ABSTRACT: Antimicrobials used in poultry production have the potential to bioaccumulate in poultry feathers but available data are scarce. Following poultry slaughter, feathers are converted by rendering into feather meal and sold as fertilizer and animal feed, thereby providing a potential pathway for reentry of drugs into the human food supply. We analyzed feather meal \( (n = 12) \) samples for 59 pharmaceuticals and personal care products (PPCPs) using EPA method 1694 employing liquid chromatography tandem mass spectrometry (LC/MS/MS). All samples tested positive and six classes of antimicrobials were detected, with a range of two to ten antimicrobials per sample. Caffeine and acetaminophen were detected in 10 of 12 samples. A number of PPCPs were determined to be heat labile during laboratory simulation of the rendering process. Growth of wild-type \( \text{E. coli} \) in MacConkey agar was inhibited by sterilized feather meal \( (p = 0.01) \) and by the antimicrobial enrofloxacin \( (p < 0.0001) \) at levels found in feather meal. Growth of a drug-resistant \( \text{E. coli} \) strain was not inhibited by sterilized feather meal or enrofloxacin. This is the first study to detect antimicrobial residues in feather meal. Initial results suggest that more studies are needed to better understand potential risks posed to consumers by drug residues in feather meal.

### INTRODUCTION

Feather meal is a byproduct made from poultry feathers (part a of Figure 1). The U.S. poultry industry raises nearly 9 billion broiler chickens and 80 million turkeys each year,\(^1\) to support a per capita consumption rate of 45 kg (100 lbs, retail weight) of poultry meat. The per capita consumption of poultry meat is greater than that of any other animal- or vegetable-derived protein source in the U.S.\(^2\) For every 3-kg (6.6 lb) chicken produced, over 1 kg (or 33% of the total mass) is inedible and has no human food market;\(^3\) as a result, the byproduct from poultry slaughtering – heads, bones, viscera, and feathers – amount to billions of kilograms of inedible chicken parts and 2 billion kg of feathers.\(^4,5\)

The rendering industry processes feathers into feather meal by methods previously reported by the authors.\(^6\) Feather meal is sold as a fertilizer,\(^7\) a raw material in biodiesel,\(^8\) an ingredient in bioplastics\(^4\) and an animal feed ingredient. As a feed ingredient, feather meal can be added to poultry,\(^9\) pig,\(^10\) ruminant,\(^11\) and fish feeds.\(^12,13\) In animal feeding studies, feather meal is assessed for palatability, nutrient content, and animal weight gain. Contaminants such as antimicrobials have only recently been shown to accumulate in raw poultry feathers,\(^14,15\) and tests of antimicrobials in feather meal products have not been described in the literature.

In the U.S., antimicrobials are introduced into the feed and water of industrially raised poultry, primarily for growth promotion. In 2010, 13.2 million kg of antimicrobials were sold to the U.S. poultry and livestock industries, which represents ~80% of all antimicrobial sales for use in humans and animals in the U.S.\(^16,17\) Whereas the types and quantities of drugs directly administered to poultry and other food animals are not reported by the U.S. Food and Drug Administration (FDA), it is generally accepted that the FDA-reported sales data for antimicrobials are a reasonable surrogate for antimicrobial consumption by food animals.\(^3\)
The present study expands upon a new finding of antimicrobial residues in chicken feathers by analyzing commercially available feather meal products for a suite of 59 pharmaceuticals and personal care products (PPCPs). Subsequently, we heated artificially spiked feather meal in an autoclave to understand the drug degradation process that may occur when feathers are converted into feather meal during rendering. We also examined the relevance of our findings to microbial ecology by exposing several E. coli strains to autoclave-sterilized feather meal and to levels of antimicrobials detected in feather meal.

**METHODS**

**Sample Collection.** Seven feather meal products labeled as fertilizer were purchased either online or in person from vendors in Arkansas (n = 2), North Carolina (n = 1), and Oregon (n = 4) in 0.5 to 22 kg bags. Five feather meal products labeled as animal feed ingredients were acquired from rendering plants, distributors or animal feed mills from California (n = 1), China (n = 2), Idaho (n = 1), and Tennessee (n = 1). Samples were collected in approximately 200 g amounts in plastic bags (Whirl-Pac, Nasco, Fort Atkinson, WI) and stored at room temperature (25 °C) in the dark.

**Detection of Pharmaceuticals and Personal Care Products in Feather Meal by Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS).** A modified version of EPA method 1694 for tissue was performed using acid extractions for group I and II analytes on 2.5 g feather meal samples. Extraction methods are described in detail in the Supporting Information. EPA 1694 modified for tissue was selected after comparison with EPA method 1694 for biosolids; the results of the methods comparison are presented in Table S1 of the Supporting Information.

Sample extracts were analyzed by LC/MS/MS (2795 HPLC and Micromass AutoSpec Quattro Ultima MS/MS, Waters, Milford, MA) run in Multiple Reaction Monitoring mode for 59 analytes including antimicrobials, fungicides, analgesics, stimulants, steroids, and other PPCPs. Samples were analyzed by positive electrospray ionization mode. Quantification was performed by recording the peak areas of the applicable parent ion/daughter ion transitions and utilizing isotope dilution when stable-isotope-labeled standards were available. Positive detection was considered to occur when the signal-to-noise ratio (S/N) of parent ion to daughter ion transition was ≥3:1, and when compound retention time fell within 0.4 min of the predicted retention times from the mean determined from the initial calibration. PPCP analysis was performed by AXYS Analytical Services Ltd., in Sidney, British Columbia, Canada, which is the laboratory that developed EPA Method 1694. All experiments were designed and conducted with quality assurance and quality controls, including methods blanks and spiked matrix samples to measure percentage recovery, as described in detail previously.

**PPCP Degradation in Simulated Rendering Process.** The potential degradation of antimicrobials by heat was determined in an autoclave similar to processes used by the rendering industry. Initially, a blank of 2.5 mL reagent water was autoclaved in a sterile polypropylene tube and analyzed by EPA method 1694 for group I and II analytes in order to confirm that the autoclave did not cross-contaminate samples. Next, six composite feather meal samples in sterile polypropylene tubes were spiked with 59 authentic compounds from EPA method 1694 at concentrations 1000 times greater (for group I) or 10 000 times greater (for group II) than their native levels in the feather meal. The rationale for spiking with above-native levels was to observe as much as a 3-log reduction in individual PPCPs; if spiking had occurred at more native/realistic levels (i.e., 1–50 ppb) it would have been challenging to determine the extent of degradation of PPCPs in the spiked samples due to the nearness of spikes to the practical quantitation limit of the method. Three of the replicates were stored at −20 °C as controls, and three replicates were autoclaved at 121 °C and 15 psi for 30 min, cooled to room temperature on wet ice, and stored at −20 °C until analysis by EPA method 1694. Statistical comparisons of antimicrobial concentrations were made between autoclaved and non-autoclaved sample populations with a t-test and a significance level (α) of 0.05.

![Figure 1](dx.doi.org/10.1021/es203970e1Environ. Sci. Technol. XXXX, XXX, XXX--XXX)
In Vitro Bacteriostatic/Bactericidal Assay. To evaluate the potential for feather meal antimicrobial concentrations to exert selective pressure, we tested the ability of a susceptible E. coli strain (ATCC 25922) and a strain of E. coli, SMS-3–5 (ATCC BAA-1743) resistant to numerous classes of antimicrobials (i.e., fluoroquinolones, sulfonamides, tetracyclines, macrolides, beta-lactams (penicillins and cephalosporins), aminoglycosides, and phenicols),21 to grow in the presence of four antimicrobials (chlortetracycline, erythromycin, enrofloxacin, and sulfadimethoxine) on MacConkey agar plates. In addition, autoclave-sterilized feather meal was mixed into MacConkey agar to determine whether feather meal itself potentially exerts selective pressure on E. coli. E. coli strain SMS-3–5 was selected because, as an environmental strain isolated from soil, it is relevant to the selection for antimicrobial resistance in the soil microbiome and therefore to microbial ecology of soils and other agricultural environments amended or contaminated with feather meal products.22 The four antimicrobials were selected based on high detection frequency and/or high concentrations found in the feather meal products tested, and antimicrobial challenge levels were at the maximum and/or minimum concentrations detected in feather meal. Chlortetracycline, an FDA-approved poultry drug, was not detected in feather meal, but its metabolite isochlortetracycline23 was detected, and chlortetracycline challenge levels were based on levels of isochlortetracycline. Mixtures of four antimicrobials, at maximum levels detected in feather meal, were also tested in E. coli challenge experiments. Detailed methods for challenging E. coli strains with antimicrobials are presented in the Supporting Information.

RESULTS AND DISCUSSION

Method Performance. The sensitivity of EPA method 1694 varied by analyte and by sample; typically, the method detection limit (MDL) ranged from 0.2 to 10 ng/g, with only norfloxacin showing an atypically high MDL of up to 71 ng/g (Table 1). Recoveries determined for matrix spiked samples varied by analyte and ranged from 74% to 110% with several outliers (23% norgestomet, 144% azithromycin, 163% 1,7-dimethylxanthine; Table 1). Precision was measured in duplicate composite feather meal samples (Table S1 of the Supporting Information), which was relatively high given the heterogeneous nature of the sample. No evidence of laboratory contamination was found in method blanks.

Degradation of PPCPs by Heat in a Simulated Rendering Process. Autoclaving at 121 °C for 30 min caused significant degradation of most antimicrobials (14 of 17) and other PPCPs (5 of 7) spiked into feather meal, as compared to nonautoclaved spiked samples, as measured by LC-MS/MS (parts a and b of Figure 2). The thermal stability of antimicrobials varied widely within drug class for sulfonamides and tetracyclines, suggesting that drug class is not necessarily a good predictor of thermal stability. Antimicrobials that did not degrade during autoclaving were sulfamethazine, sulfamethoxazole, and isochlortetracycline. Stimulants (caffeine, 1,7-dimethylxanthine) also did not degrade after autoclaving. Many of the PPCPs resistant to thermal degradation in the autoclave experiments were indeed prevalent in feather meal samples.

One of the main functions of the rendering process is the sterilization of animal byproduct to inactivate foodborne pathogens and spoilage microorganisms,44 although as we and others have shown, antimicrobial residues in feather meal and porcine (pig) meal persist.25 After simulated rendering of porcine meal spiked with antimicrobials, lincomycin, flumequine, enrofloxacin, neomycin, tylosin, and sulfamethazine retained antimicrobial activity against bacteria, whereas penicillin, amoxicillin, ampicillin, cloxacillin, oxytetracycline, sulfamethoxazole lost antimicrobial activity.25 When thermal treatment is efficacious, both an antimicrobial’s physical properties and its antimicrobial activity are modified.26

Thermal degradation of PPCPs that were not detected in feather meal samples, but were used in the simulated rendering experiments, are presented in Figure S1 of the Supporting Information. Understanding heat stability of PPCPs helps, in part, to explain their detection frequency in feather meal samples. It is important to note, however, that lack of detection of drugs in feather meal does not imply absence of exposure and bioaccumulation in poultry and feathers, respectively. No experiments were performed with fresh feathers, which is a potential limitation in understanding PPCP degradation during the conversion of fresh feathers into feather meal.

Antimicrobials in Feather Meal. To our knowledge, this study is the first to detect antimicrobial residues in commercially available feather meal. All feather meal samples (n = 12) contained antimicrobials. Between 2 and 10 antimicrobials (median = 4) were detected in each sample (Table 1). Samples from China had the greatest number of antimicrobials detected (10 antimicrobial drugs for each of two samples). For the 46 antimicrobials that were tested, more than one-third (n = 17) were detected in samples. Six drug classes were detected in samples; their detection frequencies from greatest to least were: sulfonamides (83%) > macrolides (75%) > fluoroquinolones (67%) > tetracyclines (58%) > follic acid antagonists (33%) > streptogramins (17%). Most, but not all, of the antimicrobials detected in samples are approved for use in industrial poultry production (Table S4 of the Supporting Information).27 For example, chlortetracycline, an FDA approved poultry drug, was not detected in feather meal, but its metabolite isochlortetracycline23 was detected in 6 of 12 samples.

Fluoroquinolones (enrofloxacin, norfloxacin, or ofloxacin) were detected in 6 of 10 U.S. samples, which was not expected because fluoroquinolone use in U.S. poultry production has been banned since 2005.28 These findings may suggest that the ban is not being adequately enforced or that other pathways, for example, through use of commodity feed products from livestock industries not covered by the ban, may inadvertently contaminate poultry feed with fluoroquinolones. Furthermore, if feather meal with fluoroquinolone residues is fed back to poultry, this practice could create a cycle of re-exposure to the banned drugs. Unintended antimicrobial contamination of poultry feed may help explain why rates of fluoroquinolone-resistant Campylobacter isolates continue to persist in poultry and commercial poultry meat products half a decade after the ban.29,30 To better interpret our findings, corroborating evidence in the form of antimicrobial usage practices and dosing amounts would be needed. These data, however, are not currently available to the public.31

Poultry feeding studies have characterized tissue deposition rates for a few antimicrobials, mainly to develop antibiotic withdrawal periods for edible meat. Enrofloxacin and flumequine, two antimicrobials in the fluoroquinolone class, accumulate in higher concentrations and persist longer in chicken feathers than in muscle.4,11,13 Enrofloxacin concentrations in the present study were 2.2 to 1050 ng/g, which is similar to enrofloxacin concentrations in feathers after poultry
### Table 1. Concentrations of Pharmaceuticals and Personal Care Products Detected in Feather Meal

<table>
<thead>
<tr>
<th>Drug class</th>
<th>Analyte</th>
<th># positive samples (% total)</th>
<th>United States</th>
<th>China</th>
<th>range of ratio of DV to MDL</th>
<th>matrix spike (%) recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>[ANTIMICROBIALS]</strong></td>
<td></td>
<td></td>
<td>Arkansas</td>
<td>Oregon</td>
<td>Nevada</td>
<td>Oregon</td>
</tr>
<tr>
<td><strong>[fluoroquinolone]</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ciprofloxacin</td>
<td>2 (17%)</td>
<td>&lt;9.4</td>
<td>&lt;16.7</td>
<td>&lt;18.3</td>
<td>&lt;32.6</td>
</tr>
<tr>
<td></td>
<td>enrofloxacin</td>
<td>4 (33%)</td>
<td>&lt;1.2</td>
<td>&lt;1.4</td>
<td>&lt;1.2</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>norfloxacin</td>
<td>3 (25%)</td>
<td>&lt;19.0</td>
<td>&lt;52.8</td>
<td>&lt;20.4</td>
<td>&lt;70.7</td>
</tr>
<tr>
<td></td>
<td>ofloxacin</td>
<td>5 (42%)</td>
<td>0.6</td>
<td>1.2</td>
<td>0.9</td>
<td>1.2</td>
</tr>
<tr>
<td><strong>[macrolide]</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>azithromycin</td>
<td>2 (17%)</td>
<td>&lt;0.6</td>
<td>&lt;0.6</td>
<td>&lt;0.6</td>
<td>&lt;0.6</td>
</tr>
<tr>
<td></td>
<td>erythromycin</td>
<td>7 (58%)</td>
<td>0.2</td>
<td>0.2</td>
<td>1.4</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td><strong>[sulfonamide]</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>norfloxacin</td>
<td>3 (25%)</td>
<td>&lt;0.6</td>
<td>&lt;0.6</td>
<td>&lt;0.6</td>
<td>&lt;0.6</td>
</tr>
<tr>
<td></td>
<td>ofloxacin</td>
<td>5 (42%)</td>
<td>0.6</td>
<td>1.8</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td><strong>[streptogramin]</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>virginiamycin</td>
<td>2 (17%)</td>
<td>&lt;3.8</td>
<td>&lt;7.8</td>
<td>&lt;6.2</td>
<td>&lt;4.6</td>
</tr>
<tr>
<td><strong>[tetracycline]</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>oxytetracycline</td>
<td>1 (10%)</td>
<td>&lt;0.6</td>
<td>&lt;0.1</td>
<td>&lt;0.5</td>
<td>&lt;0.6</td>
</tr>
<tr>
<td></td>
<td>thiabendazole</td>
<td>2 (17%)</td>
<td>&lt;0.6</td>
<td>&lt;0.6</td>
<td>&lt;0.6</td>
<td>&lt;0.6</td>
</tr>
<tr>
<td></td>
<td>thiabendazole</td>
<td>2 (17%)</td>
<td>&lt;0.6</td>
<td>&lt;0.6</td>
<td>&lt;0.6</td>
<td>&lt;0.6</td>
</tr>
<tr>
<td></td>
<td>[fungicide]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 1. continued

<table>
<thead>
<tr>
<th>Drug class</th>
<th>Analyte</th>
<th>United States</th>
<th>China</th>
<th>range of ratio of DV to MDL</th>
<th>matrix spike (% recovery)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Arkansas</td>
<td>Arkansas</td>
<td>Oregon</td>
<td>Oregon</td>
</tr>
<tr>
<td>analgesic</td>
<td>acetaminophen</td>
<td>&lt;6.0</td>
<td>&lt;6.1</td>
<td>18.7</td>
<td>40.4</td>
</tr>
<tr>
<td>sex hormone</td>
<td>norgestimate</td>
<td>&lt;1.2</td>
<td>&lt;1.2</td>
<td>&lt;1.2</td>
<td>&lt;1.2</td>
</tr>
<tr>
<td>stimulant</td>
<td>caffeine</td>
<td>&lt;6.0</td>
<td>&lt;6.1</td>
<td>55.8</td>
<td>13.9</td>
</tr>
<tr>
<td></td>
<td>1,7-dimethylxanthine</td>
<td>&lt;24.0</td>
<td>&lt;24.4</td>
<td>90.8</td>
<td>&lt;23.7</td>
</tr>
</tbody>
</table>

*a* Amount analyzed: 2.46–2.53 g. *b* Percent moisture: 4.5–8.2%. *c* Ormetoprim is in the folic acid antagonist drug class, but is routinely prescribed in combination with sulfadimethoxine. *d* Positive samples in bold. *DV* = detection value; MDL = method detection limit. *f* Data not available due to sample inhibition.

Figure 2. Degradation of pharmaceuticals and personal care products in a simulated rendering process. Statistical comparisons of antimicrobial concentrations were made between autoclaved and non-autoclaved sample populations with a t-test.
norgestimate, a sex hormone, were detected in four and two U.S. samples respectively. Both fluoxetine and diphenhydramine have been described in the veterinary literature for use in companion avian species to reduce anxiety. In addition, diphenhydramine has been described for clinical gastrointestinal indications in broiler poultry. Anecdotal evidence from farmers suggesting use of caffeine, acetaminophen, and diphenhydramine in poultry production is provided in the Supporting Information (Box S1).

Four PPCPs were found at levels near the MDL only; these detections, which may constitute true positives or false detections from signal interferences, are reported in Table S2 of the Supporting Information. PPCPs and antimicrobials not detected in any sample were: carbadox, carbamazepine, clarithromycin, clinafloxacin, cloc8, diltiazem, flumequine, lomefloxacin, miconazole, oxacillin, oxolinic acid, penicillin G, penicillin V, roxithromycin, sarafloxacin, sulfadiazine, sulfanilamide, sulfathiazole, trimethoprim, tylosin, and 11 drugs in the tetracycline class (anhydrochlorotetracycline, anhydrotetracycline, chlortetracycline, demeclocycline, doxycycline, 4-epianhydrotetracycline, 4-epianhydrochlorotetracycline, 4-epianhydrotetracycline, 4-epoxytetracycline, 4-epitetracycline, minocycline, tetracycline).

**In Vitro Bacteriostatic/Bacteriocidal Assay.** To characterize the biological significance of finding antimicrobials in feather meal, we performed several in vitro experiments with E. coli. Initially, we exposed E. coli to agar plates containing 1 g of feather meal to assess the bioavailability of the drugs in the feather meal matrix. This approach was unsuccessful because the background levels of organisms in feather meal grew on plates and thus precluded accurate counts. The isolation and characterization of these organisms from feather meal would be worthwhile for future research, although it was beyond the scope of the current study. Instead, one gram of autoclaved feather meal from China was used. Growth of a susceptible E. coli strain, ATCC 25922, was inhibited ($p = 0.01$) by an unidentified constituent of feather meal, whereas growth of a resistant E. coli strain, SMS-3−5, was not inhibited ($p = 0.2$) (Table S5 of the Supporting Information). These initial results suggest, but cannot prove, that the inhibiting substance may be an antibiotic/bacteriostatic. Autoclaving may have attenuated the quantity and bioavailability of antimicrobial drugs originally present.

In additional experiments, we exposed susceptible and resistant E. coli strains to agar plates containing four antimicrobials (chlortetracycline, enrofloxacin, erythromycin, and sulfadimethoxine) at the maximum levels detected in feather meal (Table 2). The resistant E. coli strain, SMS-3−5, grew in the presence of individual antimicrobials and in the presence of mixtures of all four antimicrobials (Table S3 of the Supporting Information).

Growth of a susceptible strain of E. coli was inhibited by 1.1 μg/mL enrofloxacin, regardless of inoculation density ($p < 0.0001$) and by mixture of all four antimicrobials at their maximum concentrations detected in feather meal ($p = 0.009$) (Table S3 of the Supporting Information), presumably due to the effects of enrofloxacin. Other antimicrobials individually applied in the maximum concentrations detected did not inhibit the growth of the susceptible E. coli strain. In other in vitro studies, the MIC$_{90}$ or minimum inhibitory concentration to inhibit 50% of the organisms, for strains of E. coli at 10$^5$ CFU inocula was 0.016 μg/mL for enrofloxacin. Further, the minimum concentration of enrofloxacin ($\sim 0.002$ μg/mL), representing a concentration found in a U.S. feather meal product, partially inhibited growth of the susceptible E. coli. Because neither the maximum nor the minimum detected concentrations of enrofloxacin inhibited the resistant environmental E. coli, these findings suggest enrofloxacin residues may be selective for resistant strains.

**U.S. Domestic and Imported Feather Meal.** Feather meal is a global commodity product used in terrestrial and aquatic animal feeds and fertilizer. Most of the feather meal produced in the U.S. is used domestically, which is more than 0.5 billion kg/yr (part b of Figure 1), whereas the remainder is exported to Indonesia, Canada, Vietnam, Ecuador, Honduras, Taiwan, and Thailand. The U.S. also imported feather meal; between September 2010 and September 2011, Chinese imports accounted for a quarter of the feather meal shipments received in the U.S. Imports of feather meal produced outside the U.S. may introduce residues from drugs otherwise banned or restricted in U.S. poultry production. Feather meal itself is not directly tested as part of the U.S. Department of Agriculture’s Food Safety Inspection Service and National Residue Program, but residues in feather meal may result from the same antimicrobial dosing that leads to residues found in other tissues.

We have previously described risks related to administration of medicated feed to food animals, which may promote selection for antimicrobial resistance. The presence of antimicrobials in feather meal, as determined in this study, is a previously unrecognized source of these drugs in animal feed. Because this is the first study of PPCPs in feather meal, we invite independent verification of our results by others. More work is needed to determine whether the detected levels of PPCPs in feather meal have an impact on the quality of food animal products and the safety of consumers.

### Table 2. Growth of Susceptible (ATCC 25922) and Resistant (SMS-3-5) E. coli on Agar Plates Containing Antimicrobials

<table>
<thead>
<tr>
<th>Antimicrobial treatment</th>
<th>E. coli CFU counts; $n = 3$ replicates (standard error)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ATTCC 25922 susceptible</td>
</tr>
<tr>
<td>negative control</td>
<td>$&gt;1000^a$</td>
</tr>
<tr>
<td>chlortetracycline</td>
<td>$&gt;1000$</td>
</tr>
<tr>
<td>erythromycin</td>
<td>$&gt;1000$</td>
</tr>
<tr>
<td>sulfadimethoxine</td>
<td>$&gt;1000$</td>
</tr>
<tr>
<td>enrofloxacin (0.02 μg/g)</td>
<td>$0 \ (0)^b$</td>
</tr>
<tr>
<td>enrofloxacin (1.1 μg/g)</td>
<td>$&gt;1000$</td>
</tr>
</tbody>
</table>

$^a$Standard errors are not reported where high counts ($>100$ or higher) were estimated based on visual inspection. $^b$Significant differences ($p < 0.05$, paired $t$-test) between control and antimicrobial-containing plates.

### ASSOCIATED CONTENT

#### Supporting Information

Detailed assay methods, EPA methods, methods for comparison of detection of pharmaceuticals, and so forth. This material is available free of charge via the Internet at http://pubs.acs.org.
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Notes
The authors declare no competing financial interest.

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