

We pipetted 400 µl DNA wash buffer 1 (dilute) into the spin column and centrifuged for 1 minute, then discarded the collected liquid. We repeated the above process with 700 µl DNA wash buffer 1 then 200 µl DNA wash buffer 2 (concentrated). For the next step, we transferred the spin column into a microcentrifuge tube (labeled "F"), pipetted in 100 µl nuclease-free water, and centrifuged for 1 minute to elute DNA into the tube. To remove final contaminants, we prepared an III-HRC filter with a and added another microcentrifuge tube (labeled "FF") to the bottom of the HRC. We then pipetted the liquid from tube F into the HRC and centrifuged for 3 minutes to isolate DNA into tube FF. We prepared another microcentrifuge tube (labeled "1/100"), pipetted in 198 µl nuclease-free water, then pipetted in 2 µl DNA from tube FF to create a 1/100 DNA dilution in tube 1/100. Then, we prepared 20 µl PCR tubes:

Tubes	**1**	**2**	**3**	**4**	**5**	**6**	**7**
5x PCR mix	5 µl for each tube						
18 µl primer	*16S*	*TETB*	*TETM*	*TETB*	*TETM*	*TETB*	*TETM*
2 µl DNA	1/100 sample			Nuclease free water		Positive control	

We entered the following programming for 38 cycles of PCR:

Stage	**Initial denaturation**	**Denaturation**	**Annealing**	**Extension**	**Final extension**
Temperature (°C)	94	94	61	72	72
Time (s)	120	20	20	40	60

We then casted agarose gels by combining 1 agarose tablet with 25 mL 1x TBE buffer in an Erlenmeyer flask We poured the heated mixture into a casting tray to create gels Afterwards, we loaded samples for 25 minutes of gel electrophoresis:

Lane	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Sample (µl)	10 µl DNA ladder	15 µl tube 1	15 µl tube 2	15 µl tube 3	15 µl tube 4	15 µl tube 5	15 µl tube 6	15 µl tube 7

Study Data Analysis

We observed all gels for *16S* and positive control DNA bands and ensured their bps were accurate relative to the present DNA ladder (FIGURE 1). The one band that appeared in *TETB/TETM* lanes must have had an accurate relative size to suggest the presence of resistance; additionally, negative control bands must be absent. Primer dimers and nonspecific bands from PCR may occur but didn't invalidate results.

A linear calibration curve [distance traveled (mm) vs the $\log_{10}$ of the base pairs (bp)] was plotted to verify the presence of each *TETB/TETM* band. To create the calibration curve, the bp and $\log_{10}$bp for each band in the DNA ladder were recorded in descending order. Then, the amount each DNA ladder band traveled was determined. We measured the distance between the well's center and the band's center. The distance each *TETB/TETM* band traveled was measured, converted into $\log_{10}$bp using the unique calibration curve created for each gel, then converted into bp using the antilog. Each chart received error bars for reference, a trendline, and an R^2 value. A percent error calculation was used to measure the deviation between the observed and expected bp of a band.

A densitometric analysis using ImageJ (Windows-64) software was used to determine each recorded band's density after verifying its presence. In *TETB/TETM* bands, the density of a band correlated with the amount of DNA/resistance present. Images of each agarose gel were converted into 8-bit. The subtraction tool was used to eliminate the background noise on the image of the gel.

The invert tool was then used to change the color of the bands from white-colored to black-colored, to make them more the more visible. Next, the "plot lanes" tool was used to create a wave-like plot that represented the selected bands. The line tool was used connect the first bottom corners of each wave. Each completed wave represented a band on the gel. The wand tool was used to record the area of each complete wave. Then, the "label peaks" tool was used to record the percent area intensity of each complete wave. To generate normalized values, the percent area intensity of the *TETB/TETM* band was divided by the percent area intensity of the corresponding positive control band. The greater the normalized value, the denser the band and the greater the amount of resistance.

The normalized values for all *TETB/TETM* bands were graphed to identify trends in the amount of resistance across positive locations. To identify trends, we looked for commonalities in the recorded characteristics of positive locations and searched through any prior literature to determine if related studies could affirm or provide new explanations for trends we observed.

To determine the relative efficacy of the workflow, the above process was used for all non-*TETB/TETM* bands (except DNA ladder bands) to record their areas. Additionally, the standard deviation (SD) between the areas of each type of band was calculated. The SD and CV of the percent errors recorded when verifying the presence of *TETB/TETM* bands were also calculated. This helped us determine the efficacy of the workflow in producing bands of correct bps.

SUPPLEMENT 2: Study Detailed Materials

General Laboratory Materials

- Beaker, Erlenmeyer flask, graduated cylinder
- Deionized water and bottle
- Gloves and safety goggles
- Lab tray with tray covers
- Metal spatula, sensitive balance, weight boats
- Micropipettes and tips
- Nuclease-free water
- Sharpie
- Shovel and Ziplock bags

DNA Extraction Materials

- Soil sample
- Lysis tube with lysis beads
- Lysis solution
- Vortexer

DNA Processing Materials

- Spin filter
- Spin column
- HRC filter
- DNA binding buffer
- 95% ethanol
- DNA wash buffers
- HRC preparation solution
- Centrifuge

PCR Operation Materials

- PCR tubes
- 5x PCR mix with Taq pol, nucleotides
- *16S*, *TETB*, *TETM* primers
- 1/100 DNA
- Positive control DNA
- PCR machine

Gel Electrophoresis Materials

- Agarose tables
- 1x TBE buffer
- DNA ladder
- Casting tray
- Electrophoresis machine