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Antibiotic Resistance Genes as Emerging Contaminants: Studies in Northern Colorado†

AMY PRUDEN,* RUOTING PEI, HEATHER STORTEBOOM, AND KENNETH H. CARLSON

Department of Civil and Environmental Engineering, Colorado State University, Fort Collins, Colorado 80523

This study explores antibiotic resistance genes (ARGs) as emerging environmental contaminants. The purpose of this study was to investigate the occurrence of ARGs in various environmental compartments in northern Colorado, including Cache La Poudre (Poudre) River sediments, irrigation ditches, dairy lagoons, and the effluents of wastewater recycling and drinking water treatment plants. Additionally, ARG concentrations in the Poudre River sediments were analyzed at three time points at five sites with varying levels of urban/agricultural impact and compared with two previously published time points. It was expected that ARG concentrations would be significantly higher in environments directly impacted by urban/agricultural activity than in pristine and lesser-impacted environments. Polymerase chain reaction (PCR) detection assays were applied to detect the presence/absence of several tetracycline and sulfonamide ARGs. Quantitative real-time PCR was used to further quantify two tetracycline ARGs (tet(W) and tet(O)) and two sulfonamide ARGs (sul(I) and sul(II)). The following trend was observed with respect to ARG concentrations (normalized to eubacterial 16S rRNA genes): dairy lagoon water > irrigation ditch water > urban/agriculturally impacted river sediments ($p < 0.0001$), except for sul(II), which was absent in ditch water. It was noted that tet(W) and tet(O) were also present in treated drinking water and recycled wastewater, suggesting that these are potential pathways for the spread of ARGs to and from humans. On the basis of this study, there is a need for environmental scientists and engineers to help address the issue of the spread of ARGs in the environment.

Introduction

The spread of antibiotic-resistant pathogens is a growing problem in the U. S. and around the world. Recently a 2000 World Health Organization (WHO) report (*1*) focused on antibiotic resistance as one of the most critical human health challenges of the next century and heralded the need for "a global strategy to contain resistance". According to the report, more than two million Americans are infected each year with resistant pathogens and 14 000 die as a result. The rapid growth of the problem emphasizes the need for intervention. For example, vancomycin is currently considered to be the most powerful antibiotic of "last resort", yet within 10 years the incidence of vancomycin-resistant enterococci (VRE) increased in the United States from 0% to 25% (*2*, *3*). Resistance to penicillin, the antibiotic that originally revolutionized human health 50 years ago, is now as high as 79% in *Staphylococcus pneumoniae* isolates in South Africa (*4*, *5*). Alarmingly, diseases that were once considered to be eradicated, such as tuberculosis, are now beginning to make a comeback because of antimicrobial resistance (*1*, *6*, *7*). As with other dangerous pollutants that spread in the environment and threaten human health, there is a need for environmental scientists and engineers to help address the critical problem of microbial resistance to antibiotics.

The rise of antibiotic resistance is considered to be closely linked with the widespread use of antibiotic pharmaceuticals in humans and animals. In particular, more than one-half of the antibiotics used in the U. S. are administered to livestock for purposes of growth promotion or infection treatment (*8*, *9*). In both animals and humans, up to 95% of antibiotics can be excreted in an unaltered state (*10*, *11*). Some removal has been observed in wastewater treatment plants (WWTPs); however, as is true with the larger problem of pharmaceutical compounds, WWTPs are not designed for the removal of micropollutants (*12*−*14*). Residual antibiotics thus are released into the environment where they may exert selection pressure on microorganisms. While overprescribing or other improper use/disposal of antibiotics in humans is generally considered to contribute to the problem, several studies have also linked agricultural antibiotic use with antibiotic-resistant infections in humans (*15*−*23*). For example, avoparcin, an antibiotic growth-promoter used in poultry, was recently banned in Europe because of its association with the development of vancomycin-resistant enterococci (*24*).

Because of the direct selection pressure that antibiotics exert on organisms carrying antibiotic resistance genes (ARGs), the transport pathways of antibiotic-resistant microorganisms and the ARGs that they carry are expected to be similar to the pathways of antibiotic pharmaceuticals. In fact, it is likely that ARGs persist further in the pathway, considering that in many cases they are maintained in the microbial populations even after the antibiotic selection pressure has been removed (*25*−*28*). Also, horizontal gene transfer (HGT) is a major mechanism for sharing ARGs between microbes and has been documented to occur between nonpathogens, pathogens, and even distantly related organisms, such as Gram-positive and Gram-negative bacteria (*25*, *29*−*31*). In many cases, ARGs have been discovered to occur as part of multiple antibiotic resistant (MAR) superintegrons, which may contain over 100 ARG cassettes (*32*). These MAR superintegrons cause multiple-drug resistance in organisms, meaning that even when very different antibiotics are used, one antibiotic may coselect for resistance to other antibiotics (*5*, *33*). MAR gene cassettes and ARGs are notorious for being associated with plasmids and/or transposons that facilitate HGT. Finally, even if cells carrying ARGs have been killed, DNA released to the environment has been observed to persist, to be protected from DNAse, especially by certain soil/clay compositions, and to be eventually transformed into other cells (*34*−*36*). For all of these reasons, ARGs in and of themselves can be considered to be emerging "contaminants" for which mitigation strategies are needed to prevent their widespread dissemination.

The purpose of this study was to document the occurrence of tetracycline and sulfonamide ARGs in various environmental compartments in northern Colorado. These two ARG

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* Corresponding author phone: (970)491-8814; fax: (970)491-8671; e-mail: apruden@engr.colostate.edu.

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groups were chosen because sulfonamide and tetracycline antibiotics have been previously characterized in Poudre River sediments and shown to relate to urban/agricultural activity (*37*). The breadth of the study included Cache La Poudre (Poudre) River sediments, dairy lagoon water, irrigation ditch water, a wastewater recycling plant (WRP), and two drinking water treatment plants (DWTPs). The hypothesis was that environmental compartments most directly impacted by urban/agricultural activity would have significantly higher concentrations of ARGs than less impacted and pristine environments. Irrigation ditch waters, which were directly adjacent to farms, were investigated as a potential pathway of ARGs from farms to the Poudre River, while the WRP and the DWTPs were explored as potential routes of human environmental input and consumption. The presence/absence of several ribosomal protection factor tetracycline ARGs and folic acid pathway sulfonamide ARGs was determined using a polymerase chain reaction (PCR) detection assay, and four commonly occurring ARGs were further quantified by quantitative real-time PCR (Q-PCR). Documenting the baseline occurrence of ARGs in a cross-section of environmental compartments will take a step toward understanding and modeling the fate and transport phenomena associated with these emerging contaminants.

Experimental Section

Poudre River Sediment Sampling. Because of its pristine origins and zonation corresponding to land use, the Poudre River has served as a good model for relating human and agricultural activities with the occurrence of antibiotic pharmaceuticals (*37*) and ARGs (*38*). Five sampling sites were the focus of this study, numbered sequentially in the direction of flow from west to east, with the following characteristics: site 1, pristine location at the river origin in the Rocky Mountains; site 2, light-agriculture-influenced area; site 3, urban-influenced area at the outlet of the Fort Collins Drake WWTP; site 4, heavy-agriculture-influenced area between Fort Collins and Greeley; and site 5, heavy-agriculture- and urban-influenced area just east of Greeley, which is a major center for the meat-packing industry. Over 90 confined animal feeding operations (CAFOs), dairies, and ranches are located between sites 3 and 5. Further attributes of the Poudre River watershed that contribute to its suitability for investigating the impacts of urban and agricultural activity on antibiotics and ARGs have been described previously (*37*, *38*).

Sediment samples were collected along the Poudre River at the five sites on August 18, 2005, October 27, 2005, and February 17, 2006. The flow rates on these three dates were 1.04, 14.19, and 0.14 $m^3\ s^{-1}$, respectively (U. S. Geological Survey station number 06752260, Fort Collins, CO). Sampling at three points in time provided insight into potential temporal variations in ARG concentrations, and the February 17th date is exactly 1 year later than a previously published sampling date (*38*). The upper sediments (about 5 cm) from the middle and two sides of a cross-section at each site were sampled and composited. Samples were collected using a shovel and mixed well in sterilized centrifuge tubes. Fifty-five grams of mixed sample at each site were stored at −80 °C for subsequent molecular analysis.

Bulk Water Sampling. Irrigation ditch waters were investigated as a potential pathway of ARGs from farms to the Poudre River. Grab samples of bulk water were collected in sterile containers from irrigation ditches on August 18, 2005, corresponding to the August sampling date of the Poudre River sediments. All irrigation ditches were located between site 4 and site 5 on the Poudre River within a 3.5 km × 2 km zone north of the river, and a total of ten locations were sampled. To investigate a potential source of ARGs within this zone, a microaerophillic dairy lagoon (~1 mg/L dissolved oxygen in the upper 1 m) and an anaerobic dairy lagoon (0 mg/L dissolved oxygen) from an anonymous farm located 8 km from site 5 were sampled on October 20, 2005. Finally, source water, and pre-chlorinated, and post-chlorinated bulk water were collected from two anonymous DWTPs and an anonymous WRP in northern Colorado in February, 2005. The DWTP was studied as a potential direct route of ARGs to consumers, and the WRP was considered a potential human input into the environment. To collect fine particulates from the dilute ditch water, DWTP, and WRP samples for subsequent analysis, 500 mL of well-mixed sample was filtered using a 0.45 μm glass fiber filter (Whatman). This concentration step was not required for dairy lagoon samples.

DNA Extraction. DNA was extracted from 0.5 g of composited sediment using the FastDNA Spin Kit for Soil (MP Biomedicals) and from 1.8 mL of dairy lagoon water using the Ultraclean Microbial DNA Kit (MoBio Laboratories, Inc.) according to manufacturer protocol. Both approaches employ a bead-beating procedure. For fine particulates collected on filters from bulk water, the filters were cut into small pieces and added directly to the extraction tubes. Extraction yield and the quality of the DNA were verified by agarose gel electrophoresis and spectrophotometry.

Detection and Quantification of ARGs. Polymerase chain reaction detection assays were used for broad-scale screening of the presence/absence of five ribosomal protection factor tetracycline ARGs (tet(BP), tet(O), tet(S), tet(T), and tet(W)) (*39*) and four folic acid pathway sulfonamide ARGs (sul(I), sul(II), sul(III), and sul(A)). Development and validation of sul primers was described in Pei et al. (*38*). Positive controls consisted of cloned and sequenced PCR amplicons obtained from Poudre River sediments. Both positive and negative controls were included in every run, and negative signals were confirmed by spiking positive control template into the sample to verify a signal. Forty cycles were used to improve chances of product formation from low initial template concentrations. Further details on reaction mixes and temperature programs are available in Pei et al. (*38*); note that annealing temperatures for tet primers vary from Aminov et al. (*39*). Two tetracycline ARGs (tet(W) and tet(O)) and two sulfonamide ARGs (sul(I) and sul(II)) that were commonly occurring according to the PCR presence/absence assays were further quantified by Q-PCR using a SybrGreen approach. For further details on Q-PCR methods, see Pei et al. (*38*). Eubacterial 16S rRNA genes were quantified according to the TaqMan Q-PCR method described by Suzuki et al. (*40*) so that ARGs could be normalized to the total bacterial community. This provided a means to correct for potential variations in extraction efficiencies. By quantification of 16S rRNA genes, it was also possible to compare ARGs proportionally between samples of different overall population sizes. Matrix effects associated with extraction of DNA from environmental samples were corrected for by performing spiked matrix control tests and determining template suppression factors as described in Pei et al. (*38*). All Q-PCR analyses were performed using a Cepheid SmartCycler (Sunnyvale, CA).

Statistics. The influences of the environment (sites, ditch water, and dairy lagoons) on the normalized and non-normalized copies of ARGs were analyzed using the Mixed Procedure, which fits a variety of mixed linear models to data. This provides the flexibility of simultaneously modeling means, variances, and covariances (*41*−*44*). Through the use of this test, it was thus possible to comprehensively compare overall differences between different environmental compartments with respect to ARG concentrations. For comparison of the five Poudre River sites, multiple sampling time points were treated as replicates. Mixed Procedures were conducted using SAS 9.0 (SAS Institute Inc., Cary, NC). A

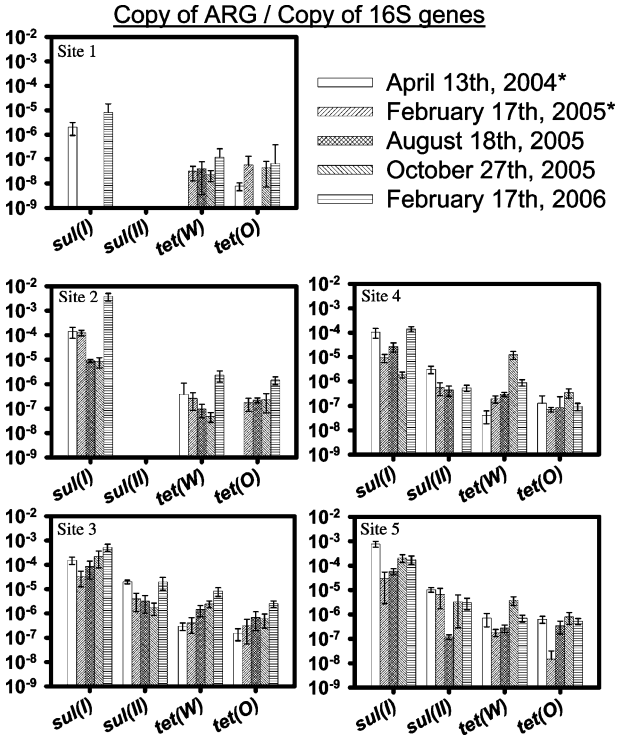

FIGURE 1. Distribution of four ARGs (sul(I), sul(II), tet(O), and tet(W)) in Poudre River sediments on three sampling dates, compared to two previously published sampling dates (April 13, 2004, and February 17, 2005 (*38*)), as determined by Q-PCR: site 1, pristine site; site 2, light agricultural activity; site 3, heavy urban activity; site 4, heavy agricultural activity; site 5, heavy urban and agricultural activity. Error bars represent the standard deviation of six measurements from three independent Q-PCR runs analyzing DNA extract from composite samples.

p-value <0.05 was considered to indicate significance. Averages and standard deviations of all data were determined using Microsoft Excel, 2003.

Results and Discussion

Occurrence of ARGs in Northern Colorado. Figure 1 summarizes the Q-PCR data obtained for the four ARGs at the five Poudre River sites, while Figure 2 summarizes the same analyses for the ditch waters and dairy lagoon water. When August 2005 data for the Poudre River sediments are compared with the dairy lagoon and ditch water, the following trend is observed with respect to ARG concentrations: dairy lagoon water > ditch water > river sediments ($p < 0.0001$), for all ARGs except sul(II), which was absent from the ditch waters. This is based on pooling of all 10 ditch water sites, the two dairy lagoons, and sites 4 and 5, which were directly adjacent to the ditch water sampling locations. Within each of these three pools, there was no statistical difference observed among the samples. Therefore, it was observed as expected that environmental compartments most directly impacted by human/agricultural activity showed higher concentrations of ARGs. This trend is even stronger in considering absolute quantities of ARGs (not normalized to 16S rRNA genes), because the concentration of cells in the dairy lagoon water was orders of magnitude higher than that of the ditch water or the sediments.

In developing a hypothetical pathway for ARGs, a trend is not as clear. The overall trend in terms of ARG concentrations of dairy lagoon water > ditch water > river sediments suggests that on-farm compartments, such as lagoons may be the source of ARGs, which are subsequently attenuated in ditch water before reaching Poudre River sediments.

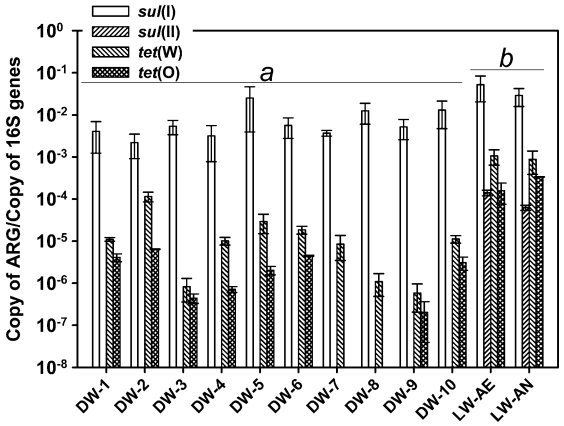

FIGURE 2. Distribution of four ARGs (sul(I), sul(II), tet(O), and tet(W)) at 10 sampling points of irrigation ditch water (DW-1−DW-10) located between site 4 and site 5 compared with that of a microaerophillic dairy lagoon (LW-AE) and an anaerobic dairy lagoon (LW-AN). DW samples were concentrated from 500 mL, and LW samples were extracted directly from 1.8 mL. All samples were normalized to the total 16S rRNA genes. Error bars represent three independent Q-PCR runs in duplicate. The labels a and b indicate that the data sets fell into two statistically different groups, according to the Mixed Procedure.

However, this trend is not supported in terms of sul(II), which is entirely absent from the ditch water and therefore cannot be the source of what is observed in the Poudre River sediments. An alternative source of the sul(II) that appears at sites 4 and 5 could instead be human inputs. This is supported by the data presented in Figure 1, in which it is observed that sul(II) is consistently present at high levels on average at site 3, which is at the point of discharge of the Drake WWTP, while consistently lower (comparing each date sampled) at site 4 (entirely absent for the October event) and equivalent or lower at site 5, which has mixed human/agricultural inputs. Because sul(II) is present in the dairy lagoon waters, it must also have agricultural sources, but it may attenuate too quickly to be transported to the ditches and subsequently to the river sediments. On the basis of this study and a previous study (*38*), it is appears that of the four ARGs quantified sul(II) is the most sensitive indicator of human/agricultural impact, and thus it is suggested that it attenuates quickly in the absence of direct inputs. The other ARGs in the Poudre River sediments at sites 4 and 5 may be of either/both human and agricultural origin, since they followed a decreasing trend from the dairy lagoon through the ditch water but were also present at site 3.

In addition to having higher concentrations of three out of four of the ARGs, the dairy lagoon water was also observed to have more different kinds of ARGs present than the irrigation ditch water according to the PCR assay (Table 1). Together with the Q-PCR results, these data further support the concept that there is some attenuation of ARGs between any linkages that may connect dairy lagoon water and irrigation ditch water. Future work should implement ARG fingerprinting/source tracking to fully characterize the potential pathways.

Temporal Variations of ARG in Poudre River Sediments. As observed in a previous study that compared a high-flow sampling point (6.8 m^3 s^{-1}, April 2004) with a low-flow sampling point (0.6 m^3 s^{-1}, February 2005), the ARG concentrations in the Poudre River sediments are variable with time (*38*). To better understand temporal variations in ARG concentrations, the Poudre River sediments were sampled at three additional time points and compared with the two previously published time points. The February sampling point in this study took place exactly 1 year after

TABLE 1. PCR Presence/Absence Assay of Various ARGs in Ditch (DW)[a] and Dairy Lagoon (LW) Water[b]

ARG	DW-1	DW-2	DW-3	DW-4	DW-5	DW-6	DW-7	DW-8	DW-9	DW-10	LW-AE	LW-AN	+ control
tet(BP)	−	−	−	−	−	−	−	−	−	−	−	−	+
tet(O)	+	+	+	+	+	+	−	−	+	+	+	+	+
tet(S)	−	−	−	−	−	−	−	−	−	−	−	−	+
tet(T)	−	−	−	−	−	−	−	−	−	−	+	+	+
tet(W)	+	+	+	+	+	+	+	+	+	+	+	+	+
sul(I)	+	+	+	+	+	+	+	+	+	+	+	+	+
sul(II)	−	−	−	−	−	−	−	−	−	−	+	+	+
sul(III)	−	−	+	+	+	−	−	−	−	−	+	+	+
sul(A)	−	−	−	−	−	−	−	−	−	−	−	−	+

[a] Collected August 18, 2005. [b] Collected October 20, 2005.

the previous February event. In support of the relationship between ARG concentration and relative environment impact observed above, the pristine site (site 1) consistently had the lowest average concentrations of ARGs with time, with sul(II) completely absent and no individual ARG consistently present at all five sampling times (Figure 1). When presence/absence of ARGs are compared, site 2 appears to be the next lowest in terms of overall impacts. For example, sul(II) is consistently absent at site 2, and tet(O) was absent in one of the five sampling events, whereas these genes were consistently present at sites 3, 4, and 5. In terms of ARG concentrations, tet(W) and tet(O) at site 2 were equal or less than site 3; however, these two genes were sometimes higher and sometimes lower than at sites 4 and 5. On the basis of ARG averages and presence/absence of ARGs, sites 1 and 2 were the least impacted, as expected.

When the Mixed Procedure was applied to the data, in which the time points were pooled as replicates, it was found that there was no statistical difference between the five sites for the 16S normalized data, except in the case of sul(II) ($p = 0.0117$). However, when the same test was performed with non-normalized data, it was found that sites 1 and 2 were statistically lower than sites 3, 4, and 5 in terms of sul(I) ($p = 0.00296$), sul(II) ($p = 0.0199$), and tet(O) ($p = 0.0102$). Though normalizing to 16S genes provides a comparison of ARGs as a proportion of the total population, arguably it may be the absolute quantities of ARGs that are more critical.

While spatial variations in ARGs could be fairly well-characterized, it is difficult to identify clear temporal patterns. Comparison of the two February sampling dates that were exactly a year apart provides some insight. All four genes were either the same on average for both events (tet(O) for sites 1 and 4 and sul(II) for sites 4 and 5) or higher in the 2006 event (all other genes, except sul(II) at sites 1 and 2, where it was not present) (Figure 1). This suggests the possibility that all ARGs are increasing in concentration with time. However, the trends in between these two dates do not support this. Only tet(W) and tet(O) at site 3 increase consistently with time. All remaining ARGs at the five sites either decrease before increasing (e.g., tet(W) at site 2 and sul(II) at site 3), are constant and then increase (e.g., tet(O) at site 2 and tet(W) at site 1), or increase and then decrease (e.g., tet(W) at sites 4 and 5) (Figure 1). Therefore, no clear trend was identified with time.

It was also attempted to analyze trends in the data with respect to river flow rate. This was of interest because flow rate directly relates to runoff and nonpoint source inputs, which were hypothesized in the previous study to play a role in the observed increase in the number of kinds of ARGs detected in Poudre River sediments (*38*). The October 2005 sampling date provided a second sampling date at high flow (14.9 $m^3\ s^{-1}$), compared to the previously published April 2004 high-flow sampling date (6.8 $m^3\ s^{-1}$). (All other dates were at or below 1.0 $m^3\ s^{-1}$.) Interestingly, all four ARGs increased on average at site 5 in comparing the high-flow October event with the immediately previous low-flow event in August (Figure 1). At site 4, tet(W) and tet(O) increased, but sul(II) stayed the same, and sul(I) decreased. There was no effect at all at site 3, which is affected primarily by point discharge rather than runoff, site 2, or site 1. However, attempts to plot ARG concentrations versus flow rate did not reveal any clear trend. Thus, it is still not possible to make a conclusive judgment on the effect of flow rate on ARG concentrations, though the role of nonpoint source inputs merits further investigation. To accomplish this, it would be necessary to gather more data with time/flow or monitor a much more controlled and smaller-scale system.

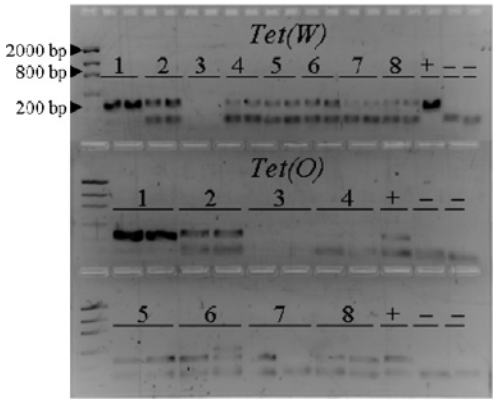

FIGURE 3. Agarose gel analysis of PCR presence/absence (in duplicate) of two ARG families, tet(W) and tet(O): + = positive control; − = negative control. The presence of a band at the same molecular weight as + indicates the presence of an ARG: 1 = WRP effluent; 2 = WRP chlorinated effluent; 3 = DWTP a influent; 4 = DWTP a treated water pre-chlorination; 5 = DWTP a treated water post-chlorination; 6 = DWTP b influent water; 7 = DWTP b treated water pre-chlorination; 8 = DWTP b treated water post-chlorination. The band appearing below 200 bp is consistent with a primer dimer.

Wastewater Recycling Plant and Drinking Water Treatment Plants. A PCR presence/absence assay was conducted on the influent, intermediate effluent, and final effluent of two drinking water treatment plants (DWTP "a" and DWTP "b") and the pre-chlorinated and chlorinated effluent of a WRP. It was observed that both tet(W) and tet(O) were present at detectable levels in all samples except the source water for DWTP "a" (Figure 3). This indicates that the same two genes that were common in various environmental compartments in northern Colorado are also present in treated recycled wastewater and bulk drinking water. These two genes also showed a response to the level of impact; e.g., they were highest in dairy lagoon water and ditch water and lowest on average at the pristine site. On the basis of the intensity of the signal, they were also higher in the recycled wastewater than in the drinking water, as would be expected. Though these two ARGs are not directly associated with any known human pathogens, they may be indicators of links

between human/agricultural activity and ARGs in drinking water. Considering that drinking water is a direct route to human consumers, this emphasizes the need to better understand the pathways by which ARGs are spread in the environment and potential ways that the spread of ARGs may be reduced. For example, vancomycin resistance genes were found in drinking water biofilms in a recent study (*45*). Considering that vancomycin is typically the antibiotic of last resort when all else fails, this underscores the need to address this issue before it is too late. One possibility may be to make simple modifications to wastewater and drinking water treatment plants to reduce the spread of ARGs.

ARGs as Emerging Contaminants. On the basis of this study it is clear that ARGs are present in various environmental compartments, including river sediments, irrigation ditch water, dairy lagoon water, DWTPs, and a WRP. Furthermore, quantitative techniques incorporating Q-PCR provide a means to compare the concentrations of ARGs associated with the known urban and agricultural impacts, which provides a more direct measure than previous culture-based methods. On the basis of this occurrence survey, it is argued that ARGs are emerging contaminants that need to be further studied in the paradigm of environmental science and engineering. The concept of ARGs as "pollutants" has also been suggested by Rysz and Alvarez (*46*).

It should be noted that besides the tetracycline and sulfonamide ARGs that were the focus of this study, there are numerous other ARGs that have been described in the literature and likely even more that have not yet been discovered, each potentially with its own unique properties. Thus, each ARG may have different behaviors with respect to fate and transport and response to physical, chemical, and/or biological treatment. In terms of defining fate and transport characteristics of ARGs in general, it is expected that their behavior will be distinct in comparison to "typical" contaminants. For example, ARGs may be sequestered with bacteria, which are themselves transported, or they may be present as naked DNA bound to clay particles (*47*). Furthermore, ARGs may actually amplify in the environment under some conditions. This is indeed a unique contaminant property. Considering the significance of the problem of the spread of antibiotic resistance, further effort by environmental researchers to better understand these emerging contaminants is well-warranted. This is especially true as the rate of discovery and development of new antibiotics is continually declining (*48*), while the corresponding development and spread of resistance is occurring at a rapid pace. On the basis of this study, understanding ARGs as emerging contaminants can add a new and important angle to helping to approach this important problem.

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Antibiotic Resistance from Wastewater Oxidation Ponds

Authors: Mispagel, Heather; Gray, Jeffrey T
Source: Water Environment Research, Volume 77, Number 7, November 2005 , pp. 2996-3002(7)
Publisher: Water Environment Federation

Abstract:
In an extensive, multiyear study of antibiotic resistance from wastewater oxidation ponds, five mobile home park wastewater oxidation ponds in Clarke and Oconee counties were shown to be discharging high numbers of antibiotic-resistant bacteria into the waterways of North Georgia. This effluent contributed to higher nitrogen, phosphorus, and fecal coliform levels in creeks downstream from the ponds. A survey of residents revealed that many people did not complete their antibiotic prescriptions, and the majority flushed leftover antibiotic medications down the toilet. In the pond discharges, resistance was found to eighteen antibiotics: amikacin, amoxicillin/clavulanic acid, ampicillin, apramycin, cefoxitin, ceftiofur, ceftriaxone, cephalothin, chloramphenicol, ciprofloxacin, gentamicin, imipenem, kanamycin, naladixic acid, streptomycin, sulphamethoxazole, trimethoprim/sulphamethoxazole, and tetracycline.

The discharged bacteria contained both integrons and plasmids, the latter being transferable to a laboratory strain of Escherichia coli (E. coli). A turtle was found living at a pond discharge site with multiply-antibiotic-resistant bacteria in its feces. Last year, RNA fingerprinting conclusively documented the survival of three multiply-resistant important pathogenic bacteria. Ceftriaxone-resistant Stenotrophomonas maltophilia and Pseudomonas aerogenosa and a ciprofloxacin-resistant E. coli were traced through oxidation pond stages and into the discharge, thus documenting that the pathogens survived the treatment process. In addition, a potential pathogen, a serotype group D Salmonella *spp.*, was found in the discharge.

In this study, tetracycline-resistance genes C and G were detected in the first and second stages of the oxidation pond and the discharge went directly into the environment. These genes are generally found in intestinal bacteria, so it can be inferred that they are from a human source. Antimicrobial residue from the beta-lactam family of antibiotics was found in all oxidation pond stages and in the creek above the pond. Tetracycline residue was found in the first and second stages of the pond. In addition to the antibiotics, genes coding for antibiotic resistance and the antibiotics themselves were documented to survive oxidation pond treatment. Tetracycline-resistant

genes were identified in the oxidation pond stages and in the discharge going into the environment.

A model was also developed to study oxidation pond function in the laboratory. A biofilm was created using a highly antibiotic-resistant Salmonella typhimurium *3/97*, and pond water was added. The biofilm was processed via a rotating disk bioreactor specifically designed to study biofilms in nature, but with conditions that were more favorable to bacterial inhibition than those in nature. Cultures revealed that, under these optimal conditions, S. typhimurium *3/97* was still present in this *in vitro* system. Thus, the competitive inhibition process that helps to remove bacteria in oxidation ponds did not effectively remove an important bacterium, S. typhimurium 3/97, in this mock oxidation pond.

The bioreactor model developed in this study can be used to further investigate discharges from oxidation ponds. From this data, it is apparent that the problem is two-fold. A cost-effective technique must be developed that inactivates antibiotic-resistant bacteria in oxidation pond discharges and also removes the antibiotics. A public awareness campaign was initiated by the author to encourage proper use and disposal of antibiotics, as flushing them is a common practice in the United States.

Keywords: wastewater; oxidation pond; antibiotic resistance; wastewater; biofilm; human waste lagoon
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Contact for Reporters:
Emily Narvaes Wilmsen
(970) 491-2336
Emily.Wilmsen@colostate.edu

COLORADO STATE RESEARCHER URGES SCIENTIFIC COMMUNITY TO TREAT ANTIBIOTIC RESISTANCE GENES AS WATER CONTAMINANTS

FORT COLLINS - Antibiotic resistance genes - a growing health problem around the world - are present in water systems in northern Colorado and should be viewed more seriously as contaminants by the scientific community, a Colorado State University civil engineering professor urges in an upcoming issue of the American Chemical Society's Environmental Science and Technology journal.

Amy Pruden, an assistant professor of civil and environmental engineering, and her team looked for the occurrence of tetracycline and sulfonamide antibiotic resistance genes in five sampling sites along the Poudre River. As expected, they found higher concentrations in more populated or heavily farmed areas, but still detected antibiotic resistance genes in all sampling areas.

Tetracycline and sulfonamide are commonly used antibiotics in people and animals.

Pruden found that treated water and wastewater also carried the genes.

"Microbes carrying these antibiotic resistance genes are not effectively killed by antibiotics, and the presence of these drugs in the environment may stimulate them to proliferate," Pruden said.

Pruden co-authored the paper with Ken Carlson, a civil engineering professor, and two graduate students, Ruoting Pei and Heather Storteboom. Funding for the research was provided through a $400,000, five-year CAREER grant awarded this year from the National Science Foundation and from the U.S. Department of Agriculture NRI Watersheds program and the USDA Agricultural Experiment Station at Colorado State.

Pruden notes that even if cells carrying the genes have been killed, the DNA still winds up in the environment and may get transferred to other cells. She stressed that testing so far only covers two classes of antibiotic resistance genes - others may also be present in the environment and have varied responses to environmental conditions.

"Antibiotic resistance genes in and of themselves can be considered to be emerging 'contaminants' for which mitigation strategies are needed to prevent their widespread dissemination," Pruden said. "This is especially true as the rate of discovery and development of new antibiotics is continually declining while the corresponding development and spread of resistance is occurring at a rapid pace."

More than 2 million Americans are infected each year with resistant pathogens; 14,000 die, according to the World Health Organization. The WHO considers antibiotic resistance to be one of the most pressing health challenges of the next century.

"This is a new class of contaminant that can have a measurable impact on human and environmental health," Pruden said. "I want to know how they're spread and develop models and find out how to treat them."

The next step of her research is to follow the path of the genes through the watershed and more closely identify the sources. At the same time, she will investigate basic modifications to water treatment to destroy the genes' DNA.

"We have some evidence of how they spread in the environment, but we need to study it in more detail to confirm the pathways," she said. "Where is it coming from, where does it end up, how does it get there?

"With environmental problems, it's often when you reach a point of no return that people start thinking about doing something," she said. "We're hoping to avoid that."

Pruden is teaching an experimental course in molecular biology for engineers this fall. The course is now a permanent part of the engineering curriculum and will help equip engineering students to tackle complex bioenvironmental problems.

She is also working with Carlson to test an early-warning security system designed to alert city utility officials when major pollutants are detected in water supplies. If installed, the real-time monitoring system, integrated by ST-Infonox of California, would help city officials respond quickly to foreign substances in the water distribution system, helping to combat any potential terrorist or natural threats.

ST-Infonox officials are working with city officials in Loveland and Fort Collins to test the technology on municipal water systems.

Pruden joined Colorado State University in 2002. She obtained her bachelor's and her doctoral degree from the University of Cincinnati.

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Accessed 12 times.

The Importance of Municipal Sewage Treatment in the Spread of Antibiotic Resistance

106th General Meeting of the American Society for Microbiology
May 21-25, 2006, Orlando, Florida
For more information on any presentation at the 106th General Meeting of the ASM contact Jim Sliwa, ASM Office of Communications at jsliwa@asmusa.org
EMBARGOED UNTIL: **Monday, May 22, 9:00 a.m. EDT**
(Session 041/Q, Paper Q-032)

Sara Firl
University of Minnesota
Minneapolis, MN, United States
Phone: **612 626 8865**
firl0002@umn.edu

Our study determined that substantial numbers of antibiotic-resistant bacteria were present in municipal wastewater, and that the existing treatment infrastructure did not adequately prevent release of antibioticresistant bacteria into the environment. Many of the bacteria found in the wastewater treatment plant and in the plant effluent were tentatively identified as potential pathogens and were also resistant to multiple antibiotics, raising public health concerns. We believe that wastewater treatment plants could be modified to further prevent the release of resistant bacteria to the environment.

Sara Firl and Leslie Onan performed this study under the supervision of principal investigator Dr. Timothy LaPara at the University of Minnesota, Department of Civil Engineering. Funding was provided by the Center for Urban and Regional Affairs at the University of Minnesota and Geomatrix Consultants, Inc. The work is being presented as a poster at the 106th General Meeting of the American Society for Microbiology in Orlando on May 22.

The spread of antibiotic-resistant bacteria is a major public health concern. Infections previously treatable are increasingly resistant to antibiotics. Scientists believe that the spread of antibiotic resistance results from both misuse of antibiotics and transfer of resistance between bacteria. A potentially large reservoir for antibiotic-resistant bacteria is municipal wastewater. People release resistant bacteria with fecal matter into the wastewater stream, which is collected and treated at municipal treatment facilities before release to the environment. The objective of this study was to investigate how many resistant bacteria were present at municipal wastewater plants and if the existing infrastructure of waste treatment was adequate to remove resistant bacteria before discharge.

In our study, the effect of effluent treatment (clarification and disinfection) and biosolids treatment (sludge digestion) on the removal of antibioticresistant bacteria was investigated at three wastewater treatment facilities.

We found substantial numbers of resistant bacteria at the wastewater treatment facilities and that, although effluent treatment reduced the numbers of bacteria, large quantities of resistant bacteria were discharged. Numerous bacteria isolated from the effluent stream were resistant to multiple antibiotics and closely related to potentially pathogenic bacteria. Our research suggests that the existing wastewater treatment infrastructure should be modified to better prevent release of these potentially dangerous bacteria to the environment.

USGS at Society of Environmental Toxicology and Chemistry: From Pharmaceuticals in Groundwater to Mercury in High U.S. Mountains

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Contact Information:
U.S. Department of the Interior, U.S. Geological Survey
Office of Communication
119 National Center
Reston, VA 20192

Catherine Puckett
Phone: 352-264-3532

The 27th Annual Meeting of the Society of Environmental Toxicology and Chemistry (SETAC) is being held November 5-9, 2006, at the Palais de Congrès in Montréal, Québec, Canada. Additional information about the conference can be found online at http://montreal.setac.org/home.asp While the conference is occurring, please contact Catherine Puckett for information about specific presentations.

A Happy Medium? Antidepressants in Aquatic Systems: Wastewater treatment plants do a remarkable job at removing the bulk of chemicals from the waste stream. But recent USGS studies have shown that a wide range of pharmaceuticals and other human-caused waste compounds remain despite wastewater treatment and are discharged to receiving waters across North America. Antidepressants are a commonly used class of pharmaceuticals whose pharmacological effects may extend beyond humans to aquatic organisms present in surface water systems that receive treated wastewater discharge. Yet few methods exist to detect antidepressants in the environment, and their effects on aquatic organisms are only beginning to be understood. Recently, USGS researchers developed a method to study the distribution and fate of antidepressants and their breakdown products in aquatic environments, including municipal wastewater and surface water. Venlafaxine (Effexor) was the predominant antidepressant researchers found in wastewater and river-water samples from Colorado, Iowa, and Minnesota, though other antidepressants were found as well. Typical concentrations of individual antidepressants ranged from a few nanograms per liter to thousands of nanograms per liter (for Venalfaxine) in wastewater. This indicates that wastewater is a point source of antidepressants into the environment, at concentrations that may impact aquatic life. **For more information, please contact Edward T. Furlong, USGS, at efurlong@usgs.gov or 303-236-3941; or Melissa Schultz, College of Wooster, Wooster, OH at mschultz@wooster.edu or 330-263-2645. SETAC presentation is Monday, Nov. 6, 8:20 a.m., Room 517A, Palais de Congrès.**

Pharmaceuticals in Long Island's Groundwater: Pharmaceuticals can infiltrate groundwater systems in areas susceptible to wastewater contamination. In studies by Stony Brook University and the U.S. Geological Survey of ground-water wells in Suffolk County, Long Island, NY, near permitted wastewater treatment facilities discharging to ground water, scientists detected pharmaceuticals in concentrations generally 1-200 ng/L (parts per trillion).These vanishingly small concentrations are several orders of magnitude below the concentrations where any effects have been observed or predicted for the compounds measured in this study. Acetaminophen, caffeine, carbamazepine (anti-epileptic), cotinine (human metabolite of nicotine), paraxanthine (human metabolite of caffeine), and sulfamethoxazole (antibiotic) were found most often in both studies. However compounds were more frequently detected in the shallower wells. These occurrences, and laboratory studies, suggest that of these compounds, caffeine, carbamazepine, paraxanthine, and sulfamethoxazole are more persistent in groundwater and have the most potential for transport in the subsurface. **For more information, contact Mark J. Benotti at mbenotti@usgs.gov or 631-736-0783 x126. SETAC presentation is Thursday, Nov. 9, 4:50 p.m., Room 516AB, Palais de Congrès.**

After the Hurricanes - The Contaminants Left Behind in New Orleans: USGS researchers measured numerous semivolatile organic compounds in street floodwater mud and Lake Pontchartrain sediment samples collected in September and October 2005 after the levee breaches caused by Hurricane Katrina and subsequent flooding from Hurricane Rita. These compounds include compounds organochlorine pesticides, PCBs, PAHs, current-use pesticides, anthropogenic indicator compounds (AICs), and pharmaceuticals (in the mud only). Contaminant concentrations in street mud varied substantially and for some - including PAHs, some AICs, and four termiticides - were highest at several sites near downtown New Orleans when compared with other locations (Chalmette, Ninth Ward, Slidell, Rigolets). USGS researchers found that the highest concentrations of urban-related compounds (such as chlordane and PAHs) in lakebed sediments exceeded average concentrations in U.S. urban lakes and sediment quality guidelines, but were not markedly dissimilar to historical values or to those reported from other urban areas. The highest concentrations were limited to within a few hundred meters of the 17th Street Canal outlet into the lake. This research suggests that the impacts of the hurricanes on the sediment history of Lake Pontchartrain are most likely transitory and confined to a relatively small geographic region. **For more information, contact W. T. Foreman at wforeman@usgs.gov or 303-236-3942. SETAC presentation is on Thursday, Nov. 9, 10:40 a.m., Room 516C, Palais de Congrès.**

Rocky Mountain High -- Mercury in Cold Environments of the Western United States: Atmospheric deposition of mercury in remote areas

in the Western United States is sufficient to pose a risk to human and ecosystem health at sites favorable for methylation, a process in which mercury in the environment is converted into a highly toxic form that accumulates in organisms and is amplified up the food chain. USGS researchers and partners measured mercury in snowpack samples during 2003-2005 as part of the National Park Service Western Airborne Contaminants Assessment Project. Eight high-altitude, high-latitude sites were selected for study in or near national parks in Colorado, Montana, California, Oregon, and Alaska. Mercury levels were lowest in the North Cascades, highest in the Rocky Mountains, and were related to the amount of particulate carbon in the snow, with both found at higher levels in forested sites than in open meadows. Seasonal variations were lowest in Denali National Park and highest in Olympic National Park. Mercury concentrations were higher during the warm season than the snow season. Total annual fluxes of mercury were as high as 10 mg m-2 at some sites in the Rocky Mountains, which receive mercury deposition equal to that in the Upper Midwest or Northeast. Global and regional sources of mercury emissions contribute to its deposition, with regional sources likely contributing more in the Rocky Mountains, where there are more upwind sources of emissions. **For more information, contact Don Campbell at Donald.Campbell@usgs.gov or 303-236-4882, ext. 298. SETAC presentation is on Thursday, Nov. 9, 8 a.m.- 7 p.m., Exhibit Hall, Palais de Congrès. Poster #934.**

Contaminants Lower Reproductive Health of Gila River Fish: Downstream of Phoenix, southern Arizona's Gila River is primarily recharged by irrigation return water, storm water, and wastewater treatment plant effluent, and fish and aquatic invertebrate habitats are degraded. Largemouth bass, common carp and channel catfish from the Gila had elevated levels of organochlorine pesticides, many of which have been associated with estrogen-like effects in fish. Reproductive biomarkers, including gonad size and hormone concentrations, were notably different in fish from the Gila River when compared to fish from the Colorado River, indicating that organochlorine contaminants may be affecting the reproductive health of fish populations in the Gila River downstream of Phoenix. **For more information, contact Jo Ellen Hinck at jhink@usgs.gov or 573-876-1808. SETAC presentation is on Thursday, Nov. 9, 2:30 p.m., Room 517B, Palais de Congrès.**

Aquatic Herbicides May Benefit Invasive Aquatic Species: Aquatic plants are frequently exposed to low-levels of agricultural herbicides at concentrations less than those known to cause adverse effects in the laboratory. Laboratory studies have shown that low levels of herbicide exposure can actually increase growth rates of aquatic plants due to physiological stress adaptations. Scientists from the U.S. Geological Survey and the University of Manitoba studied whether low levels of herbicides in aquatic systems may actually aid non-native invasive aquatic plants, allowing them to out-compete or displace more desirable native aquatic plant species. Researchers studied the effects of atrazine herbicide on both native and non-native, invasive aquatic plants in experimental ponds over a period of 42 days. Results confirmed that lower levels of atrazine could actually stimulate growth of some invasive species. Some invasive aquatic plant species were less sensitive to atrazine than a common native aquatic plant species. Although the invasive species did not totally displace the native species, the results indicate that some herbicides may have the unintended consequence of benefiting non-native invasive species that may compete with native aquatic plant species. Aquatic plants are critical components of aquatic ecosystems by providing habitat and energy sources for many fish and invertebrates. **For more information, contact James F. Fairchild at jfairchild@usgs.gov or 573-876-1871. SETAC presentation is on Wednesday, Nov. 8, 10:20 a.m., Room 516AB, Palais de Congrès.**

Wastewater Issues Get Wormy: Recent research indicates that earthworms may be an important initial step by which organic contaminants could enter the terrestrial food web**.** Wastewater treatment plants process millions of gallons of mixed solid and liquid human waste daily, returning treated effluent to surface and ground water and disposing of the residual sludge. Roughly half of the many thousands of dry tons of treated sludge (usually referred to as biosolids) generated annually in the U.S. are applied to agricultural soils as a nutrient-rich soil amendment. Recent USGS research has identified a wide variety of organic contaminants (such as disinfectants, pharmaceuticals, synthetic fragrances, and plasticizers) that can be present in biosolids, often in concentrations tens to thousands of times higher than found in treated liquid waste. One concern related to the practice of land application of biosolids is whether any of these organic contaminants find their way into soil-dwelling organisms. To address this concern, USGS and Eastern Washington University scientists collaborated on a study of earthworms collected from agricultural soils in the Midwest and Western United States that had been exposed to land-applied biosolids. The samples were analyzed for a diverse array of pharmaceuticals and other organic contaminants (77 target compounds were measured). Soil and earthworm samples were collected from select agricultural fields early and late in the growing season. Thirty-one compounds including triclosan (household disinfectant), several fragrances, caffeine, and fluoxetine (the antidepressant Prozac) were detected in earthworms from biosolid-applied fields, with tissue concentrations ranging from 100's to 1000's of micrograms per kilogram (parts per billion). These results demonstrate that earthworms can accumulate a range of these chemically diverse organic contaminants within their tissues, and may be an important initial step by which these compounds could enter the terrestrial food web**. For more information contact Ed Furlong, USGS, at efurlong@usgs.gov or 303-236-3941, and Chad Kinney, Eastern Washington University, at ckinney@mail.ewu.edu or 509-359-7932. SETAC presentation is on Thursday, Nov. 9, 4:10 p.m., Room 516 AB, Palais de Congrès.**

Toxicity Tests for Endangered Mussels: The United States is home to more mussel species than any other country in the world. Despite the diversity of mussels found in the country, no other widespread group of animals in North America is as imperiled or has faced as many extinctions. The abundance and variety of mussels have declined sharply over the past century, but the cause of mussel decline is not well understood. Researchers at the USGS-Columbia Environmental Research Center, in cooperation with other government agencies, academia, and private industry, are developing the first standardized toxicity tests using several life stages of freshwater mussels to assess the effects that pollution may have on these declines. Mussels are filter feeders that readily accumulate toxins. Results of this ground-breaking work indicate

that water quality criteria for individual chemicals established for the protection of aquatic organisms may not be adequately protective of sensitive stages of freshwater mussels. **For more information, contact Ning Wang, nwang@usgs.gov or 573-441-2946. SETAC presentation is on Thursday, Nov. 9, 8 a.m.-7 p.m., Exhibit Hall, Palais de Congrès. Poster # 1056**

Toxic Tango: Interactions of Mercury and Selenium on Bird Embryos: Mercury and selenium are common environmental contaminants that sometimes occur together at elevated levels in bird eggs. Both have been associated with reproductive impairment in birds, in particular by embryonic death and deformities. Although a lot is known about the toxicity of these two contaminants by themselves in eggs, little is known about potential toxic interactions when they occur in the same egg. USGS research indicates that combining the contaminants had a worse effect on mallard embryos than either one did separately. Follow-up studies, however, revealed that combined effects of these two contaminants may vary by species and exposure amount. **For more information, contact Gary Heinz at gary_heinz@usgs.gov or 301-497-5711. The SETAC presentation is on Wednesday, Nov. 8, 10 a.m., Room 511AD, Palais de Congrès.**

Contaminants Affect Over-Winter Survival of Swallows: The effects of chronic contaminant exposure on over-winter survival of birds are largely unknown. These studies are difficult to carry out because suitable bird species may not occur in contaminated locations, there may be insufficient number of breeding birds, they may be difficult to capture, or the species may to too long-lived to study within a reasonable time. Tree swallows overcome many of these research problems. Large numbers of breeding birds can be attracted to a site because they will readily nest in man-made nest boxes. They are also relatively easy to capture, return to the same breeding site year after year, and are short lived. The Housatonic River in western Massachusetts is extremely contaminated with polychlorinated biphenyls (PCBs) and PCB concentrations in swallow eggs are associated with decreased reproductive success. USGS researchers studied the effect on over-winter survival of chronic exposure to PCBs for 5 years on the Housatonic River to determine if adult swallow survival was reduced in this highly contaminated environment. Researchers found that annual over-winter survival was reduced significantly by about 5 percent in females that nest at the most contaminated sites. **For more information contact Christine M. Custer, ccuster@usgs.gov or 608-781-6247. SETAC presentation is on Monday, Nov. 6, 2:10 p.m., Room 517 B, Palais de Congrès.**

Fungicides: Analysis, Fate, and Toxicity: The recent spread of Asian soybean rust to North America has increased interest in fungicides to combat this scourge. Despite decades of agricultural and urban use, relatively little data are available on the fate and effects of fungicides in the aquatic environment. One of the most used fungicides in the United States, chlorothalonil, has been used for over 50 years for a variety of applications. Other fungicides (azoxystrobin, myclobutanil, propiconazole, pyraclostrobin, and tebuconazole) have been recently registered for treatment of soybean rust and are rapidly increasing in use. Some of these fungicides are highly toxic to fish and other aquatic life. Fungicides are often not included in monitoring programs, although fungicides and their degradates have been detected in water, sediments, air and rainfall at concentrations that can cause adverse effects to aquatic organisms. Effective monitoring of fungicide concentrations is required to understand if increasing use will result in increasing stream concentrations. The focus of this session will range from older fungicides such as chlorothalonil to newer fungicides such as the triazoles and strobilurins. Topics will include analysis of fungicides and their degradates, environmental occurrence, degradation pathways, modes of action, and toxicity to aquatic organisms. **For more information, contact Kathryn Kuivila at kkuivila@usgs.gov or 916-278-3054. The symposium, which is sponsored by the U.S. Geological Survey and Mississippi State Chemical Laboratory, will take place on Wednesday, Nov. 8, in room 510 BD, Palais de Congrès, from 8:00 - 11:40 a.m.**

How Much is Too Much? Mercury Thresholds for Common Loon Eggs: Assessing the ecological risk of mercury exposure to fish-eating wildlife is a priority issue for federal and state resource management agencies. Atmospheric mercury deposition has increased due to industrial activities exposing fish-eating wildlife populations in New England, coastal Atlantic states, the Southeast and the Upper Midwest to elevated mercury in their prey. The USGS, Wisconsin Department of Natural Resources, and the University of Wisconsin have conducted research to generate a scientifically defensible common loon/mercury risk assessment model. The work focused on the common loon because this species is sensitive to the toxic effects of mercury and has the greatest risk of mercury exposure among wildlife species on inland (non-marine) North American aquatic systems. A critical component of the model is determining the level of mercury in loon eggs that poses a population level risk. In 2005 and 2006, researchers conducted a study to better characterize methylmercury exposure in eggs of Wisconsin common loons and to determine the level of exposure in eggs that reduces fitness and survival of loon embryos and resultant chicks. Blood mercury levels in a sample of Wisconsin loon chicks indicated mercury exposure in some chicks rivaled that of adult birds during the breeding season. Blood mercury concentrations rapidly declined in growing chicks, such that by six weeks of age blood mercury levels were about 6 percent of levels at hatch. Reduced embryo survival was evident at an egg content concentration of mercury that is representative of what is often found on low pH lakes in northern Wisconsin, although sample sizes are small. **For more information, contact Kevin Kenow at kkenow@usgs.gov or 608-781-6278. SETAC presentation is on Wednesday Nov. 8 at 8:40 a.m., Room 511 AD, Palais de Congrès.**

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Municipal Wastewater Treatment: A Novel Opportunity to Slow the Proliferation of Antibiotic-Resistant Bacteria?

by Timothy M. LaPara, Sara J. Firl, Leslie J. Onan, Sudeshna Ghosh, Tao Yan, and Michael J. Sadowsky

Photo © The Regents of the University of Minnesota, 2006. Used with permission of the Metropolitan Design Center.

Located on the Mississippi River in St. Paul, the Metropolitan Wastewater Treatment Facility is the largest treatment plant in Minnesota, averaging 180 million gallons of wastewater daily from 62 communities and 800 industries.

The discovery of antibiotics and their subsequent application to clinical medicine is one of the outstanding scientific achievements of the twentieth century. The tale of how antibiotics were discovered is one of scientific legend: Sir Alexander Fleming astutely recognized that a contaminated Petri dish actually contained a bacteria-killing mold. For his discovery of penicillin, Fleming shared the 1945 Nobel Prize in physiology/medicine with Sir Howard Florey and Ernst B. Chain.

The unique feature of penicillin (and other antibiotics) is not merely that it kills bacteria—there are many compounds that have such a capability—but that it specifically affects bacteria. This key feature is absolutely critical for the medical application of antibiotic therapy. Antibiotics administered to humans are lethal to disease-causing bacteria but do not impact the patient. This is possible because antibiotics act on features of the bacterial cell that are absent in humans. For example, penicillin prevents the formation of new bacterial wall materials; human cells do not even contain a cell wall.

During the last half-century, antibiotics have become pervasive in human medicine. Since the discovery of penicillin, a plethora of new antibiotics, semi-synthetic antibiotics, and synthetic antibiotics (antibacterials) have been discovered or developed (Table 1). These new drugs target different features of bacterial physiology, thus expanding the range of bacterial species that can be successfully treated with antibiotics. Antibiotics are also used extensively in agriculture and for other non-medical purposes. Low doses of antibiotics are often included in animal feed to promote growth and increase weight gain, as well as prevent the onset of

Table 1. Major Classes of Antibiotics and Antibacterials, and Representative Drugs in Each Class

Class	Representative Drug(s)
β-lactams	Penicillin, Amoxicillin, Methicillin
Aminoglycosides	Streptomycin, Neomycin, Kanamycin, Gentamicin
Macrolides	Tylosin, Erythromycin
Ketolides	Telithromycin
Tetracycline	Tetracycline, Oxytetracycline
Lincosamides	Clindamycin
Ansamycins	Rifampin
Glycopeptides	Vancomycin
Quinolones/fluoroquinolones	Nalidixic acid, Ciprofloxacin
Sulfonamides	Sulfamethoxazole

disease. Although reliable estimates are difficult to obtain, most scientists believe that approximately 70% of all antibiotics are used for agricultural purposes.

In this article, we report on a research project that investigated the role of municipal wastewater treatment facilities in the spread or control of antibiotic-resistant bacteria. The project was supported by a grant from CURA's Faculty Interactive Research Program, as well as grants from the Undergraduate Research Opportunity Program at the University of Minnesota. We hypothesized that the disinfection processes most treatment facilities use would adequately inactivate antibiotic-resistant bacteria in wastewater. However, our research suggests that treatment facilities, which are primarily designed to protect water quality, do not adequately prevent resistant bacteria from being released into the environment. We conclude that relatively simple changes in the design, operation, and regulation of municipal wastewater treatment facilities could substantially reduce the release of these bacteria and, we hope, slow the proliferation of antibiotic resistance among bacteria appearing in clinical patients.

A Brief History of Antibiotic Resistance

Antibiotic-resistant bacteria were discovered soon after the medical use of penicillin began. At the time, the development of resistant bacteria was largely viewed as inconsequential. If a patient had an infection that a resistant bacterium caused, then an alternative antibiotic was always available for effective treatment. However, some foresighted scientists warned of the pending problem of antibiotic resistance. In his Nobel acceptance speech, Alexander Fleming himself cautioned doctors about the danger of giving an "underdosage" of penicillin, noting: "It is not difficult to make microbes resistant to penicillin in the laboratory by exposing them to concentrations not sufficient to kill them, and the same thing has occasionally happened in the body . . . Moral: If you use penicillin, use enough."

The pioneering work of Stuart Levy in the 1970s was also informative. Levy was concerned that antibiotic use in agriculture at subtherapeutic concentrations could lead to the proliferation of antibiotic resistance. His research demonstrated that tetracycline-resistant bacteria were present in the droppings of chickens within one week after tetracycline was included in their feed. More alarming, however, the bacteria in chickens that were fed only tetracycline became resistant to multiple antibiotics within two weeks. Finally, multiple-antibiotic-resistant bacteria dominated the fecal material of farmers working with these chickens within five months, even though the farmers had received no antibiotics during the study.

It was not until the 1980s, however, when a multiple-drug-resistant form of tuberculosis emerged, that scientists became concerned about antibiotic resistance. Multiple-drug resistance soon appeared among other pathogens, particularly among nosocomial (hospital-acquired) infections. Today, 40% to 60% of nosocomial *Staphylococcus aureus* infections are methicillin resistant. The problem of antibiotic-resistant bacteria is particularly significant for immunodeficient patients, who are susceptible to a broader array of pathogens, many of which are multiple-drug resistant.

Many believe that the problem is linked to excessive antibiotic use in hospitals, making them a "hot spot" for resistant bacteria. Unfortunately, mounting evidence refutes this perspective. Community-acquired methicillin-resistant *Staphylococcus aureus* is becoming far more prevalent, particularly at public gymnasiums, where insufficiently sanitized towels are prevalent.

The Development of Antibiotic Resistance in Bacteria

The simplest method by which bacteria become resistant to antibiotics is via a *point mutation* of the deoxyribonucleic acid (DNA) within their genome. Point mutations are typically lethal to the bacterium or have no effect, but on rare occasions these mutations are beneficial (from the bacterium's perspective) and allow the organism to become resistant to antibiotics. Point mutations, however, are not the major concern with respect to antibiotic resistance. This form of bacterial evolution is slow and random, and it is unlikely that bacteria could rapidly achieve resistance to multiple antibiotics via point mutations alone.

Ultimately, the proliferation of antibiotic resistance is caused by the propagation of specific genes that allow bacteria to defy the lethal effects of antibiotics. These *antibiotic resistance genes* are probably not new, but likely result from millions of years of evolution, during which time bacteria have developed many mechanisms to survive the dangers that the world thrusts upon them. Certainly, many of these genes were specifically developed to counteract antibiotics, which are, after all, naturally occurring compounds. Many antibiotic resistance genes, however, likely are subtle adaptations of genes that provide protection against other toxic compounds. For example, there is a strong correlation between genes that encode for resistance to heavy metals and antibiotic resistance genes.

The existence of antibiotic resistance genes, however, is insufficient to explain the global proliferation of resistance. Bacteria also harbor other genes that are specifically designed to help bacteria rapidly evolve—genes designated as *evolution genes* by 1978 Nobel Prize winner Werner Arber. Evolution genes allow bacteria to rapidly develop new genes (usually by manipulating preexisting genes) and to spread them

throughout the bacteria population. The evolution genes that allow *lateral gene transfer* are perhaps the most important class of evolution genes with respect to antibiotic resistance. Lateral gene transfer is the exchange of genetic material between different bacteria; it allows bacteria to share their abilities to resist antibiotics. This is believed to be the principal mechanism by which similar resistance genes are found throughout the world among many different species of bacteria.

During the last 20 years, scientists have also recognized the importance of *integrons*, another type of evolution gene. Integrons are responsible for integrating resistance genes into the genomes of bacteria, and then controlling the expression of these resistance genes. Because of this unique ability, integrons can be viewed as a genetic "luggage rack" in which different genes can be kept until they are needed. Integrons are a key component in the development of multiple-antibiotic-resistant bacteria because they allow bacteria to easily accumulate numerous genes.

Responding to Antibiotic Resistance

Although scientists have known about antibiotic-resistant bacteria for almost as long as they have known about antibiotics, the assumption was that new antibiotics would be discovered or developed faster than bacteria could become resistant. The discovery of new antibiotics, however, has slowed substantially since the 1960s. In fact, most "new" antibiotics are merely subtle modifications of previously existing ones and have little impact on bacteria that are already resistant.

During the last decade, therefore, there has been a considerable effort to restrict antibiotic use to only those applications where antibiotics are appropriate. Physicians are now reminded to avoid prescribing antibiotics for viral infections such as influenza and the common cold. Likewise, patients are carefully instructed to follow prescription guidelines so that enough of the drug is administered to limit the development of resistant bacteria. There is also increasing pressure to limit or eliminate non-medical use of antibiotics and antibacterials. As noted above, a substantial fraction of all antibiotics are used in agriculture at subtherapeutic concentrations. Although the United States appears to be far from prohibiting this practice, the European Union is banning subtherapeutic antibiotic use in agriculture in 2006. Although more controversial, many scientists—led by the Alliance for the Prudent Use of Antibiotics—are recommending the elimination of triclosan and other antibacterials from liquid hand soap, toothpaste, and other common household items.

A New Paradigm: Resistance Control?

The current situation with respect to antibiotic resistance is bad and the future is bleak. The discovery of new drugs has slowed to a trickle—a problem that will only worsen as pharmaceutical companies devote a greater fraction of their research and development budgets to less essential drugs (e.g., Botox, Viagra). Simultaneously, the ever-increasing use and misuse of antibacterials in common household products can only exacerbate the problem.

From our perspective, current efforts to reduce the spread of antibiotic resistance are an excellent first step. Certainly, our historically indiscriminate use of antibiotics needs to end. The more important issue is to identify novel approaches to limit the spread of antibiotic resistance. Our intention in undertaking this research, therefore, was to take a different approach to solving the problem of antibiotic resistance. We started by asking some simple yet fundamental questions about the proliferation of antibiotic-resistant bacteria.

First, where do the majority of antibiotic-resistant bacteria originate? Certainly, many bacteria are naturally resistant, but the majority of antibiotic-resistant bacteria result from antibiotic use. Therefore, people and animals taking antibiotics are most likely the primary source of antibiotic-resistant bacteria.

Second, how do resistant bacteria spread throughout the world after they originate inside a person? Humans actually contain about 10 times more bacterial cells in their bodies than they do human cells. The overwhelming majority of these bacterial cells reside in our gastrointestinal tracts, and most are released from the body during defecation.

Having asked and answered these two simple questions, we then inferred that municipal wastewater treatment plants, which handle virtually all human toilet waste in large municipalities (in rural areas, septic systems are more commonly used), would be critical in reducing the spread of antibiotic resistance. We hypothesized that municipal wastewater treatment facilities could adequately control the release of antibiotic-resistant bacteria to the world.

Municipal Wastewater Treatment Facilities: How Do They Work?

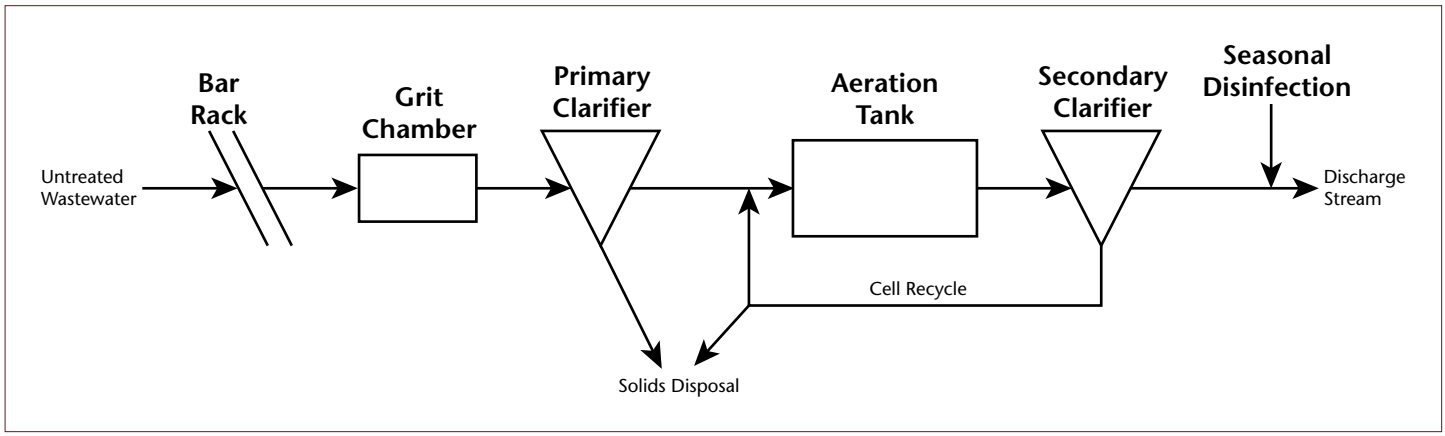
Municipal wastewater treatment facilities are primarily designed and operated to protect the environment. Municipal wastewater treatment facilities remove readily biodegradable compounds from sewage. Although there is relatively little in human sewage that is toxic, these biodegradable compounds are of environmental concern because if they were released untreated, they would biodegrade in the environment, resulting in oxygen depletion leading to septic conditions. Municipal wastewater treatment facilities, therefore, allow surface waters to maintain high dissolved oxygen levels, improving their aesthetic and recreational use value, as well as their ability to support healthy populations of fish and other aquatic fauna.

Although all municipal wastewater treatment facilities are unique, most are similar in design and involve a common series of unit operations (Figure 1). The first few unit operations, called primary treatment, are designed to remove particles from the wastewater. The bar rack removes large particles (greater than 1 inch), whereas the grit chamber removes sand and other dense, rapid-settling particles. The primary clarifier is a quiescent settling zone that allows organic particles to settle or float so that they can be removed. These primary treatment operations account for about 50% of the treatment that occurs.

The next unit operation, the aeration tank, is designed to remove dissolved organic compounds (which are readily biodegradable) from the wastewater by creating conditions favorable for the growth of bacteria. The tank works by bubbling air through the wastewater, allowing bacteria to metabolize pollutants that are present. Because these bacteria grow in excessive quantities, they must be removed from the wastewater. This is accomplished by the next unit operation, which is a quiescent settling chamber called the secondary clarifier. The combination of the aeration tank and the secondary clarifier is called the activated sludge process, which is the most common technology for the secondary treatment of wastewater.

Following primary and secondary treatment, the quality of municipal wastewater is quite good—not yet potable (i.e., safe to drink), but often

Figure 1. Schematic Diagram of a Conventional Municipal Wastewater Treatment Process



Note: Individual unit operations are labeled in bold lettering.

as good as or better than the quality of many lakes and rivers. This treated wastewater, however, still contains pathogenic bacteria that could make people sick if they accidentally ingested the water. Municipal wastewater treatment facilities, therefore, perform a final treatment step in which the treated wastewater is disinfected to help reduce the number of disease-causing microbes. Disinfection is required only when recreational use of the receiving stream is a reasonable expectation. In Minnesota, for example, wastewater treatment facilities usually disinfect their wastewater only from April to November.

In addition to treating the wastewater, municipal wastewater treatment facilities must deal with the solid residues that the primary and secondary clarifiers collect. These solid residues are readily biodegradable organic materials that are most commonly treated by a process called *anaerobic digestion*. The conventional anaerobic digestion process, which largely mimics our gastrointestinal tracts (hence the "digestion" nomenclature), is kept free of oxygen and operated at 98.6°F. Following digestion,

Photo courtesy of Timothy LaPara

An aeration tank at a municipal wastewater treatment plant. The tank removes dissolved organic compounds by bubbling air through the wastewater, creating favorable conditions for the growth of bacteria that are capable of metabolizing pollutants.

the treated wastewater solids are either applied to farmland as a fertilizer and soil conditioner, or sent to a landfill for disposal. The former alternative is preferred as a "sustainable" practice, whereas landfill space is finite.

Because anaerobic digestors operate at conditions similar to the human body, they are not particularly good at eliminating human pathogens. Numerous alternative treatment technologies, therefore, have been developed to better treat wastewater solids. All of these alternative treatment technologies are more expensive, however, and thus municipalities do not frequently use them. Perhaps the most attractive treatment alternative is thermophilic anaerobic digestion, which operates almost identically to conventional anaerobic digestion, except that it operates at sufficiently high temperatures (greater than 110°F) to kill most human pathogens.

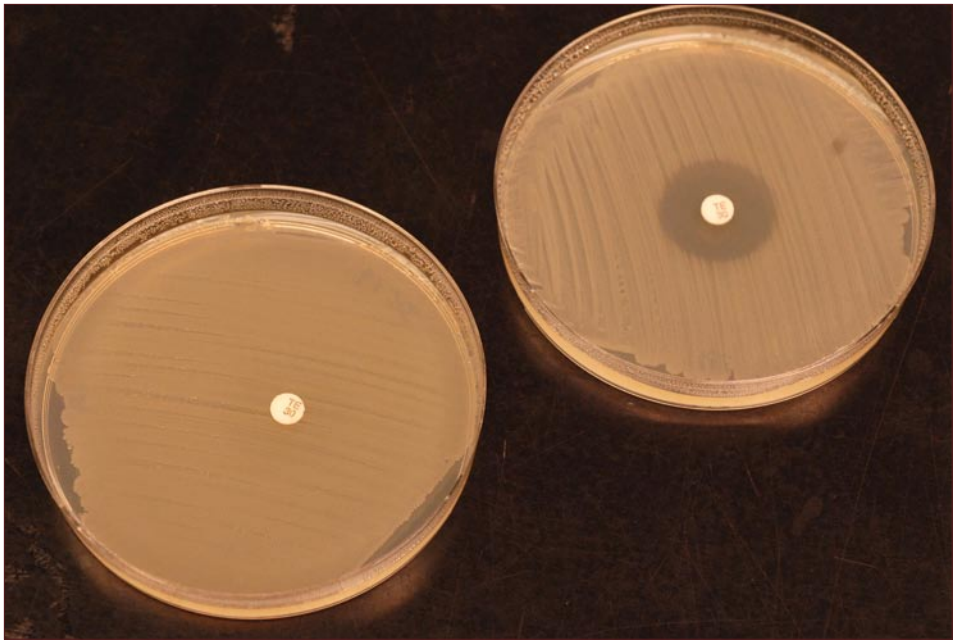


Photo © Steve Schneider, 2006

Bacteria growing on petri dishes that include disks treated with the antibiotic tetracycline. The bacteria growing on the left petri dish were obtained from treated wastewater and are resistant to tetracycline, as shown by the ability of the microbes to grow near the white disk. The bacteria growing on the right petri dish are a tetracycline-sensitive strain of* E. coli*. The circular ring around the disk shows that these microbes cannot grow in the presence of tetracycline.

Methodology and Analysis

The first goal of our project was to determine the extent to which municipal wastewater treatment facilities prevent the release of antibiotic-resistant bacteria. There are two potential paths by which antibiotic-resistant bacteria can escape a municipal wastewater treatment facility. The most obvious is in the treated wastewater. Our research, therefore, investigated the importance of secondary clarification and disinfection in preventing the release of antibiotic-resistant bacteria from the aeration tank. Resistant bacteria could also be released in the solids collected during primary treatment and from the secondary clarifier. Our research, therefore, compared the effectiveness of two variations of conventional anaerobic digestion and thermophilic anaerobic digestion at destroying resistant bacteria.

We investigated the efficacy of wastewater disinfection at the Metropolitan Wastewater Treatment Facility in St. Paul. This facility is very large, treating an average of 180 million gallons of sewage each day. Typically, the quality of treatment from the Metropolitan plant is top-notch, and the facility regularly wins state and national awards for operational excellence. Throughout the year, we quantified about 100,000 (10^5) tetracycline-resistant bacteria per milliliter of water in the aeration tanks at the Metropolitan plant. From the treated wastewater, we quantified about 300 tetracycline-resistant bacteria per milliliter in the winter (i.e., when disinfection was not performed) and about 30 tetracycline-resistant bacteria per milliliter during the summer (i.e., during the disinfection period). That is, about 99.6% and 99.97% of the resistant bacteria in the aeration tanks are removed in the winter and summer, respectively. Although this removal efficiency might seem sufficient, 30 bacteria per milliliter translates to more than 10 trillion (10^{13}) tetracycline-resistant bacteria released each day from this treatment facility into our waterways.

We also investigated the efficacy of anaerobic digestion at the Western Lake Superior Sanitary District (thermophilic process) and the Empire Wastewater Treatment Facility (conventional process), which are located in Duluth and Farmington, respectively. Both of these plants have also earned awards for operational excellence. We again detected about 100,000 (10^5) tetracycline-resistant bacteria per milliliter in the waste stream entering the anaerobic digestors at each of these treatment facilities. However, we were unable to detect any tetracycline-resistant bacteria in the waste stream leaving the anaerobic digestors at these two treatment facilities, in part because the research method we used is unable to detect levels of tetracycline-resistant bacteria below 1,000 (10^3) per milliliter of sludge solids. However, this suggests that both anaerobic digestion processes were able to inactivate at least 99% of antibiotic-resistant bacteria. We are currently attempting to develop an alternative technique to measure the efficiencies by which these anaerobic digestors inactivate antibiotic-resistant bacteria.

The second goal of our research was to characterize the antibiotic-resistant bacteria in sewage. From the three treatment facilities, we isolated and identified 173 bacterial strains that were resistant to tetracycline. All of these bacterial strains were pathogenic (disease-causing—e.g., *Shigella* or *Klebsiella* spp.), possibly pathogenic (e.g., *Escherichia coli*), or non-pathogenic but related to pathogens (e.g., *Citrobacter* spp.). In more than 50% of these bacteria, we also detected at least one gene encoding for tetracycline resistance.

Based on these initial data, we then studied 14 different tetracycline-resistant bacterial strains in more detail. All 14 of these strains contained an integron and were resistant to at least three different antibiotics (we tested resistance to amoxicillin, ampicillin, chlortetracycline, enrofloxacin, erythromycin, sulfamethoxazole, trimethoprim, and tylosin). We also tested these bacteria for lateral gene transfer. Although this work is still ongoing, many of these bacterial strains are capable of

exchanging with other bacteria a gene encoding for tetracycline resistance.

Following our work on tetracycline-resistant bacteria, we isolated an additional 65 different bacteria that were resistant to ciprofloxacin. Ciprofloxacin is a relatively new antibiotic and there is not much known about bacterial resistance to it. Once again, we found that all of these bacterial strains were pathogenic, possibly pathogenic, or related to pathogens. We then focused our efforts on 11 of these strains, all of which were resistant to at least four different antibiotics. About half of these strains contained an integron or a gene encoding for resistance to tetracycline. Although this work is also ongoing, our analysis revealed that several of these strains were capable of laterally exchanging genes encoding for resistance to ciprofloxacin.

Conclusion and Policy Recommendations

Our research has demonstrated that extremely high numbers of antibiotic-resistant bacteria are released from municipal wastewater treatment plants, even when disinfection is performed. Our original hypothesis was that disinfection would adequately inactivate antibiotic-resistant bacteria in treated municipal wastewater, and that an outcome of our work would be to encourage the implementation of year-round disinfection. Instead, we learned that although a 99% inactivation looks encouraging, 1% of a very large number (10^{15}, or 1 quadrillion) still represents a very large number (10^{13}, or 10 trillion) of antibiotic-resistant bacteria that are released from the Metropolitan Wastewater Treatment Facility each day.

The bacteria that we studied were all pathogens or related to pathogens and all were resistant to multiple antibiotics. A substantial fraction of these bacteria (greater than 50%) harbored genes encoding for tetracycline resistance. These bacteria frequently harbored integrons (genes that allow bacteria to accumulate multiple genes for antibiotic resistance) and some of them were capable of transferring their resistance to other bacteria. The frequency of lateral gene transfer of ciprofloxacin resistance, which occurred in more than 40% of the strains we studied, is particularly worrisome because this trait is typically very rare (less than 1%) among clinical strains of ciprofloxacin-resistant *E. coli*. Simply put, the bacteria that we detected in municipal wastewater are some of the most resistant bacteria ever studied. There is a substantial need, therefore, to prevent these organisms from reaching the environment.

At first glance, the most obvious solution to the problem of antibiotic-resistant bacteria in treated municipal wastewater would be to require more stringent disinfection. The majority of municipal wastewater is disinfected using chlorine, which poses a security risk (chlorine gas is very dangerous) and generates disinfection by-products that are known or suspected carcinogens. Although we recommend a policy shift to include year-round wastewater disinfection, we do not recommend that more stringent disinfection regulations be imposed because of these unwanted consequences.

Instead, we recommend that wastewater effluents be passed through a sand filter prior to disinfection. Sand filters can physically remove antibiotic-resistant bacteria from treated wastewater, but without the use of potentially dangerous chemicals. At the present time, sand filters are rarely used in wastewater treatment, but they are commonly used at drinking water treatment facilities, so the technology is well-developed and well-understood. Additional research is needed, however, to optimize the removal/inactivation of antibiotic-resistant bacteria by our proposed combination of sand filtration and effluent disinfection.

Although our research on the fate of antibiotic-resistant bacteria in anaerobic digestors was inconclusive due to the limitations of our research method, we suspect that our ongoing research will demonstrate that thermophilic anaerobic digestors achieve substantially better inactivation efficiencies than conventional technologies. This ongoing research is particularly pertinent because of a recent shift in policy that emphasizes the application of treated wastewater solids to land rather than putting these residues into landfills—that is, the "environmental friendly" practice of applying wastewater solids to land may have unexpected and undesirable consequences in terms of the proliferation of antibiotic-resistant bacteria.

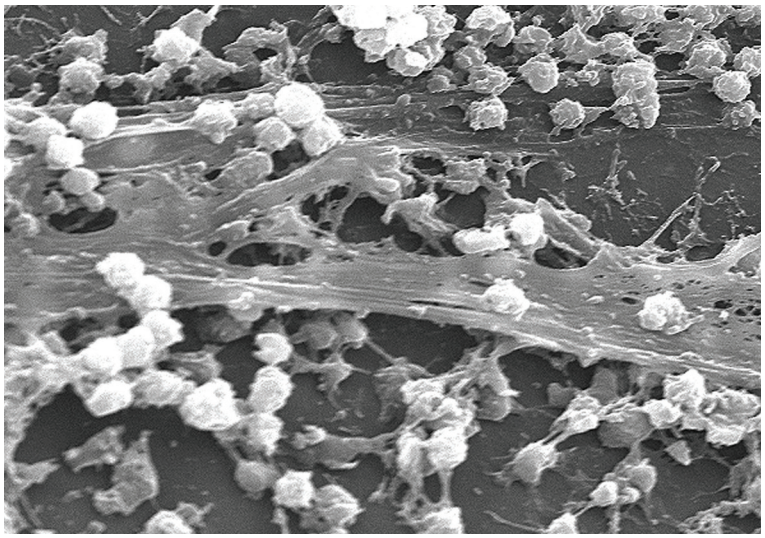
Timothy M. LaPara is associate professor in the Department of Civil Engineering at the University of Minnesota. His research focuses on the microbial ecology of wastewater treatment. **Sara J. Firl** was a graduate student in the Department of Civil Engineering at the University of Minnesota during this study. She currently works for Barr Engineering. **Leslie J. Onan** was an undergraduate student in the College of Biological Sciences at the University of Minnesota during this study. She is currently attending law school at the University of Michigan. **Sudeshna Ghosh** is a doctoral candidate in the Department of Civil Engineering at the University of Minnesota. **Tao Yan** is a post-doctoral research associate in the Biotechnology Institute at the University of Minnesota. **Michael J. Sadowsky** is Distinguished McKnight Professor in the Department of Soil, Water, and Climate and the Biotechnology Institute at the University of Minnesota. His research focuses on the genetics, genomics, and biochemistry of bacteria of environmental importance.

This study was supported by a grant from CURA's Faculty Interactive Research Program. The program was created to encourage University faculty to carry out research projects that involve significant issues of public policy for the state and that include interaction with community groups, agencies, or organizations in Minnesota. These grants are available to regular faculty members at the University of Minnesota and are awarded annually on a competitive basis. Additional support was provided by grants from the Undergraduate Research Opportunity Program (UROP) at the University of Minnesota.

A Microscopic Arms Race:
The Battle Against Antibiotic Resistance

LAURA STERNICK '08

In 1928, bacteriologist Alexander Fleming noticed that a culture of staphylococcus bacteria had been contaminated by mold. This rather mundane event stirred Fleming's curiosity when he noticed that the mold was dissolving the bacteria it touched. Fleming cultured the mold and found that it produced a substance that could kill several disease-causing bacteria (1), including pneumococci, streptococci, meningococci, and gonococci (2). He named the substance–the first antibiotic–penicillin, after the *Penicillium* mold that produced it (3). It would be the first of many antibiotics to revolutionize the field of medicine. After the discovery, Howard Florey and Ernst Chain studied penicillin's properties and developed the first antibiotic drugs. Penicillin was in mass production by the 1940s (1). Fleming, Florey, and Chain shared the Nobel Prize in Medicine in 1945 "for the discovery of penicillin and its curative effect in various infectious diseases" (4). The development of antibiotics is widely considered to be one of the greatest public health achievements of the past century. The use of antibiotics has led to a massive improvement in the treatment of infectious bacterial diseases and has made invasive surgical procedures much safer to perform. However, the efficacy of antibiotics has encouraged overuse. Doctors would prescribe them to treat minor infections, for which antibiotics are unnecessary. Moreover, livestock breeders give antibiotics to their animals in order to protect them from disease. The overuse of antibiotics has led to the evolution of resistant strains of bacteria. Strains now exist that elude even the strongest antibacterial agents. The World Health Organization estimates that two million people in the United States are infected with antibiotic-resistant bacteria, resulting in the deaths of 14,000 each year (5). Once an indispensable weapon in the fight against disease, antibiotics have ushered in a new public health threat.



Electron micrograph depicting large numbers of *Staphylococcus aureus* bacteria, found on the luminal surface of an indwelling catheter. The sticky looking substance is composed of polysaccharides, and is known as "biofilm." This biofilm has been found to protect the bacteria from attacks by antimicrobial agents such as antibiotics. Image magnified 2363x.

Image Couresy of CDC/Rodney M. Donlan, Pd.D.; Janice Carr

A recent article in *Scientific American* describes a report from Colorado State University about the presence of DNA that promotes antibiotic resistance in drinking and non-drinking water. Amy Pruden, the primary author of the study, chose to look for the genes that create antibiotic resistance, rather than for antibiotics themselves since this approach provided more accurate information about the amount of antibiotic resistance in these water sources. DNA samples were obtained from bacteria found in bodies of water in northern Colorado. The researchers looked for genes that conferred resistance to tetracycline and sulfonamide to bacterial hosts. According to the report, bodies of water in close proximity to urban areas or farming regions had levels of drug-resistance genes that were hundreds to thousands of times higher than those of isolated bodies of water. However, antibiotic resistance genes were found in every water source tested (5).

The study links the increase in antibiotic resistance to the overuse of drugs in humans and animals. According to *Scientific American*, "up to 95 percent of antibiotics are excreted unaltered, seeping into the environment and possibly encouraging antibiotic resistance there" (5). Microbes that are able to survive the dose of antibiotics that they encounter will be selected for survival.

The presence of antibiotic resistance genes in drinking water is particularly troubling. Water filtration systems are designed to remove bacteria and toxic minerals, rather than DNA, which can pass through undetected. Pruden and her team are currently working to develop water treatment methods using ultraviolet light, peroxides, or both to clean up contaminated water (5). Others are working to prevent antibiotics from reaching water sources in the first place. A movement to end the use of antibiotics in livestock is gaining momentum. In Europe, a January 2006 law made it illegal to give antibiotics to animals for nontherapeutic purposes. The antibiotic avoparcin, a relative of vancomycin, has been

outlawed since its overuse was correlated significantly with an increase in vancomycin resistance in microbes in the human intestine (6).

But antibiotic resistance threatens more than our water supplies. Hospitals have become breeding grounds for antibiotic-resistant bacteria. These strains are associated with increased lengths of stay, higher costs, and mortality (7). According to data from the Centers for Disease Control and Prevention (CDC), the proportion of infections that are resistant to antibiotics has increased (7). One such strain is known as methicillin-resistant *Staphylococcus aureus* (MRSA), which has become more common in recent years. In 1974, MRSA infections represented 2% of the total number of staphylococcus infections; in 1995 it was 22%; in 2004 it was about 63% (8). Ironically, it was *S. aureus* that was killed by penicillin in Fleming's original culture (3).

The most common sources of MRSA in hospital settings are patients who are infected with MRSA but who do not display symptoms. It is transmitted to others most commonly by healthcare workers who come into contact many patients and families each day. Individuals most susceptible to MRSA infection are those with severe disease, particularly those with compromised immune systems. Groups with the highest infection rates include: individuals recovering from surgery, individuals who have internal medical devices, such as urinary catheters, and hospitalized patients. (7).

In terms of pathology, MRSA produces different symptoms in patients compared to methicillin-susceptible *S. aureus* (MSSA) strains. Individuals with MRSA infections are more likely to develop symptomatic infections. Higher fatality rates are also associated with MRSA infections, compared to MSSA infections. A number of studies offer explanations for these observations. One suggested that the delayed delivery of vancomycin, which is considered the "last-hope treatment" for MRSA (6) might be responsible. The same study also mentioned the possibility that vancomycin has decreased in efficacy against the strains of MRSA in question (7). Another study hypothesized that the particular bacteremia associated with the strains of MRSA involved in the study was particularly lethal (7).

Interestingly, genetic analyses suggest that only a small proportion of the MRSA strains found in hospitals contained mutations that promote peculiar aggressiveness or persistence, which could allow them to transmit to other individuals easier. It was also found that most MRSA strains were genetically unique compared with strains prevalent in hospitals. This suggests that the MRSA strains may have been the product of MSSA strains that acquired genes for antibiotic resistance (7).

The CDC has taken steps to monitor and reduce the presence of MRSA in healthcare settings. It conducts extensive surveillance of MRSA transmission rates and has published guidelines for hospitals on how to deal with MRSA infections. CDC also conducts epidemiologic and laboratory research on MRSA in hope of finding ways to actively treat infections (9).

Some believe that the hospitals and public health organizations in the U.S. are not doing enough to prevent MRSA transmission. Experiments with new containment measures in European hospitals have succeeded. In Denmark and the Netherlands, hospitals isolate all incoming high-risk patients until lab results confirm that they are not infected with MRSA. Currently, less than 1% of staphylococcus infections in Dutch hospitals are MRSA, compared to 64% of staphylococcus infections in the U.S. (6). According to *Scientific American*, American hospitals have been hesitant to follow the European example, citing the high costs of such intense screening. However, considering that MRSA infections increase costs of care, it might be economically viable to implement an extensive screening process (6).

Physicians and researchers are engaged in an arms race with bacteria, and bacterial evolution has begun to outstrip human scientific progress. At the moment, the best defense against antibiotic-resistant bacteria is prevention of transmission. Public health agencies around the world are working to find more aggressive treatments, but unless a means of stalling evolution is found, the battle with bacteria is far from over.

CDC conducts MRSA surveillance, prevention, epidemiologic and laboratory research, and outbreak and laboratory support. For more information, see http://www.cdc.gov/ncidod/dhqp/ar_mrsa_CDCactions.html

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Prescription drugs found in soil sludge used for lawns and gardens

by Jerome Douglas

(NewsTarget) According to research by Chad Kinney, assistant professor of chemistry and biochemistry at Eastern Washington University, fertilizer made from sewage sludge may be adding pharmaceuticals, flame retardants and other chemicals to the land.

Kinney's research showed that no less than nine different biosolid products were produced by municipal wastewater treatment plants in seven different states -- Washington, Arizona, Wisconsin, Kansas, Colorado, Texas and Iowa. These biosolid products were analyzed for 87 different organic wastewater contaminants, which represents a cross section of medicinal, industrial and household compounds.

These compounds are able to enter wastewater treatment plants and may be discharged without being completely metabolized or degraded -- causing them to show up in the sludge that is then processed into certain garden and yard fertilizers. In fact, 55 of the contaminants were detected in at least one biosolid product sold as lawn and garden enhancements, and 25 compounds were found in every single one of the samples.

Kinney went on to say that "No matter what biosolid we looked at, there were some of these compounds in it." His research was published in online edition of the journal Environmental Science and Technology. Kinney, who is a postdoctoral fellow at the United States Geological Survey (USGS), has the support of the USGS's Toxic Substance Hydrology Program as well, who supports his research.

Government regulators and health officials say there is no immediate risk to public health; however, the study's authors called for more research on the long-term impact on the environment. Thomas Burke -- a professor of public health policy at Johns Hopkins University in Baltimore -- believes that Kinney's research is a sobering reminder for the Environmental Protection Agency, which has promoted biosolids for decades because they contain the same nutrients found in fertilizers.

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Executive Summary

Chemicals of Concern

Iowa's First Field Research Area for Emerging Contaminants

Douglas Schnoebelen
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Larry Barber
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The Iowa Policy Project
318 2nd Avenue North, Mount Vernon, Iowa 52314
319-338-0773
www.iowapolicyproject.org

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Chemicals of Concern
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By Douglas Schnoebelen, Dana Kolpin, Larry Barber, Edward Furlong, Michael Meyer and Mary Skopec

Emerging Contaminants: Chemicals of Concern

As Americans, we use a wide variety of chemicals in our homes and our jobs, whether we work at factories, on farms, or in offices. Recent research has shown compounds not previously considered contaminants are present in the environment.[1] These include human and veterinary prescription drugs, diagnostic agents, hormones, cosmetics, dyes, preservatives, detergents, and numerous other organic compounds. There are increasing concerns about the potential environmental effects that may occur from such "emerging contaminants" (ECs). ECs are defined as:

> Any synthetic or naturally occurring chemical or any microorganism that is not commonly monitored in the environment, but has the potential to enter the environment and can cause suspected adverse ecological and/or human health effects. In some cases, release of emerging chemical or microbial contaminates to the environment has likely occurred for a long time, but may not have been recognized until new detection methods were developed. In other cases, synthesis of new chemicals or changes in use and disposal of existing chemicals can create new sources of emerging contaminates.[2]

Most ECs are not routinely monitored. Indeed, water-quality monitoring in the United States is largely driven by regulations of the Clean Water Act and Safe Drinking Water Act. Over the last three decades, much of the water-quality monitoring work has focused almost exclusively on the conventional "priority pollutants," however this is only one piece of the larger environmental puzzle.[3] Recently ECs have begun to be examined in limited studies using newly developed laboratory analytical methods and techniques allowing detection at much lower levels. Furthermore, the possibility that environmental contaminants may be complex mixtures that can interact synergistically or antagonistically has increased the need to understand ECs.

In order to minimize ecologic effects from ECs, it is essential to understand how a contaminant moves and is altered in the environment. Investigations of processes influencing transport (e.g. sorption, dispersion, degradation, etc.) require a systematic evaluation of a variety of hydrologic, landscape and anthropogenic factors. The purpose of this paper is to provide a short synopsis of ECs as potential contaminants of concern and to highlight an 8-km reach of Fourmile Creek in central Iowa as an ideal research site to investigate the transport, fate and effects from an urban source of ECs.

Possible Effects of ECs: Endocrine Disruption and Antibiotic Resistance

The potential toxicological behavior from the environmental occurrence of ECs and mixtures of ECs are largely unknown. In particular, the effects of ECs on aquatic organisms are difficult to measure because concentrations of these compounds are generally low (nanogram per liter range) and, over the life of the organism produce no acutely toxic effects. However, detrimental effects to organisms from ECs may be subtle and go unnoticed until some cumulative threshold is reached. In recent years, the presence and effects of endocrine disrupting compounds (EDCs) in the environment has become an important issue.[4] The endocrine system is the "key control system" of most organisms.

The presence of low concentrations of some chemicals in the environment (e.g. natural and synthetic hormones, alklyphenols, pesticides, solvents and pharmaceuticals) could affect or damage the function of the endocrine system.[5] For example, nonylphenol (a detergent degradation product found in laundry and dish detergents), and AHTN (a polycyclic musk found in perfumes, laundry products, air fresheners and cosmetics) have been shown to disrupt reproduction and growth in fish by affecting endocrine systems.[6] A variety of ECs have been shown to bioaccumulate in fish tissue.[7] Data from laboratory experiments suggest that EDCs in the aquatic environment may impact the reproductive health of fish populations.[8] Linking EDCs to observed changes in fish populations, however, remains an open challenge.[9] As the ecological risk assessment of EDCs is in its infancy stage, less is known about potential effects to other aquatic species, yet early research suggests effects to aquatic organisms are possible.[10]

Antibiotics are an important class of pharmaceuticals and their prevalence in the last 60 years has brought dramatic and often even "miraculous" progress in fighting bacterial infections in humans and animals. In livestock farming, sub-therapeutic doses of antibiotics are often used to promote more rapid animal growth.[11] Despite their widespread use, antibiotics have only recently received attention as environmental contaminants. However, the increase of resistant bacterial strains and the spread of bacterial resistance have become a worldwide concern.[12] Concerns also exist for antibiotic use and increasing antibiotic resistance in livestock confined feeding operations.[13] Many antibiotics are only partially metabolized after administration to humans or animals.[14] Concentrations of select antibiotics in animal manure have been reported at milligrams per liter levels (they are typically reported at parts per billion levels).[15]

Antibiotics can reach streams and ground water via a variety of mechanisms and the potential for the aquatic environment to promote or maintain antibiotic resistance is largely unknown. Some chemicals, such as triclosan (an antimicrobial disinfectant found in many liquid soaps, dishwasher powders and plastics), are suspected of increasing the antibiotic resistance of bacteria in the environment,[16] reducing algae diversity in streams,[17] and affecting natural ecosystem functions such as soil microbial activity.[18] In addition, research has shown effects of mixtures of antibiotics to aquatic organisms.[19]

Evolution of Fourmile Creek as a Research Site for ECs

Following a national stream reconnaissance study,[20] water samples were collected in 2001, upstream and downstream of select towns and cities in Iowa during low-, normal- and high-flow conditions to determine the contribution of urban centers to concentrations of ECs in streams under varying

flow conditions.[21] This study found the number of ECs detected decreased as streamflow increased from low- (51 ECs detected) to normal- (28) to high-flow (24) conditions. Fourmile Creek near Ankeny, Iowa, was initially sampled for ECs during this study and results showed a strong gradient in EC detections during low-flow conditions between samples collected upstream of Ankeny (three ECs detected) compared to samples collected downstream (31 EC detected).

The initial EC results from Fourmile Creek,[22] led to including this stream as part of collaborative research between the U.S. Geological Survey (USGS) and the U.S. Environmental Protection Agency to better understand the fate of ECs following their discharge from wastewater treatment plants (WWTPs).[23] This research involved collecting four samples at each of 10 WWTPs across the nation: upstream of the WWTP, at the WWTP where effluent was being discharged into the stream, at a location in close proximity downstream of the WWTP, and at a location farther downstream from the WWTP. All samples were measured for 110 different ECs. Between 28 and 50 ECs were found in treated wastewater effluent being discharged to streams.[24] The similarity in chemical concentrations between WWTP effluent and proximal downstream sampling points clearly shows the contribution of WWTPs to EC concentrations in streams. Additional knowledge gained from Fourmile Creek during this study included:

1. the ECs detected in Fourmile Creek during the previous study[25] were primarily derived from the Ankeny WWTP (see Figure 1),
2. there are significant reductions of the number of ECs detected and total EC concentrations through the 8.4 km study reach (Figure 1),
3. ECs vary in their type of transport (conservative versus nonconservative) through the study reach (see Table 1),
4. at low-flow conditions, greater than 90 percent of the streamflow is derived from WWTP discharge.[26]

Figure 1. Compounds and Concentrations of Emerging Contaminants at Various Locations of Fourmile Creek

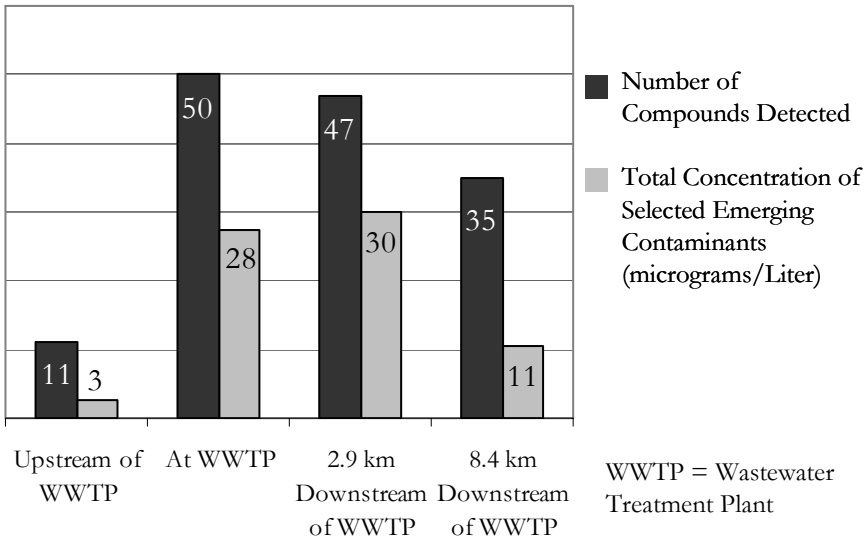

Table 1. Selected Compounds Detected, Primary Use, Reporting Level, and Concentration from Samples Collected at Various Locations of Fourmile Creek

Compound	Primary Use	Reporting Level (μg/L)	Upstream of WWTP	WWTP Effluent	8.4 km Downstream of WWTP
			(concentration in (μg/L)		
Cimetidine	Antacid	0.012	undetected	0.123	0.107
Dehydronifedipine	Antianginal	0.015	undetected	0.202	0.018
Diltiazem	Antihypertensive	0.016	undetected	0.053	0.029
Diphenhydramine	Antihistamine	0.015	undetected	0.218	undetected
Sulfamethozole	Antibiotic	0.064	undetected	0.589	0.321
Tonalide (AHTN)	Fragrance, musk	0.500	undetected	2.300	0.700
Trimethoprim	Antibiotic	0.013	undetected	0.353	0.093

In 2003, the USGS EC Project[27] was searching for a real-world setting to investigate the complex in-stream processes (e.g. dilution, sorption, degradation, dispersion, etc.) that can affect ECs following their discharge from a WWTP and determining if such input is having an effect on the aquatic ecosystem. Such research requires the integration of multi-disciplinary efforts at a carefully selected field site. Knowledge gained from previous research[28] and other unique aspects of Fourmile Creek led to its selection as a field setting to help answer these important research questions. Critical aspects of Fourmile Creek include the following:

1. A single source WWTP effluent-dominated stream. This allows for the examination of EC concentrations as water moves downstream without complications from additional inputs.
2. Data documented the input of a wide variety of ECs from WWTP discharge. Previous research found between 3 and 10 ECs present upstream of the WWTP and between 30 and 50 downstream.[30]
3. Small basin size (less than 160 km^2 size). This facilitates an increased understanding of the transport and fate of environmental contaminants.
4. Relatively simple flow system. Little to no ground-water or surface-water inputs to streamflow exist in Fourmile Creek during normal flow conditions. Thus, any changes in EC concentrations observed downstream can be attributed to in-stream processes.
5. Data documented that ECs vary in their type of transport. Undefined processes are taking place within the stream that affect EC concentrations.
6. The WWTP uses a treatment technology (conventional activated-sludge) typical of many towns and cities across the United States. Thus, the source is representative of many similar sources in the United States.
7. The hydrogeologic setting (low-gradient stream, glaciated deposits, rowcrop agriculture) is typical of the Midwest.
8. A low-head dam exists approximately 2 km upstream of the WWTP outfall. The dam provides a physical barrier to fish migration. Thus, comparisons in fish community structure and fish health assessment can be made to more accurately determine potential effects from the input of ECs by the WWTP. Research has found a range of abnormalities in fish populations (vitellogenin induction in males and juvenile females, development of oocytes in testes, etc.) downstream of WWTPs.[31]

9. A major change is anticipated to the primary source of ECs in the system. Around 2010, the WWTP is scheduled to close. This closure provides a unique opportunity to examine how a stream and aquatic biota react to the removal of the primary source of ECs and allows a novel "before" and "after" assessment not been previously available in EC research.

Future Work

Future work on ECs will involve not only the occurrence of these compounds, but also their fate, transport and possible effects in the environment. Several large-scale studies in the United States by the USGS Toxic Substances Hydrology Program have already documented the occurrence of ECs in the environment.[32] These studies have shown that a wide variety of ECs are commonly detected in streams, streambed sediment, and ground water as complex mixtures of compounds. Other studies have documented the occurrence of ECs globally.[33] Many of these same EC compounds have been detected in a study of Iowa's streams.[34] Indeed, the data on ECs collected at Fourmile Creek are consistent with similar national studies. However, the effects of long-term, low-level exposure to these mixtures of emerging contaminants on aquatic life and humans are currently unknown. Research on the effects of ECs in the environment is only in the beginning stages.

The field research site established at Fourmile Creek will continue to build a framework for better understanding of the transport, fate, and effects of ECs in the environment. One goal of the field research site at Fourmile Creek is to move beyond documenting the occurrence of these compounds to examine what happens to these compounds once they enter the environment and their potential effects to aquatic ecosystems.

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The case against land application of sewage sludge pathogens

Maureen Reilly BA *University of Toronto, Toronto, Ontario*

There is currently a public debate about whether health, agricultural and environmental authorities should continue to allow sewage sludge to be spread on farmland. Some of the concern in the debate is about the pathogen content of sewage sludge. This concern was heightened by the tragedy at Walkerton where *Eschericha coli* 0157:H7 and other pathogens contaminated the drinking water supply of this Ontario town. The Canadian public were reminded how vulnerable they can be to disease when agricultural practices ajoin population centres without adequate health and environmental controls.

But pathogens are not the only contaminants of concern in sewage sludge. Sewage sludge also contains potentially harmful levels of toxic metals and environmentally persistent chemicals such as polychlorinated biphenyls and dioxins (1).

In the past, farm application was often not the favoured method of disposal. Sludge was incinerated or landfilled, and only a small percentage was applied to farmland. With air quality concerns increasing and landfill capacity at a premium, municipalities have moved to farm disposal of these waste sludges, especially in Ontario, where almost all wastewater sludges are applied on farms.

Have the risks to human health been adequately evaluated? Should government promote the transfer of these wastes to the countryside? Is it reasonable to ask rural residents to live next to sites where human excrement mixed with industrial waste is stockpiled and spread on the land?

Background

Flush toilets were invented almost 200 years ago, yet we as a society are still trying to manage the health and environmental impact of our own waste. The whole idea of 'public health' developed in response to the contagion created by allowing urban streets to flow with septic wastes. Eventually, the crises in pests, disease and plagues led to the creation of an infrastructure of sewers to remove these wastes and confine them away from the public in a sewage treatment plant.

Over the past 20 years, with the public desire to protect surface waters from sewage contamination, sewage treatment plants (accurately renamed wastewater treatment plants) are designed to separate the water in the sewer system from the solids and contaminants, and return the water fraction the lake, river or ocean in a relatively purified form. Sewage sludge, sometimes called 'biosolids', is the solid fraction of this waste. Wastewater treatments plants are not designed to treat the the sludge effectively; they are primarily designed to clean the water fraction.

What 'treatment' is required?

The industries that promote the placement of sewage on rural and agricultural land like to speak of 'treated' sewage, 'treated' sludge or 'biosolids'. However, there is little in provincial statutes that sets out what 'treatment' may be required before sewage is placed on land. Requirements for

sewage treatment vary from one wastewater facility to the next and may or may not be detailed in a Certificate of Approval or permit from the provincial Ministry of the Environment.
In Ontario, septage, which is untreated sewage waste pumped out of septic tanks and abbatoir waste, is also allowed to be spread or sprayed on rural land. No treatment is required.
Often, the Ontario government adopts the American requirement for 'treatment' that the sludge should contain less that two million fecal coliforms colony forming units/g. However, not all sewage treatment plants achieve this level. The Ashbridges Bay Treatment Plant in Toronto, Ontario processes sludge for about 1.7 million people. There are not enough digesters to allow for the recommended minimum 15 to 30 days of digestion of sludge in anaerobic tanks. Therefore, Toronto sewage sludge may receive as little as eight to 10 days of digestion (personal communication, Interim Compliance and Monitoring Committee, City of Toronto, Toronto, Ontario). The Ontario Ministry of the Environment has facilitated the spreading of this sludge with elevated pathogens by allowing the City of Toronto to average the test results according to a specific formula, so rural residents have no assurance that specific standards of disinfection have been met by sludge delivered to farms.
The fecal coliform levels in the City of Toronto sewage sludge have often exceeded even the above permissive standards. In 1999, levels of fecal coliform exceeded two million colony forming units/g, even when the city was allowed to average the test results over several weeks.
Toronto sewage has been trucked to a facility in Halton, Ontario where it was stored in an open pit the size of a football field. Neighbours of this facility have protested because foul odours have stopped them from being able to leave the house or open the windows. Residents near the facility have become seriously ill with enteric viruses, which they are concerned may be the result of their exposure to the sludge (personal communication, Laura Eagles).

Do wastewater treatment plants 'treat' sludge?

Scientists at the University of Ottawa Faculty of Medicine prepared a research report for the City of Ottawa on whether the Ottawa Pickard Centre sewage treatment plant was effective in reducing pathogens in Ottawa sewage sludge (2). Their report concluded that "it is clear that the pathogen content of biosolids from the Pickard Centre may be relatively high.". The report also concluded that there was no reduction on giardia cyst levels, only a 49.9% decrease in cryptosporidium oocyst densities and only slightly reduced levels of somatic coliphages, suggesting a similarly small reduction in enteric virus loading in the land applied sewage (3).
A more detailed report on the health risks from pathogens associated with land application of sewage sludge can be found in "Hazards from pathogenic microorganisms on land-disposed sewage sludge" (4), which outlines the range of pathogens found in sludge and their longevity in the soil.
A study of sewage workers at the Toronto Ashbridges Bay Treatment Plant showed increased incidence of disease com-pared with a similar group of workers not exposed to biosolids (5). The United States National Institute for Occupational Safety and Health has posted a health alert for those who work with biosolids (6). *The Journal of Agromedicine* also published a report linking odours with health risks (7). The research has implications for those workers, farmers and rural residences who are exposed to sewage sludge.

RISKS FROM SEWAGE SLUDGE

Venues for pathogen transfer: Land application of sewage sludge can lead to the transport of pathogens through bioaerosols downwind of sludge storage or spreading sites, through contamination of ground water, drinking water wells, stockponds and surface waters, or through food contamination from eating food grown in sludge spread land. Pathogens can be transported to

humans who walk through sludge spread fields. Wild animals, farm animals, birds, rodents and pets may become infected by or transmit sludge pathogens. There is no requirement to post signs that sludge has been spread or stored on fields in Canada. Therefore, children, family members and others may access and traverse sludge spread sites unknowingly.

Bioaerosol risk: A recent study from the University of Arizona (8**)** shows that the risk of windborne pathogens is significant within a 10 km radius of a sludge spread field. In Canada, the relatively cooler and wetter climate may make this risk even higher.

Risk to water: Sewage sludge contains a high concentration of wastes; indeed, a 100-acre spread of sewage sludge represents the annual fecal output of approximately 1400 people plus the industrial waste component. To compare sludge with manure, a field of similar size would support 100 cattle.
Some provinces have 'guidelines' or other criteria that are intended to address the environmental and health issues related to the land application of sewage sludge. However, these 'guidelines' are not always enforceable, and most are not legally binding. Ontario has one of the most detailed documents on the land application of sewage sludge and other organic waste. However, the *Guidelines for the Use of Biosolids and Other Wastes on Agricultural Land* (9) are only a 'guidelines'. They are not statutes or regulations. In Ontario, even after the Walkerton tragedy, reductions were allowed in the the separation distances between sludge spreading sites and drinking wells, lakes, rivers and surface waters. Ottawa and Toronto are currently allowed to spread sludge as close as 15 m to a drilled drinking water well and as close as 15 m to surface water despite provincial law that stipulates greater, more protective distances (10).

Risk from food: The Ontario Ministry of the Environment and the Ministry of Agriculture recommend that farmers observe a waiting period between sludge spreading and the harvesting of some crops, and recommend that farmers restrict farm animals from fields recently spread with sludge. These recommendations are not enforceable under current legislation. Therefore, sludge spread fields can be used to grow small fruits such as strawberries or pasture livestock, and thus expose the consumer to contaminated produce and meats.

Animal transfer: Farm animals and pets such as cats and dogs can be expected to continue to travel through fields, even after the fields have been spread with sludge, and return to their owner's homes, spreading sewage sludge to the family home. Birds may also visit sludge spread fields, and spread viruses, bacteria and parasites in their subsequent droppings.

Issues of concern

Lack of research: The Environmental Protection Agency in the United States, the Ontario government and the Water Environment Association of Ontario have all acknowledged that pathogen risks from land application of sewage sludge have not been adequately evaluated. In both Canada and the United States, policies concerning land application are under review for this reason. In the United States, the National Academy of Science is conducting a risk assessment on the land application of sewage sludge. In Ontario, just a few months after Walkerton crisis, the Ontario Ministry of the Environment announced a review of the regulations concerning the land application of sewage sludge, septage and all biosolids.

Lawsuits in the United States: It is thought that several deaths in the United States have been attributed to the land application of sewage sludge, and lawsuits have been brought (11). One of these cases, concerning the death of Shayne Connor, who lived adjacent to a sewage sludge

spreading site, is currently making its way through the New Hampshire courts. Other lawsuits involving livestock deaths have also been filed in Georgia.

Regrowth: A study conducted in Australia in 1997 looked at pathogen regrowth in soils that had been spread with sewage biosolids (12). Researchers found that during hot, dry months, there were reduced levels of pathogens (fecal coliforms and salmonellae) in stored and spread sewage sludge, but that the concentrations of pathogens rose during the wet cool months that followed. In some instances, the pathogen regrowth was to levels higher than found in the initial tests.

No informed consent: Farmers and rural residents are told that the sludge is treated for pathogen reduction. There is no requirement to explain the health risks to farmers or those who live near sludge spreading or storage sites.
Occupational health and safety issues related to sewage sludge are starting to be addressed with the recent Health Alert from the National Institute for Health and Safety. Documents like the Safety Alert issued by the Spokane Regional Health District in the State of Washington are increasingly common in the United States and Great Britain. (13). However, action on public health education on sludge lags behind the occupational health and safety requirements for sludge handlers. The public needs to be informed of the pathogenic risk posed by sludge transporatation, storage and spreading.

No storage: When rainy weather makes sludge spreading particularly risky for runoff and ground water contamination, most municipalities do not have adequate disposal alternatives for sewage sludge. The City of Toronto sludge has been evicted from the Halton storage facility, and the City of Toronto hauler is now storing sewage sludge in open piles in Ontario farmfields. The Ontario Ministry of the Environment has allowed the Toronto sewage sludge to be stored in huge open piles on farmland in Northumberland County near Warkworth (14).

CONCLUSIONS

Open field storage of sludge and sludge spreading near wells and surface water, increase the risk that sewage sludge pathogens will be transported to workers, farmers and neighbours, and increases the environmental risks of this wastedisposal practice. It seems unreasonable to offer the farm community 'free fertilizer' and promote its use when the public health risks associated with the land application of sludge have not been addressed. The case against land application does not stop with the issues posed by pathogens; other heath risks are associated with the elevation of heavy metals in the soils and foods, the release of mercury into the atmosphere from sludge spreading and the presence of priority pollutants in the land-applied sludge.
We seem to have forgotten the public health lessons of the 1800s and the underlying reason for sewage treatment plants themselves. The pathogen levels in sewage sludge are high and can even increase over time once the sludge is stored or applied to land. Land application allows sludge pathogens to be transported by weather events, and facilitates the spread of diseases to animals and humans. It is unreasonable to expect rural residents to tolerate exposure to these diseases in land, air and water. The Canadian Infectious Disease Society should be applauded for its call for a moratorium on sludge spreading until the risks to public health and the integrity of food are thoroughly evaluated.

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Lea Brooks, a spokeswoman for the state Department of Public Health, said recycled water is treated but can contain bacteria and viruses that cause diarrhea and other illnesses.

News | San Diego

Otay water district officials investigate water foul-up

Stores may reopen if samples test clean

By Anne Krueger
UNION-TRIBUNE STAFF WRITER

August 23, 2007

CHULA VISTA Otay Water District officials spent the day yesterday trying to determine how a Chula Vista business park had treated sewage flowing from its water taps for two years, and how to ensure it doesn't happen anywhere else.
“We thought we had a good process,” said Mark Watton, Otay's general manager. “Obviously, there's a gap.”
The 17 stores at Fenton Business Center in Eastlake were ordered Friday not to drink or wash their hands with tap water after tests from a private lab showed they were getting recycled water, or treated sewage, instead of drinkable water.
Otay officials are investigating, but it appears a mis-marked pipe was the cause.
Two food-related businesses “ the Candy Bouquet, which creates candy baskets, and Dream Dinners, a meal-preparation store “” were shut down by the county.
If recent water samples are clean, the state Department of Public Health is expected to declare the water safe today, allowing the stores to reopen.
Otay serves about 190,000 people in a 125-square-mile district ranging from El Cajon to eastern Chula Vista.
Watton said the district's approximately 500 meters for recycled water will be inspected. All pipes carrying recycled water are in eastern Chula Vista along thoroughfares, on public property or in business areas, not in residential sections.
Recycled water flows through a separate system of distribution pipes, colored purple to distinguish them from pipes carrying drinking water.
Watton said when the business park was built in 2002, a purple plastic sleeve placed over the copper recycled-water pipe somehow slipped below ground when the pipe was installed. Whoever hooked the pipe to the water meter didn't realize the pipe carried recycled water.
The district still hasn't determined how its inspectors or supervisors for the contractor on

the project could have overlooked the error, he said.
Some business owners are concerned a former Otay inspector who pleaded guilty to a bribery charge could be at fault.
In 2005, Otay inspector William Cooper pleaded guilty to taking $5,000 in exchange for overlooking deficiencies in underground water and sewer lines on two Chula Vista residential developments.
Watton confirmed Cooper was one of the inspectors on the Fenton Business Park project. He said he has not determined whether that is related to the bad connection.
When the business park opened in July 2005, Otay blended recycled water with drinkable water. In May, Otay reached a deal to buy recycled water from the city of San Diego, and began flowing 100 percent recycled water through its purple pipes.
When the district first received a complaint about the business park's water July 27, Otay employees tested the water at the main line on the street. The district doesn't usually test water at the tap in response to a complaint, Watton said.
The main line carried drinkable water, so the tests showed nothing wrong. Watton said Otay employees thought the water might be stagnant because the park was new and many of the offices unoccupied.
The property manager sent the water to a private lab, and the results that came back Friday showed the water contained coliform bacteria, which indicates contamination.
Recycled water is safe for full body contact, though it's meant for irrigation and manufacturing, not consumption.
Lea Brooks, a spokeswoman for the state Department of Public Health, said recycled water is treated but can contain bacteria and viruses that cause diarrhea and other illnesses.

Jane Ballard, who owns a sign company in the business park, said she's told her 10 employees to see their doctors. Watton said the district would pay for the exams and compensate businesses for their losses.

“We just want to know that we're all OK,” Ballard said. “Everybody is just kind of a little tense waiting.”

Anne Krueger: (619) 593-4962; **anne.krueger@uniontrib.com**

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MORE PHOTOS

Cary has 'weird' water mix-up

TOBY COLEMAN, Staff Writer

CARY - The bitter water pouring from Vinay Jain's tap was a water conservation measure gone foul.

For nearly five months, the Jain family home in Cary has been connected to the town's reclaimed-water system. That is the treated wastewater the town considers clean enough for suburban lawns but not good enough to drink.

"I had a suspicion because when we moved, I said, 'The water tastes weird,' " said Jain, a 37-year-old information technology worker. "My wife said, 'You always complain.' "

Jain lived with the funny-tasting water until Monday. Then, suddenly, the water in the house went off. Somebody had shut off the neighborhood's irrigation pipe.

Cary water workers discovered the goof after Jain's neighbors, who had water inside, realized that their sprinklers weren't spraying. Cary officials then started scrambling around town to see whether any of the other 500 homes with irrigation systems served by reclaimed water had reversed water connections.

By Tuesday afternoon, Public Works head Mike Bajorek seemed confident that the Jains were the only ones in Cary with treated wastewater coming out of their taps and drinking water coming out of their sprinklers.

"We believe that this is a unique situation," he said.

Just to be sure, though, Cary workers were completing a house-by-house check Tuesday.

Cary is the only town in the Triangle that pipes reclaimed water into residential neighborhoods to help conserve drinking water. Residents connected to the system do not have to obey outdoor watering restrictions, and they pay less for the water they use to quench their lawns. But they're never, ever supposed to wash with the stuff, much less drink it.

Cary officials say the risk from reclaimed water is low. By one estimate, you would have to drink 12 gallons of reclaimed water in a single sitting to get an infectious dose of coliform bacteria. But it is serious: State regulations ban water systems from distributing reclaimed water for consumption.

The Jains have been unnerved by the revelation. Jain and his wife, Priyanka, wonder whether their children's dinnertime claims of bellyaches were legitimate reactions to low-quality water and not the childlike ploys to avoid eating unwanted food, as they originally suspected.

"In a place like Cary, it never even occurred to me that this might even be a possibility," Vinay Jain said Tuesday as Cary workers walked through his house testing taps. "This gives the impression of a Third World country. At least in India, we knew the water was bad, and we boiled it."

The town is putting the Jains up in a hotel while the problem is repaired. The Jains said they will be able to move back in Thursday, once the town has completed follow-up testing and inspections.

It might take longer to figure out how the foul-up occurred. Currently, water workers think somebody switched the Jains' black drinking-water main and their purple reclaimed-water main between their water main installation and their final home inspection.

But why would somebody do that? And why didn't anybody catch the mistake earlier?

"That," Bajorek said, "is what we're trying to determine."

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From: http://www.livescience.com/health/061023_antibiotic_spread.html

DNA Found in Drinking Water Could Aid Germs

By Charles Q. Choi, Special to LiveScience

posted: 23 October 2006 08:28 am ET

DNA that helps make germs resistant to medicines may increasingly be appearing as a pollutant in the water.

This DNA was found "even in treated drinking water," researcher Amy Pruden, an environmental engineer at Colorado State University in Fort Collins, told *LiveScience*.

The spread of this DNA could exacerbate the already growing problem of drug resistance among potentially infectious microbes. Diseases once considered eradicated, such as tuberculosis, are making alarming comebacks. Currently, more than two million Americans are infected each year by resistant germs, and 14,000 die as a result, the World Health Organization reports.

"I personally have known people with antibiotic-resistant infections, and they can be very scary," Pruden said.

Resistant microbes

While antibiotics kill off many germs that have no resistance against them, they also prompt the spread of microbes that are resistant. The over-prescription or other improper use of these drugs helps these resistant infections emerge, but experts also note that up to 95 percent of antibiotics are excreted by humans and animals unaltered, seeping into the environment and encouraging antibiotic resistance there.

Pruden's new research did not focus on the presence of antibiotics in the environment. Instead, she looked for the presence of genes that help confer drug resistance to the germs in the first place. Bacterial genes are encoded as DNA, and microbes often swap genes with each other. In principle, antibiotic-resistance genes could persist and spread long after the drugs they target have dissipated.

"The spread of antibiotic-resistance genes in the environment is undesirable, just

as is that of any other pollutant, such as PCBs or mercury," Pruden said.

Pruden and her colleagues focused on genes conferring resistance against two antibiotics, tetracycline and sulfonamide, which are linked to urban and farm activity. They investigated a range of northern Colorado waters, from relatively pristine river sediments to water from dairy lagoons to irrigation ditches. They also looked at water from drinking-water treatment plants and effluents from a wastewater recycling plant.

Everywhere

The levels of antibiotic-resistance genes were hundreds to thousands of times higher in waters directly impacted by urban or farm activity than in relatively pristine waters. Still, the researchers discovered the presence of antibiotic-resistance genes in all the waters they investigated.

"Wastewater treatment systems are not designed to treat antibiotic-resistance genes. The treated effluent is usually chlorinated, but even though this inactivates bacteria, it does not destroy DNA," Pruden explained. The DNA they found likely is inside dead or living cells, although it is possible it is floating in the water outside cells.

The researchers will further investigate what other antibiotic-resistance genes are present in the environment, such as ones against vancomycin, often considered the most powerful antibiotic of last resort. They will also explore ways to modify wastewater treatment plants to help them destroy DNA.

Pruden and her colleagues reported their findings online this month via the journal *Environmental Science & Technology*.

- Top 10 Mysterious Diseases
- Study Details Chemicals in Tap Water
- Bottled Water Sales Soar as Tap Water Safety Questioned
- Rise of Deadly Superbugs should 'Raise Red Flags' Everywhere
- All About DNA