Protecting Our Water Environment

Metropolitan Water Reclamation District of Greater Chicago

RESEARCH AND DEVELOPMENT DEPARTMENT

REPORT NO. 06-32

THE EFFECT OF SECONDARY SEWAGE TREATMENT ON THE TOTAL NUMBERS AND PERCENTAGES OF ANTIBIOTIC RESISTANT FECAL COLIFORMS IN RAW SEWAGE ENTERING THE SEVEN WATER RECLAMATION PLANTS OF THE METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

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THE EFFECT OF SECONDARY SEWAGE TREATMENT ON THE TOTAL NUMBERS AND PERCENTAGES OF ANTIBIOTIC RESISTANT FECAL COLIFORMS IN RAW SEWAGE ENTERING THE SEVEN WATER RECLAMATION PLANTS OF THE METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

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Dr. Cecil Lue-Hing of Cecil Lue-Hing and Associates, Dr. James Patterson of Patterson Environmental Consultants, and Mr. Richard Lanyon, Director of Research and Development, are acknowledged for recognizing the need to conduct this study. Mr. Lanyon is acknowledged for authorizing this study. All microbiological analyses were conducted by Andrea Maka, Laboratory Technician II and Kathy Jackowski, Laboratory Technician II. Both Kathy and Andrea made many helpful suggestions during the course of this study. The findings in this report will be presented at the 106th General Meeting of the American Society of Microbiology in Orlando, FL, May 20-25, 2006.

Mr. Lanyon, and Dr. Catherine O'Connor, Research Scientist IV, are acknowledged for initiating efforts in the District to reduce pharmaceuticals in the District's water environment. The District's cooperative efforts with the Food and Drug Administration, the American Medical Association, the Pharmaceutical Research and Manufacturers Association, and the Illinois Environmental Protection Agency were discussed by Dr. O'Connor at the first joint conference of the Illinois section of the American Water Works Association and the Illinois Water Environment Association held in Springfield, IL, March 13-16, 2006.

After reviewing the data and the findings presented in this report, Mr. Lanyon authorized a three-year study of antibiotic resistant bacteria in the Chicago Area Waterways (CAWs), as recommended by Drs. Lue-Hing and Patterson and the authors of this report. The CAWs study was begun in the winter of 2005-2006 and is proceeding on schedule. This report was typed by Ms. Rhonda Griffith.

DISCLAIMER

Mention of proprietary equipment and chemicals in this report does not constitute endorsement by the Metropolitan Water Reclamation District of Greater Chicago.

LIST OF ACRONYMS

Acronym	Full Phrase
AML	Analytical Microbiology Laboratory
AMP	Ampicillin
FC	Fecal Coliforms
FC_{AMP-R}	Ampicillin Resistant Fecal Coliforms
$FC_{AMP/TET/GEN-R}$	Ampicillin/Tetracycline/Gentamicin Resistant Fecal Coliforms
FC_{GEN-R}	Gentamicin Resistant Fecal Coliforms
FC_{TET-R}	Tetracycline Resistant Fecal Coliforms
FE	Final Effluent
GEN	Gentamicin
HB_{AMP-R}	Ampicillin Resistant Heterotrophic Bacteria
$HB_{AMP/TET/GEN-R}$	Ampicillin/Tetracycline/Gentamicin Resistant Heterotrophic Bacteria
HB_{GEN-R}	Gentamicin Resistant Heterotrophic Bacteria
HB_{TET-R}	Tetracycline Resistant Heterotrophic Bacteria
RS	Raw Sewage
TC	Total Coliform
TC_{AMP-R}	Ampicillin Resistant Total Coliforms
$TC_{AMP/TET/GEN-R}$	Ampicillin/Tetracycline/Gentamicin Resistant Total Coliforms
TC_{GEN-R}	Gentamicin Resistant Total Coliforms
TC_{TET-R}	Tetracycline Resistant Total Coliforms
TS	Treated Sewage
TET	Tetracycline
WRP	Water Reclamation Plant

SUMMARY AND CONCLUSIONS

Morozzi et al. (1988) and Andersen (1993) reported finding higher percentages of multiple-antibiotic-resistant bacteria including fecal coliform (FC) in treated sewage (TS) compared with raw sewage (RS). The results suggested that the environments in sewage treatment plants may actually be conducive to the propagation of multiple-antibiotic-resistant bacteria. In 2004 the Metropolitan Water Reclamation District of Greater Chicago (District) adapted the method of Guardabassi and Dalsgaard (2002) to enumerate the total number and percentages of antibiotic resistant FC in RS entering and final effluents (FE) discharged from its seven water reclamation plants (WRPs). The densities of antibiotic resistant FC were determined on m-FC agar containing ampicillin, sodium salt, (AMP) (16 μg/mL), gentamicin (GEN) (8 μg/mL), tetracycline (TET) (8 μg/mL), or all three antibiotics. A small percentage of the antibiotic resistant isolates in RS (25) and FE (16) were identified with the BD BBL™ Crystal™ ID System.

$FC_{AMP\text{-}R}$ and $FC_{TET\text{-}R}$ were found in RS and FE from all seven WRPs. $FC_{GEN\text{-}R}$ were found in RS from all seven WRPs and in FE from the Stickney, Calumet, North Side, Lemont, and Hanover Park WRPs. $FC_{AMP/TET/GEN\text{-}R}$ were found in RS from all seven WRPs, and in FE from the North Side WRP. The numbers of $FC_{AMP\text{-}R}$, $FC_{TET\text{-}R}$, $FC_{GEN\text{-}R}$, and $FC_{AMP/TET/GEN\text{-}R}$ observed in RS ranged from 2.0×10^5 (Calumet WRP) to 1.1×10^7 (James C. Kirie WRP), 9.5×10^4 (Calumet WRP) to 2.2×10^6 (James C. Kirie WRP), 95 (Lemont WRP) to 1.5×10^4 (Hanover Park WRP), and 90 (Calumet, North Side, and Hanover Park WRPs) to 9.5×10^3 (James C. Kirie WRP) per 100mL, respectively. The percentages of antibiotic resistant FC observed in RS followed the trend: $FC_{AMP\text{-}R}$ (11.6 to 46.8) > $FC_{TET\text{-}R}$ (5.8 to 35.7) > $FC_{GEN\text{-}R}$, (<0.01 to 0.29) and $FC_{AMP/TET/GEN\text{-}R}$ (<0.01 to 0.06). Ninety-six percent of the antibiotic resistant isolates from RS (24 of 25 isolates) were identified as *E. coli*.

Secondary sewage treatment without disinfection was shown to reduce the number of antibiotic resistant FC (FC_{AMP-R}, FC_{TET-R}, FC_{GEN-R}, and $FC_{AMP/TET/GEN-R}$) by two to three orders of magnitude. The numbers of FC_{AMP-R}, FC_{TET-R}, FC_{GEN-R}, and $FC_{AMP/TET/GEN-R}$ observed in non-disinfected FE ranged from 1.3×10^2 (John E. Egan WRP) to 6.4×10^3 (North Side WRP), 1.1×10^2 (John E. Egan and James C. Kirie WRPs) to 4.1×10^3 (North Side WRP), 9 (Calumet, North Side, and Hanover Park WRPs) to 15 (North Side and Stickney WRPs), and <10 (six of seven WRPs) to <20 (Lemont WRP) per 100mL, respectively. The percentages of antibiotic resistant FC observed in FE followed the same trend observed in RS: FC_{AMP-R} (9.0 to 28.4) > FC_{TET-R} (5.3 to 21.9) > FC_{GEN-R} (0.03 to <1.05) and $FC_{AMP/TET/GEN-R}$ (0.03 to <1.05). Only one $FC_{AMP/TET/GEN-R}$ organism was found in this study (North Side WRP) indicating that $FC_{AMP/TET/GEN-R}$ was virtually eliminated by secondary sewage treatment. Eighty-seven and one-half percent of the antibiotic resistant isolates (14 of 16 isolates) from FE were identified as *E. coli*.

Equations to predict FC_{AMP-R}, FC_{TET-R}, FC_{GEN-R}, and $FC_{AMP/TET/GEN-R}$ concentrations, on the basis of the total FC concentration, were derived for both RS and FE using multivariate and univariate regression analysis. Testing the slopes of the respective equations to predict FC_{AMP-R}, FC_{TET-R}, FC_{GEN-R}, and $FC_{AMP/TET/GEN-R}$ concentrations in RS versus FE for equality showed that the percentages of all of these antibiotic resistant FC in the FE from all 7 District WRPs were lower than the percentages of these organisms in RS ($p = <0.01$). These results support the conclusion that secondary sewage treatment in the District reduces the numbers and percentages of FC_{AMP-R}, FC_{TET-R}, FC_{GEN-R}, and $FC_{AMP/TET/GEN-R}$ in the FE and that the environments in the District's seven WRPs are not conducive to the propagation or survival of these antibiotic resistant organisms. This conclusion contrasts with the suggestions in the literature cited above that sewage treatment may increase the numbers and percentages of antibiotic resistant bacteria. This conclusion is in general agreement with the finding of Guardabassi and Dalsgaard (2002) that relative numbers of antibiotic resistant total coliforms (TC) and acinetobacters in RS from two large-scale sewage treatment plants in Denmark were not increased by sewage treatment.

INTRODUCTION

The annual usage of 88 specific antibiotics (or anti-microbial compounds) in the United States has been estimated at almost sixteen and one-half tons (Lachmayr and Ford, 2004). The widespread use of antibiotics in human and veterinary medicine, aquaculture, and agriculture has resulted in the contamination of environmental waters and in the emergence of antibiotic resistant bacteria. Antibiotics and antibiotic resistant bacteria make their way into environmental waters by numerous routes, including via sewer systems and wastewater treatment plants (Arvanitidou et al., 2001; Ash et al., 2002; Hirsch et al., 1999; Iwane et al., 2001; Koplin et al., 2002; Lobova et al., 2002; McKeon et al., 1995; and Murdyk, 2002). Since bacteria can transfer genetic information horizontally, especially in nutrient rich environments, there are concerns that antibiotic resistance genes are being transferred to pathogenic bacteria in the environment, including sewage.

These concerns have raised the issue as to whether wastewater treatment plants are adequately reducing the burden of antibiotics and antibiotic resistant bacteria present in sewage. It has even been suggested that conditions may exist within wastewater treatment plants which increase the number of antibiotic resistant bacteria through the wastewater treatment process (Morozzi et al., 1988; Andersen, 1993). Since wastewater treatment plants have not been designed to remove antibiotics and antibiotic resistant bacteria from sewage, this issue should be closely studied before large expenditures for new engineering controls are mandated by regulatory agencies. This study was undertaken to determine whether secondary sewage treatment at the District's seven WRPs is reducing adequately the numbers and percentages of $FC_{AMP\text{-}R}$, $FC_{TET\text{-}R}$, $FC_{GEN\text{-}R}$, and $FC_{AMP/TET/GEN\text{-}R}$ in the FE and whether the environments in the District's seven WRPs are conducive to the survival and propagation of these antibiotic resistant organisms.

OBJECTIVES

To determine the total number and percentage of antibiotic resistant FC in RS and FE at each of the District's WRPs and to analyze the data statistically to assess the effect of secondary sewage treatment at each WRP on the prevalence of antibiotic resistant FC in FE.

MATERIALS AND METHODS

Sampling Plan

One RS and one FE sample were collected at each of the District's seven WRPs in the spring, summer, and fall of 2004 and in the winter of 2004-2005. The locations of the District's seven WRPs are shown in Figure 1. The dates on which these samples were collected are shown in Tables AI-1 through AI-4 and Tables AII-1 through AII-4.

Sample Collection, Transport, and Receiving

Samples were collected in sterile 175 mL capacity polypropylene plastic bottles containing the following sterile reagents: 0.30 mL of a 15 percent solution of the disodium salt of ethylenediaminetetraacetic acid, and 0.1 mL of a 10 percent solution of sodium thiosulfate. All samples were collected by Maintenance and Operations personnel or Analytical Microbiology Laboratory (AML) personnel (MWRDGC, 2003 a, b, c, d, e, f, and g). After collection, all samples were placed on ice and transported to the District's AML. All samples were processed within 6 h.

Enumeration of the Total Number and Percentage of Antibiotic Resistant FC in RS and FE

The methodology described by Guardabassi and Dalsgaard (2002) was adapted to enumerate the total number and percentages of antibiotic resistant FC bacteria in RS and FE. FC densities were determined by membrane filtration following the methodology outlined in SM 9222D (APHA, 1992a). Samples were filtered through 0.45 μm pore size membrane filters, which retained the bacteria. The membrane filtration method was performed on a series of dilutions; five times for each sample. Each membrane filter was incubated on m-FC agar (control) at 44.5 ± 0.2°C for 22 to 26 h. The densities of antibiotic resistant FC were determined on the same medium (m-FC agar) containing ampicillin, sodium salt, (AMP) (Sigma A 9518) (16 μg/mL),

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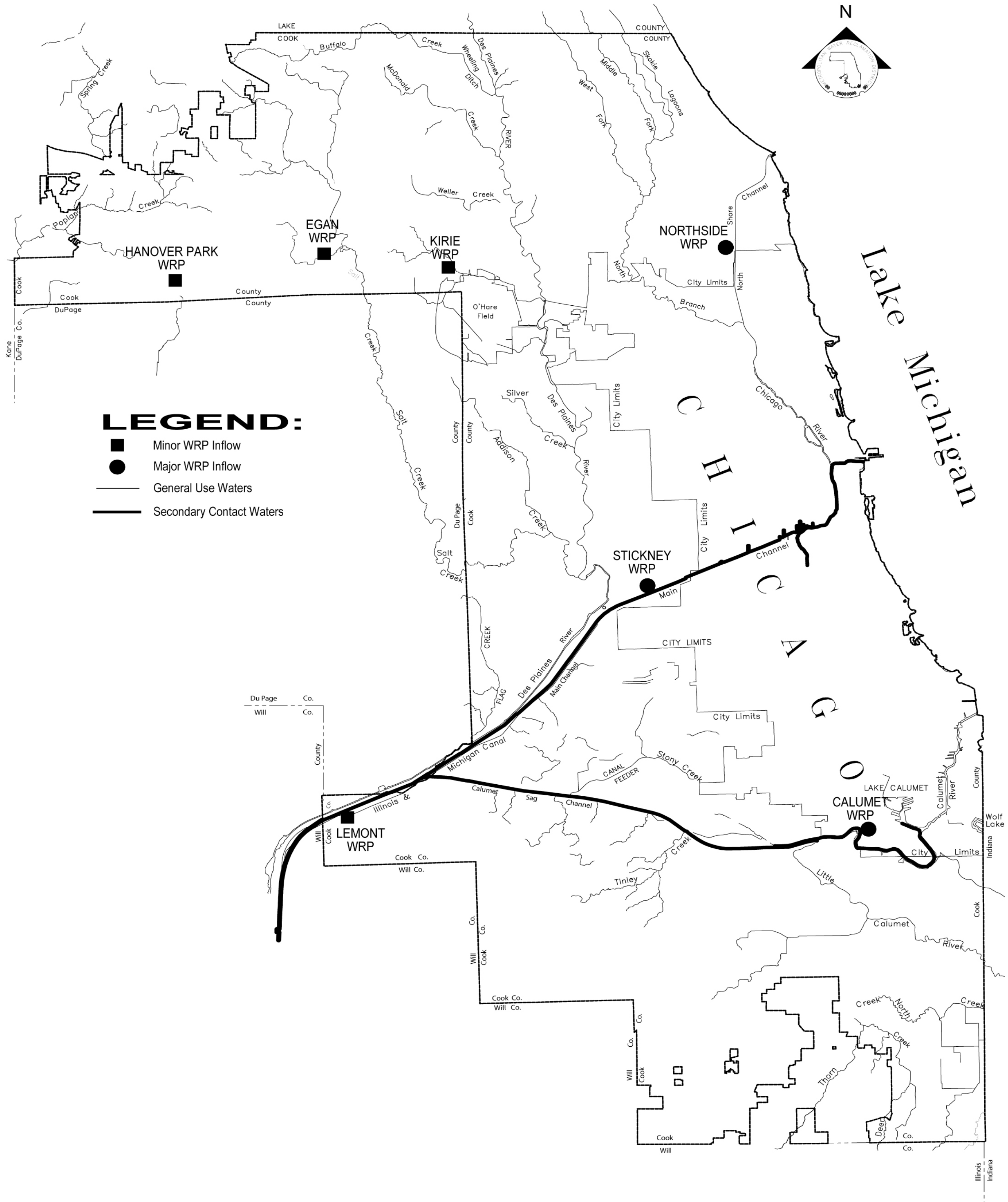
FIGURE 1

LOCATION OF THE DISTRICT'S SEVEN WRPs



gentamicin (GEN) (Sigma G 3632, gentamicin sulfate salt, approximately 60 percent GEN) (8 μg GEN/mL [13.3 μg gentamicin sulfate salt/mL]), tetracycline (TET) (Sigma T 3258) (8 μg/mL), or all three antibiotics combined following the same procedure outlined above. Blue colonies (presumptive FC isolates) from membrane filters containing 20 to 60 colonies were counted, and the density of FC/100 mL was calculated. Preparation of the stock antibiotic solutions used to prepare the antibiotic m-FC plates is outlined in Table 1. The percentages of antibiotic resistance were calculated for each sample as the number (CFU/100 mL) of resistant FC divided by the total number of FC multiplied by 100.

Enumeration of the Total Number and Percentage of Antibiotic Resistant Heterotrophic Bacteria in RS and FE

The methodology described by Guardabassi and Dalsgaard (2002) was also adapted to enumerate the total number and percentages of antibiotic resistant heterotrophic bacteria (HB) in RS and FE. The HB densities were determined by membrane filtration following the methodology outlined in SM 9215D (APHA, 1992b). The methodology was essentially the same as that described above for the antibiotic resistant FC with the following exceptions. Each membrane filter was incubated on m-HPC agar at 35°C for 48 ± 3 h and the total number of HB were counted after incubation. The density of HB/mL was calculated.

Identification of Antibiotic Resistant FC Isolates

Isolates of FC_{AMP-R}, FC_{TET-R}, FC_{GEN-R}, and $FC_{AMP/TET/GEN-R}$ were identified using the BBL™ Crystal™ ID System and BBL Crystal MIND Software V5.02E (Becton, Dickinson and Company, Sparks, Maryland).

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TABLE 1

STOCK ANTIBIOTIC SOLUTIONS USED TO PREPARE ANTIBIOTIC m-FC AGAR PLATES

Antibiotic	Sigma Product Number of Antibiotic	Wt. (mg) of Antibiotic	Volume (mL) of Solvent	Solvent	Final Concentration of Antibiotic (mg/mL) [1,2,3]
Ampicillin (AMP)	A 9518	160.0	10	Milli-Q Water	16
Gentamicin (GEN) Potency = 60%	G 3632	133.3	10	Milli-Q Water	8
Tetracycline (TET)	T 3258	80.0	10	1:1 Milli-Q Water-Ethanol	8

[1]Stock solutions were prepared no more than 2 days before needed and stored at 1-4°C.
[2]1.0 mL of stock solution was added to 1 L of m-FC media to prepare antibiotic m-FC agar plates.
[3]Antibiotic containing m-FC agar plates were stored no longer than one week at 1-4°C.

<u>Statistical Analysis</u>

The collected data (untransformed and log transformed $FC_{AMP\text{-}R}$, $FC_{TET\text{-}R}$, $FC_{GEN\text{-}R}$, and $FC_{AMP/TET/GEN\text{-}R}$) were tested for normality by the Kolmogorov-Smirnov (K-S) test (Gibbons and Chakraborti, 1992). Bartlett's test for homogeneity of variances (Walpole and Meyers, 1989) was performed on log transformed $FC_{AMP\text{-}R}$, $FC_{TET\text{-}R}$, $FC_{GEN\text{-}R}$, and $FC_{AMP/TET/GEN\text{-}R}$ data for which there was no reason to question the assumption of normality. Pearson product moment correlation coefficients, r, were calculated to identify any linear relationship between any two antibiotic resistant FC bacteria from the list of $FC_{AMP\text{-}R}$, $FC_{TET\text{-}R}$, $FC_{GEN\text{-}R}$, and $FC_{AMP/TET/GEN\text{-}R}$. Correlation matrices were constructed to identify any linear relationships among $FC_{AMP\text{-}R}$, $FC_{TET\text{-}R}$, $FC_{GEN\text{-}R}$, and $FC_{AMP/TET/GEN\text{-}R}$ that should not be ignored. The K-S test was used to test for multivariate normality.

Concentrations of $FC_{AMP\text{-}R}$, $FC_{TET\text{-}R}$, $FC_{GEN\text{-}R}$, and $FC_{AMP/TET/GEN\text{-}R}$ were considered as the dependent or response variables, Y_1, Y_2, Y_3, and Y_4 respectively, and FC concentration in the control was considered as the independent or explanatory variable X. Since each Y_i (i = 1,2,3,4) and X has n observations, the matrix Y has n rows and 4 columns, and X has n rows and 2 columns. The entries in the first column of X are all 1 if there is an intercept in the model.

The objective was to predict Y on the basis of X by regression method for univariate (Rao, 2002) and multivariate (Anderson, 1984) analysis and to determine whether the predictions of Ys are the same for the RS and FE by testing the equality of the regressions. The regression equation in any case can be written as

$$Y = X\beta + \varepsilon \tag{1}$$

where Y, X, β, and ε are, respectively, $n \times p, n \times (k+1), (k+1) \times p,$ and $n \times p$ matrices. The number of observations, number of response variables, and the number of independent or explanatory variables in the regression model, are represented by *n*, *p*, *k*, respectively.

In the case of univariate regression, the dimensions of the matrices for *Y*, *X*, β, and ε are $n \times 1$, $n \times (k+1)$, $(k+1)$, and $n+1$, respectively. When there is no intercept in the model the number of columns in *X* and the number of rows in β are each reduced by 1. The least square estimate of β (univariate or multivariate) is given by

$$\hat{\beta} = (X'X)^{-1} X'Y \qquad (2)$$

Regression analyses (univariate and multivariate) were performed on $FC_{AMP\text{-}R}$, $FC_{TET\text{-}R}$, $FC_{GEN\text{-}R}$, and $FC_{AMP/TET/GEN\text{-}R}$ in RS and FE for each WRP. The estimate of $\hat{\beta}$ in FE and RS are denoted by $\hat{\beta}_1$ and $\hat{\beta}_2$, respectively. Standard parametric analysis of variance (ANOVA) (Khattree and Naik, 1999), and the method of Rao (2002) were adopted to test the hypothesis $H_0 : \beta_1 = \beta_2$, the equality of regressions.

It should be mentioned that if there is an intercept in the model then $\beta_1 = \begin{pmatrix} \beta_{10} \\ \beta_{11} \end{pmatrix}$ is not a matrix of slope but a matrix of intercept β_{10} and slope β_{11} as shown above. All statistical analyses were performed using SAS software.

RESULTS AND DISCUSSION

The total numbers and percentages of FC_{AMP-R}, FC_{TET-R}, FC_{GEN-R}, and $FC_{AMP/TET/GEN-R}$ in samples of RS entering the District's seven WRPs are shown in Tables 2-5, respectively. The complete data are shown in Tables AI-1 through AI-4. The total numbers and percentages of FC_{AMP-R}, FC_{TET-R}, FC_{GEN-R}, and $FC_{AMP/TET/GEN-R}$ in FE from the District's seven WRPs are shown in Tables 6-9, respectively. The complete data are shown in Tables AII-1 through AII-4.

FC_{AMP-R} and FC_{TET-R} were found in RS and FE from all seven WRPs. FC_{GEN-R} were found in RS from all seven WRPs and in FE from the Stickney, Calumet, North Side, Lemont, and Hanover Park WRPs. $FC_{AMP/TET/GEN-R}$ were found in RS from all seven WRPs, and in FE from the North Side WRP. The numbers of FC_{AMP-R}, FC_{TET-R}, FC_{GEN-R}, and $FC_{AMP/TET/GEN-R}$ observed in RS ranged from 2.0×10^5 (Calumet WRP) to 1.1×10^7 (Kirie WRP), 9.5×10^4 (Calumet WRP) to 2.2×10^6 (Kirie WRP), 95 (Lemont WRP) to 1.5×10^4 (Hanover Park WRP), and 90 (Calumet, North Side, and Hanover Park WRPs) to 9.5×10^3 (Kirie WRP) per 100mL, respectively. The percentages of antibiotic resistant FC observed in RS followed the trend: FC_{AMP-R} (11.6 to 46.8) > FC_{TET-R} (5.8 to 35.7) > FC_{GEN-R}, (<0.01 to 0.29) and $FC_{AMP/TET/GEN-R}$ (<0.01 to 0.06).

Secondary sewage treatment without disinfection was shown to reduce the number of the antibiotic resistant FC by two to three orders of magnitude. The numbers of FC_{AMP-R}, FC_{TET-R}, FC_{GEN-R}, and $FC_{AMP/TET/GEN-R}$ observed in non-disinfected FE ranged from 1.3×10^2 (John E. Egan WRP) to 6.4×10^3 (North Side WRP), 1.1×10^2 (John E. Egan and James C. Kirie WRPs) to 4.1×10^3 (North Side WRP), 9 (Calumet, North Side and Hanover Park WRPs) to <15 (North Side and Stickney WRP), and 9 (North Side WRP) to <10 (all seven WRPs) per 100mL, respectively. The percentages of antibiotic resistant FC observed in FE followed the same trend

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TABLE 2

NUMBER AND PERCENTAGE OF FC_{AMP-R} PER 100 mL IN RAW SEWAGE (RS)

WRP	Range	Percentage[1]
Stickney	$4.0 \times 10^5 – 3.9 \times 10^6$	13.5 – 34.3
Calumet	$2.0 \times 10^5 – 6.9 \times 10^5$	12.7 – 15.8
North Side	$2.5 \times 10^5 – 1.2 \times 10^6$	11.6 – 25.1
Lemont	$2.6 \times 10^5 – 8.6 \times 10^5$	12.7 – 21.2
John E. Egan	$8.4 \times 10^5 – 3.0 \times 10^6$	19.2 – 46.8
Hanover Park	$2.8 \times 10^5 – 2.1 \times 10^6$	16.8 – 31.1
James C. Kirie	$4.3 \times 10^5 – 1.1 \times 10^7$	20.4 – 35.4

[1](FC_{AMP-R} in RS/Total FC in RS) x 100.

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TABLE 3

NUMBER AND PERCENTAGE OF FC_{TET-R} PER 100 mL IN RAW SEWAGE (RS)

WRP	Range	Percentage[1]
Stickney	$2.2 \times 10^5 – 1.9 \times 10^6$	6.9 – 17.6
Calumet	$9.5 \times 10^4 – 5.8 \times 10^5$	7.3 – 12.8
North Side	$2.9 \times 10^5 – 7.2 \times 10^5$	9.2 – 35.7
Lemont	$1.2 \times 10^5 – 4.6 \times 10^5$	7.4 – 11.4
John E. Egan	$4.5 \times 10^5 - 9.3 \times 10^5$	13.0 – 18.2
Hanover Park	$2.0 \times 10^5 – 1.0 \times 10^6$	11.6 – 19.8
James C. Kirie	$3.4 \times 10^5 – 2.2 \times 10^6$	5.8 – 27.9

[1](FC_{TET-R} in RS/Total FC in RS) x 100.

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TABLE 4

NUMBER AND PERCENTAGE OF FC_{GEN-R} PER 100 mL IN RAW SEWAGE (RS)

WRP	Range	Percentage[1]
Stickney	1.7×10^3 - 5.6×10^3	0.03 - 0.06
Calumet	3.0×10^2 - 2.2×10^3	0.02 - 0.04
North Side	2.5×10^2 - 9.5×10^3	<0.01 - 0.29
Lemont	9.5×10^1 - 2.5×10^2	<0.01
John E. Egan	7.6×10^2 - 3.2×10^3	0.02 - 0.06
Hanover Park	1.0×10^2 - 1.5×10^4	<0.01 - 0.20
James C. Kirie	4.0×10^2 - 9.5×10^3	0.01 - 0.05

[1](FC_{GEN-R} in RS/Total FC in RS) x 100.

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TABLE 5

NUMBER AND PERCENTAGE OF $FC_{AMP/TET/GEN-R}$ PER 100 mL IN RAW SEWAGE (RS)

WRP	Range	Percentage[1]
Stickney	2.0×10^2 - 2.0×10^3	<0.01 – 0.02
Calumet	9.0×10^1 - 4.0×10^2	<0.01 – 0.01
North Side	9.0×10^1 – 2.4×10^3	<0.01 – 0.04
Lemont	9.5×10^1	<0.01
John E. Egan	3.0×10^2 - 1.4×10^3	0.01 – 0.02
Hanover Park	9.0×10^1 – 1.5×10^3	<0.01 – 0.06
James C. Kirie	3.0×10^2 – 9.5×10^3	<0.01 – 0.06

[1]($FC_{AMP/TET/GEN-R}$ in RS/Total FC in RS) x 100.

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TABLE 6

NUMBER AND PERCENTAGE OF FC_{AMP-R} PER 100 mL IN FINAL EFFLUENT (FE)

WRP	Range	Percentage[1]
Stickney	$4.1 \times 10^2 – 4.7 \times 10^3$	14.0 – 27.1
Calumet	$4.2 \times 10^2 - 2.0 \times 10^3$	9.0 – 20.5
North Side	$1.2 \times 10^3 – 6.4 \times 10^3$	15.3 – 28.4
Lemont	$1.9 \times 10^3 – 2.9 \times 10^3$	11.9 – 20.0
John E. Egan	<10[a] – 6.9×10^2	13.2[b] – 16.3[b]
Hanover Park	<10[a] – 2.5×10^3	14.5[b] – 22.3[b]
James C. Kirie	<10[a] – 2.0×10^2	11.9[b] – 19.1[b]

[1](FC_{AMP-R} in FE/Total FC in FE) x 100.
[a]Chlorination season.
[b]Non-chlorination season.

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TABLE 7

NUMBER AND PERCENTAGE OF FC_{TET-R} PER 100 mL IN FINAL EFFLUENT (FE)

WRP	Range	Percentage[1]
Stickney	$2.3 \times 10^2 – 2.4 \times 10^3$	7.9 – 17.2
Calumet	$3.3 \times 10^2 – 7.7 \times 10^2$	7.2 – 12.6
North Side	$7.2 \times 10^2 – 4.1 \times 10^3$	11.5 – 21.9
Lemont	$8.5 \times 10^2 – 3.4 \times 10^3$	5.3 – 20.9
John E. Egan	<10[a] – 5.9×10^2	11.1[b] – 14.1[b]
Hanover Park	<10[a] – 2.4×10^3	14.4[b] – 21.8[b]
James C. Kirie	<10[a] – 2.2×10^2	8.1[b] – 21.0[b]

[1](FC_{TET-R} in FE/Total FC in FE) x 100.
[a]Chlorination season.
[b]Non-chlorination season.

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TABLE 8

NUMBER AND PERCENTAGE OF FC_{GEN-R} PER 100 mL IN FINAL EFFLUENT (FE)

WRP	Range	Percentage[1]
Stickney	<10 - <15	0.07 – 0.34
Calumet	9 - <10	0.09 – 0.22
North Side	9 - <15	0.03 – 0.19
Lemont	<10	0.06 – 0.08
John E. Egan	<10[a]	<0.24[b] – <1.05[b]
Hanover Park	9[a] - <10[a]	0.08[b] – <0.25[b]
James C. Kirie	<10[a]	<0.77[b] – <0.95[b]

[1](FC_{GEN-R} in FE/Total FC in FE) x 100.
[a]Chlorination season.
[b]Non-chlorination season.

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TABLE 9

NUMBER AND PERCENTAGE OF $FC_{AMP/TET/GEN-R}$ PER 100 mL IN FINAL EFFLUENT (FE)

WRP	Range	Percentage
Stickney	<10	0.05 – 0.34
Calumet	<10	0.10 – 0.22
North Side	9[a] - <10	0.03 – 0.20
Lemont	<10	0.06 – 0.08
John E. Egan	<10[b]	<0.24[c] – <1.05[c]
Hanover Park	<10[b]	<0.09[c] – <0.25[c]
James C. Kirie	<10[b]	<0.77[c] – <0.95[c]

[1]($FC_{AMP/TET/GEN-R}$ in FE/Total FC in FE) x 100.
[a]One colony.
[b]Chlorination season.
[c]Non-chlorination season.

observed in RS: $FC_{AMP\text{-}R}$ (9.0 to 28.4) > $FC_{TET\text{-}R}$ (5.3 to 21.9) > $FC_{GEN\text{-}R}$ (0.03 to <1.05) and $FC_{AMP/TET/GEN\text{-}R}$ (0.03 to <1.05). Only one $FC_{AMP/TET/GEN\text{-}R}$ organism was found in FE (North Side WRP) indicating that $FC_{AMP/TET/GEN\text{-}R}$ was virtually eliminated by secondary sewage treatment.

Results of the K-S test (not shown) indicated that log transformed $FC_{AMP\text{-}R}$, $FC_{TET\text{-}R}$, $FC_{GEN\text{-}R}$, and $FC_{AMP/TET/GEN\text{-}R}$ data were normally distributed. Therefore, log transformed values of the explanatory variables were used in the regression analysis to predict log transformed values of the response variables. Results showed that there is no intercept in any regression model. The basic statistics calculated using univariate regression analysis are shown in Tables 10 and 11 and Table 12 contains slopes $\hat{\beta}$ and R^2 values. In each case the slope of the regression equation to predict an antibiotic resistant FC density in FE was less than the slope of the regression equation to predict the same antibiotic resistant FC density in RS. In each case the difference in the compared slopes was shown to be highly significant by two statistical methods. These results showed that the numbers and percentages of all of these antibiotic resistant FC in the FE from all 7 District WRPs were significantly lower than the numbers and percentages of these organisms in RS (p =< 0.01).

Parenthetically, in a number of instances the results of analyses for $FC_{GEN\text{-}R}$ and $FC_{AMP/TET/GEN/\text{-}R}$ gave less than values instead of actual values (Appendices AI and AII). The less than values were not used in the regression analyses. Therefore, as indicated in footnotes to Tables 10 and 11, in some cases there were insufficient data to perform the regression analyses.

Calculated values of the correlation coefficients, r values, are shown in Tables 13 (RS) and 14 (FE). The absolute values of r are all greater than zero, indicating that the response variables for RS and for FE are significantly correlated. Results of the K-S test shown in Tables 13

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TABLE 10

BASIC STATISTICS[1]: FC_{AMP-R}, FC_{TET-R}, FC_{GEN-R} and $FC_{AMP/TET/GEN-R}$ CONCENTRATIONS IN RAW SEWAGE (RS)

WRP	Measured	Obs.	Mean[2]	s[a,2]
Calumet	FC_{AMP-R}	8	12.684	0.494
	FC_{TET-R}	8	12.244	0.756
	FC_{GEN-R}	5	6.688	0.925
John E. Egan	FC_{AMP-R}	8	14.063	0.528
	FC_{TET-R}	8	13.433	0.344
Hanover Park	FC_{AMP-R}	8	13.648	0.801
	FC_{TET-R}	8	13.188	0.691
	FC_{GEN-R}	9	7.196	1.813
James C. Kirie	FC_{AMP-R}	8	14.560	1.409
	FC_{TET-R}	8	13.710	0.918
Lemont	FC_{AMP-R}	8	12.959	0.495
	FC_{TET-R}	8	12.372	0.605
	FC_{GEN-R}	5	4.840	0.646
	$FC_{AMP/TET/GEN-R}$	5	4.563	0.058
North Side	FC_{AMP-R}	8	13.262	0.709
	FC_{TET-R}	8	13.052	0.456
	FC_{GEN-R}	6	6.933	1.633
	$FC_{AMP/TET/GEN-R}$	5	5.834	1.780
Stickney	FC_{AMP-R}	8	13.658	0.953
	FC_{TET-R}	8	12.960	0.927
	FC_{GEN-R}	5	7.942	0.630

[1]Calculated using univariate regression. In some cases insufficient data were collected to perform the calculations. These cases were omitted from the table.
[2]Ln values.
[a]Standard deviation.

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TABLE 11

BASIC STATISTICS[1]: FC_{AMP-R}, FC_{TET-R}, FC_{GEN-R} and $FC_{AMP/TET/GEN-R}$ CONCENTRATIONS IN FINAL EFFLUENT (FE)

WRP	Measured	Obs.	Mean[2]	s[a,2]
Calumet	FC_{AMP-R}	8	6.857	0.625
	FC_{TET-R}	8	6.346	0.372
	FC_{GEN-R}	8	2.276	0.049
John E .Egan	FC_{AMP-R}	4	5.665	1.008
	FC_{TET-R}	4	5.516	0.998
Hanover Park	FC_{AMP-R}	4	7.073	0.841
	FC_{TET-R}	4	7.067	0.827
	FC_{GEN-R}	4	2.250	0.061
James C. Kirie	FC_{AMP-R}	4	5.169	0.170
	FC_{TET-R}	4	5.010	0.464
Lemont	FC_{AMP-R}	8	7.812	0.199
	FC_{TET-R}	8	7.401	0.584
	FC_{GEN-R}	8	2.450	0.340
	$FC_{AMP/TET/GEN-R}$	8	2.476	0.321
North Side	FC_{AMP-R}	8	7.875	0.845
	FC_{TET-R}	8	7.570	0.764
	FC_{GEN-R}	8	2.337	0.271
	$FC_{AMP/TET/GEN-R}$	8	2.289	0.037
Stickney	FC_{AMP-R}	8	7.200	1.084
	FC_{TET-R}	8	6.660	1.053
	FC_{GEN-R}	8	2.376	0.253

[1]Calculated using univariate regression. In some cases insufficient data were collected to do the calculations. These cases were omitted from the table.
[2]Ln values.
[a]Standard deviation.

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TABLE 12

RESULTS OF UNIVARIATE REGRESSION[1] TO PREDICT FC_{AMP-R}, FC_{TET-R}, FC_{GEN-R}, AND $FC_{AMP/TET/GEN-R}$ IN FINAL EFFLUENT (FE) (β_1) AND RAW SEWAGE (RS) (β_2) BASED UPON TOTAL (CONTROL) FC CONCENTRATIONS AND THE SIGNIFICANCE PROBABILITY OF EQUAL SLOPES ($\beta_1 = \beta_2$)

WRP	Predicted[2] Value	FE		RS		Significance Probability of Equal Slopes ($\beta_1 = \beta_2$)	
		β_1[a]	R-square[3]	β_2[b]	R-square[3]	T-Test[4]	Rao[4]
Calumet	Y_1	0.7842	0.9966	0.8691	0.9998	0.001	0.001
Calumet	Y_2	0.7250	0.9987	0.8394	0.9988	0.001	0.001
Calumet	Y_3	0.2595	0.9972	0.4468	0.9921	0.001	0.001
John E. Egan	Y_1	0.7497	0.9968	0.9188	0.9992	0.001	0.001
John E. Egan	Y_2	0.7302	0.9965	0.8777	0.9999	0.001	0.000
Hanover Park	Y_1	0.8058	0.9976	0.9044	0.9996	0.002	0.001
Hanover Park	Y_2	0.8051	0.9980	0.8738	0.9998	0.006	0.001
Hanover Park	Y_3	0.2545	0.9935	0.4737	0.9578	0.001	0.001
James C. Kirie	Y_1	0.7335	0.9968	0.9160	0.9997	0.001	0.001
James C. Kirie	Y_2	0.7095	0.9871	0.8603	0.9991	0.007	0.001
Lemont	Y_1	0.8046	0.9992	0.8789	0.9997	0.001	0.001
Lemont	Y_2	0.7627	0.9955	0.8395	0.9996	0.001	0.001
Lemont	Y_3	0.2529	0.9881	0.3170	0.9845	0.001	0.001
Lemont	Y_4	0.2556	0.9900	0.2990	0.9996	0.001	0.001
North Side	Y_1	0.8389	0.9987	0.8843	0.9996	0.001	0.001
North Side	Y_2	0.8060	0.9986	0.8693	0.9987	0.001	0.001
North Side	Y_3	0.2475	0.9857	0.4607	0.9638	0.001	0.001
North Side	Y_4	0.2420	0.9927	0.3869	0.9396	0.002	0.001

TABLE 12 (Continued)

RESULTS OF UNIVARIATE REGRESSION[1] TO PREDICT FC_{AMP-R}, FC_{TET-R}, FC_{GEN-R}, AND $FC_{AMP/TET/GEN-R}$ IN FINAL EFFLUENT (FE) (β_1) AND RAW SEWAGE (RS) (β_2) BASED UPON TOTAL (CONTROL) FC CONCENTRATIONS AND THE SIGNIFICANCE PROBABILITY OF EQUAL SLOPES ($\beta_1 = \beta_2$)

WRP	Predicted[2] Value	FE		RS		Significance Probability of Equal Slopes ($\beta_1 = \beta_2$)	
		β_1[a]	R-square[3]	β_2[b]	R-square[3]	T-Test[4]	Rao[4]
Stickney	Y_1	0.8224	0.9973	0.8937	0.9990	0.001	0.001
Stickney	Y_2	0.7609	0.9959	0.8480	0.9986	0.001	0.001
Stickney	Y_3	0.2691	0.9899	0.5043	0.9991	0.001	0.001

[1]Final Effluent: $\text{Ln } y = \beta_1(\ln x) = x^{\beta 1}$; Raw Sewage: $\text{Ln } y = \beta_2(\ln x) = x^{\beta 2}$.
Where Y = predicted concentration of antibiotic resistant FC and X = control (total) FC concentration.

[2]$Y_1 = FC_{AMP-R}$; $Y_2 = FC_{TET-R}$; $Y_3 = FC_{GEN-R}$; $Y_4 = FC_{AMP/TET/GEN-R}$.

[a]β_1 = slope of the regression equation for FE.

[b]β_2 = slope of the regression equation for RS.

[3]R-square = coefficient of determination. The *R-square* value is an indicator of how well the model fits the data (e.g., an *R-square* close to 1.0 indicates that we have accounted for almost all of the variability with the variables specified in the model).

[4]P values.

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TABLE 13

RESULTS OF TESTS FOR MULTIVARIATE NORMALITY: FC_{AMP-R}, FC_{TET-R}, FC_{GEN-R}, AND $FC_{AMP/TET/GEN-R}$ CONCENTRATIONS IN FINAL EFFLUENT (FE)

WRP		Correlation Matrix[1] (*r* Values)				Significance Probability of Multivariate Normality[2]
		Y_1	Y_2	Y_3	Y_4	(Y_1, Y_2, Y_3, Y_4)
Calumet	Y_1	1.000	0.914	-0.418	ID[3]	
Calumet	Y_2	0.914	1.000	-0.332	ID	
Calumet	Y_3	-0.418	-0.332	1.000	ID	0.881
John E. Egan	Y_1	1.000	0.580	ID	ID	
John E. Egan	Y_2	0.580	1.000	ID	ID	0.829
Hanover Park	Y_1	1.000	0.969	0.710	ID	
Hanover Park	Y_2	0.969	1.000	0.702	ID	
Hanover Park	Y_3	0.710	0.702	1.000	ID	0.709
James C. Kirie	Y_1	1.000	0.966	ID	ID	
James C. Kirie	Y_2	0.966	1.000	ID	ID	0.822
Lemont	Y_1	1.000	0.855	-0.293	-0.725	
Lemont	Y_2	0.855	1.000	-0.005	-0.601	
Lemont	Y_3	-0.293	-0.005	1.000	0.461	
Lemont	Y_4	-0.725	-0.601	0.461	1.000	0.999

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TABLE 13 (Continued)

RESULTS OF TESTS FOR MULTIVARIATE NORMALITY: FC_{AMP-R}, FC_{TET-R}, FC_{GEN-R}, AND $FC_{AMP/TET/GEN-R}$ CONCENTRATIONS IN FINAL EFFLUENT (FE)

WRP		Correlation Matrix[1] (*r* Values) Y_1	Y_2	Y_3	Y_4	Significance Probability of Multivariate Normality[2] (Y_1, Y_2, Y_3, Y_4)
North Side	Y_1	1.000	0.867	-0.046	0.135	
North Side	Y_2	0.867	1.000	-0.258	-0.011	
North Side	Y_3	-0.046	-0.258	1.000	0.176	
North Side	Y_4	0.135	-0.011	0.176	1.000	0.971
Stickney	Y_1	1.000	0.948	-0.448	ID	
Stickney	Y_2	0.948	1.000	-0.573	ID	
Stickney	Y_3	-0.448	-0.573	1.000	ID	0.985

[1]Calculated values of *r*, the Pearson product moment correlation coefficients, are shown in the matrix. The rows and columns correspond to the variables indicated (Y_1, Y_2, Y_3, *and* Y_4). All of the absolute values of *r* greater than zero are significant. Values of *r* for the same variable are by definition unity (1.000). In the cases of the Calumet, John E. Egan, Hanover Park, James C. Kirie, and Stickney WRPs the correlation matrix is asymmetrical because there were insufficient data to calculate all values of *r*.
[2]Results of the K-S test. If this probability is > 0.05, the data are from a multivariate normal population.
[3]ID = Insufficient data to calculate *r* values.

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TABLE 14

RESULTS OF TESTS FOR MULTIVARIATE NORMALITY: $FC_{AMP\text{-}R}$, $FC_{TET\text{-}R}$, $FC_{GEN\text{-}R}$, AND $FC_{AMP/TET/GEN\text{-}R}$ CONCENTRATIONS IN RAW SEWAGE (RS)

WRP		Correlation Matrix[1] (*r* Values)				Significance Probability of Multivariate Normality[2]
		Y_1	Y_2	Y_3	Y_4	(Y_1, Y_2, Y_3, Y_4)
Calumet	Y_1	1.000	0.904	-0.715	ID[3]	
Calumet	Y_2	0.904	1.000	-0.472	ID	
Calumet	Y_3	-0.715	-0.472	1.000	ID	0.998
John E. Egan	Y_1	1.000	0.985	ID	ID	
John E. Egan	Y_2	0.985	1.000	ID	ID	0.639
Hanover Park	Y_1	1.000	0.964	-0.980	ID	
Hanover Park	Y_2	0.964	1.000	-0.996	ID	
Hanover Park	Y_3	-0.980	-0.996	1.000	ID	0.553
James C. Kirie	Y_1	1.000	0.688	ID	ID	
James C. Kirie	Y_2	0.688	1.000	ID	ID	0.993
Lemont	Y_1	1.000	-0.595	0.455	0.438	
Lemont	Y_2	-0.595	1.000	0.295	0.329	
Lemont	Y_3	0.455	0.295	1.000	0.991	
Lemont	Y_4	0.438	0.329	0.991	1.000	0.642

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TABLE 14 (Continued)

RESULTS OF TESTS FOR MULTIVARIATE NORMALITY: FC_{AMP-R}, FC_{TET-R}, FC_{GEN-R} , AND $FC_{AMP/TET/GEN-R}$ CONCENTRATIONS IN RAW SEWAGE (RS)

WRP		Correlation Matrix[1] (*r* Values) Y_1	Y_2	Y_3	Y_4	Significance Probability of Multivariate Normality[2] (Y_1, Y_2, Y_3, Y_4)
North Side	Y_1	1.000	0.906	0.282	-0.425	
North Side	Y_2	0.906	1.000	0.409	-0.369	
North Side	Y_3	0.282	0.409	1.000	0.207	
North Side	Y_4	0.425	0.369	0.207	1.000	0.996
Stickney	Y_1	1.000	0.982	0.424	ID	
Stickney	Y_2	0.982	1.000	0.312	ID	
Stickney	Y_3	0.424	0.312	1.000	ID	0.999

[1]Calculated values of *r*, the Pearson product moment correlation coefficients, are shown in the matrix. The rows and columns correspond to the variables indicated (Y_1, Y_2, Y_3, *and* Y_4). All of the absolute values of *r* greater than zero are significant. Values of *r* for the same variable are by definition unity (1.000). In the cases of the Calumet, John E. Egan, Hanover Park, James C. Kirie, and Stickney WRPs the correlation matrix is asymmetrical because there were insufficient data to calculate all values of *r*.
[2]Results of the K-S test. If this probability is > 0.05, the data are from a multivariate normal population.
[3]ID = Insufficient data to calculate *r* values.

and 14 indicate that Y_1, Y_2, Y_3, and Y_4 come from one multivariate normal population for RS and for FE. The results of multivariate regression analysis are shown in Table 15 (slopes $\hat{\beta}$s and R^2 values). In each case the slope of the regression equation to predict an antibiotic resistant FC density in FE was less than the slope of the regression equation to predict the same antibiotic resistant FC density in RS. In each case the difference in the compared slopes was shown to be highly significant by ANOVA. These results showed that the numbers and percentages of all of these antibiotic resistant FC in the FE from all seven District WRPs were significantly lower than the numbers and percentages of these organisms in RS ($p =< 0.01$). Since the sample sizes for testing multivariate normality were very small for some WRPs, there could be some questions as to whether the results of multivariate regression analysis are as reliable as those of univariate regression analysis. However, the results of multivariate regression analysis are in complete agreement with the results of univariate regression analysis.

The numbers of $FC_{AMP\text{-}R}$, $FC_{TET\text{-}R}$, $FC_{GEN\text{-}R}$, and $FC_{AMP/TET/GEN\text{-}R}$ observed in RS and FE from the District's seven WRPs by season are shown in Figures 2-5. Visual inspection of the data plotted in Figures 2 through 5 suggests that the seasons may have some effect on the numbers or relative percentages of antibiotic resistant FC observed in RS or FE. However, these data are limited, so no statistical analysis was performed, and no conclusion can be drawn from the data regarding confounding effects of seasonal variation.

Identities of antibiotic resistant FC isolates from RS and FE are shown in Table 16. Ninety-six percent of the antibiotic resistant isolates from RS (24 of 25 isolates) were identified as *E. coli*. The one non *E. coli* isolate from RS was identified as *Klebsiella oxytoca*. Eighty-seven identified as *E. coli*. Of the two non-*E. coli* isolates from FE, one was identified as *Klebsiella*

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TABLE 15

RESULTS OF MULTIVARIATE REGRESSION[1] TO PREDICT FC_{AMP-R}, FC_{TET-R}, FC_{GEN-R}, AND $FC_{AMP/TET/GEN-R}$ IN FINAL EFFLUENT (FE) (β_1) AND RAW SEWAGE (RS) (β_2) BASED UPON TOTAL (CONTROL) FC CONCENTRATIONS AND THE SIGNIFICANCE PROBABILITY OF EQUAL SLOPES ($\beta_1 = \beta_2$)

WRP	Predicted[2] Value	FE		RS		Significance Probability of Equal Slopes ($\beta_1 = \beta_2$) T-Test[4]
		β_1[a]	R-square[3]	β_2[b]	R-square[3]	
Calumet	Y_1	0.7842	0.9966	0.8748	0.9998	0.001
Calumet	Y_2	0.7250	0.9987	0.8611	0.9997	0.001
Calumet	Y_3	0.2595	0.9972	0.4343	0.9931	0.001
John E. Egan	Y_1	0.7497	0.9968	0.9188	0.9992	0.001
John E. Egan	Y_2	0.7302	0.9965	0.8777	0.9999	0.001
Hanover Park	Y_1	0.8058	0.9976	0.8984	0.9997	0.003
Hanover Park	Y_2	0.8051	0.9980	0.8725	0.9997	0.006
Hanover Park	Y_3	0.2545	0.9935	0.4435	0.9676	0.001
James C. Kirie	Y_1	0.7335	0.9968	0.9123	0.9998	0.001
James C. Kirie	Y_2	0.7095	0.9871	0.8650	0.9992	0.006
Lemont	Y_1	0.8046	0.9992	0.8845	0.9997	0.001
Lemont	Y_2	0.7627	0.9955	0.8534	0.9999	0.001
Lemont	Y_3	0.2529	0.9881	0.3242	0.9834	0.007
Lemont	Y_4	0.2556	0.9900	0.3013	0.9997	0.001
North Side	Y_1	0.8389	0.9987	0.8924	0.9999	0.001
North Side	Y_2	0.8060	0.9986	0.8886	0.9986	0.001
North Side	Y_3	0.2475	0.9857	0.4468	0.9766	0.002
North Side	Y_4	0.2420	0.9927	0.4241	0.9627	0.004

TABLE 15 (Continued)

RESULTS OF MULTIVARIATE REGRESSION[1] TO PREDICT $FC_{AMP\text{-}R}$, $FC_{TET\text{-}R}$, $FC_{GEN\text{-}R}$, AND $FC_{AMP/TET/GEN\text{-}R}$ IN FINAL EFFLUENT (FE) (β_1) AND RAW SEWAGE (RS) (β_2) BASED UPON TOTAL (CONTROL) FC CONCENTRATIONS AND THE SIGNIFICANCE PROBABILITY OF EQUAL SLOPES ($\beta_1 = \beta_2$)

WRP	Predicted[2] Value	FE		RS		Significance Probability of Equal Slopes ($\beta_1 = \beta_2$) T-Test[4]
		β_1[a]	R-square[3]	β_2[b]	R-square[3]	
Stickney	Y_1	0.8224	0.9973	0.9049	0.9987	0.001
Stickney	Y_2	0.7609	0.9959	0.8674	0.9987	0.001
Stickney	Y_3	0.2691	0.9899	0.5021	0.9990	0.001

[1]Final Effluent: $\text{Ln } y = \beta_1 (\ln x) = x^{\beta 1}$; Raw Sewage: $\text{Ln } y = \beta_2 (\ln x) = x^{\beta 2}$.
Where Y = predicted concentration of antibiotic resistant FC and X = control (total) FC concentration.

[2]$Y_1 = FC_{AMP\text{-}R}$; $Y_2 = FC_{TET\text{-}R}$; $Y_3 = FC_{GEN\text{-}R}$; $Y_4 = FC_{AMP/TET/GEN\text{-}R}$.

[a]β_1 = slope of the regression equation for final effluent.

[b]β_2 = slope of the regression equation for raw sewage.

[3]R-square = coefficient of determination. The *R-square* value is an indicator of how well the model fits the data (e.g., an *R-square* close to 1.0 indicates that we have accounted for almost all of the variability with the variables specified in the model).

[4]P values.

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FIGURE 2

NUMBER OF FC_{AMP-R} PER 100 mL OF RAW SEWAGE (RS) AND FINAL EFFLUENT (FE) FROM THE DISTRICT'S SEVEN WRPs

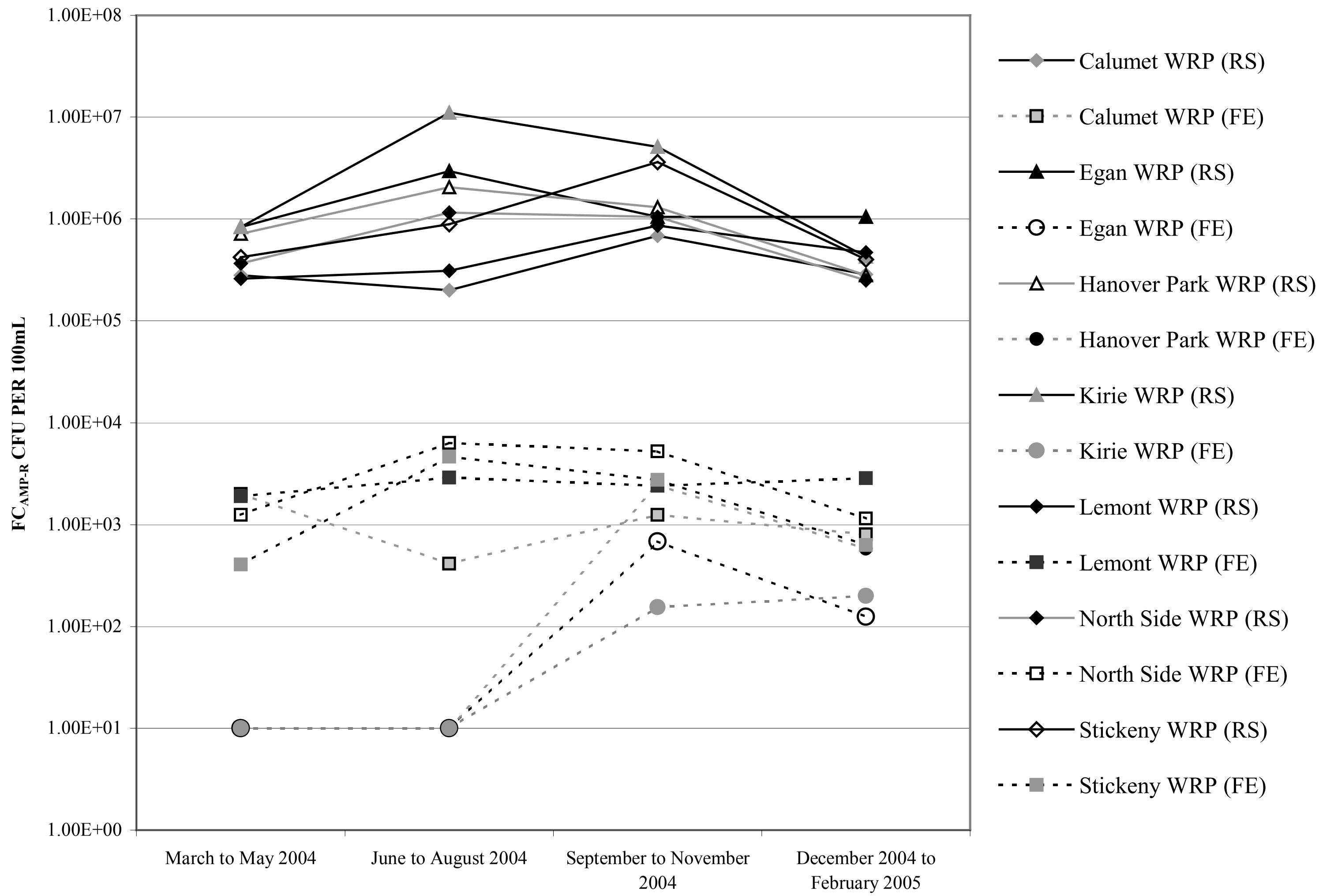

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FIGURE 3

NUMBER OF FC_{TET-R} PER 100 mL OF RAW SEWAGE (RS) AND FINAL EFFLUENT (FE) FROM THE DISTRICT'S SEVEN WRPs

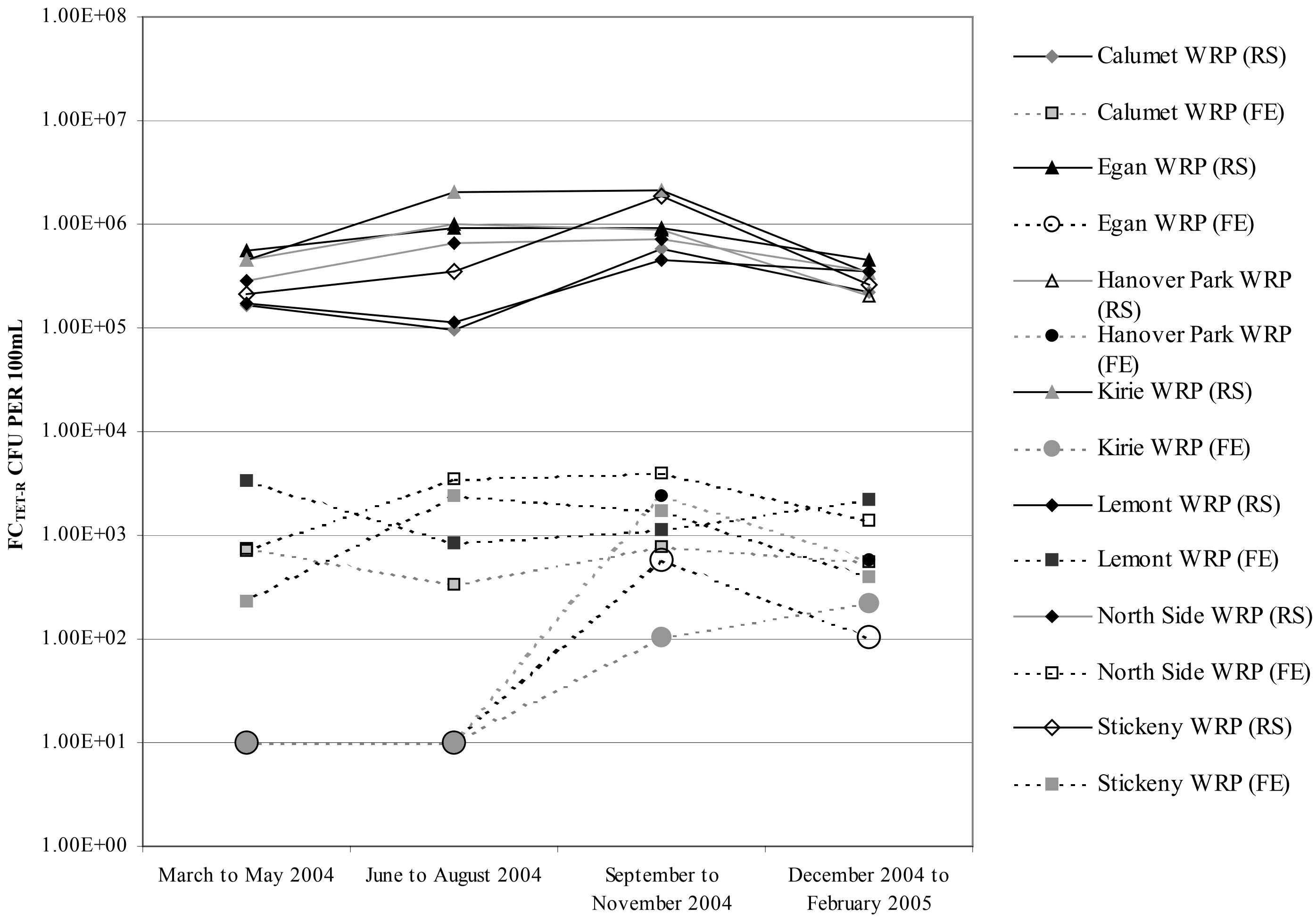

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FIGURE 4

NUMBER OF FC_{GEN-R} PER 100 mL OF RAW SEWAGE (RS) AND FINAL EFFLUENT (FE) FROM THE DISTRICT'S SEVEN WRPs

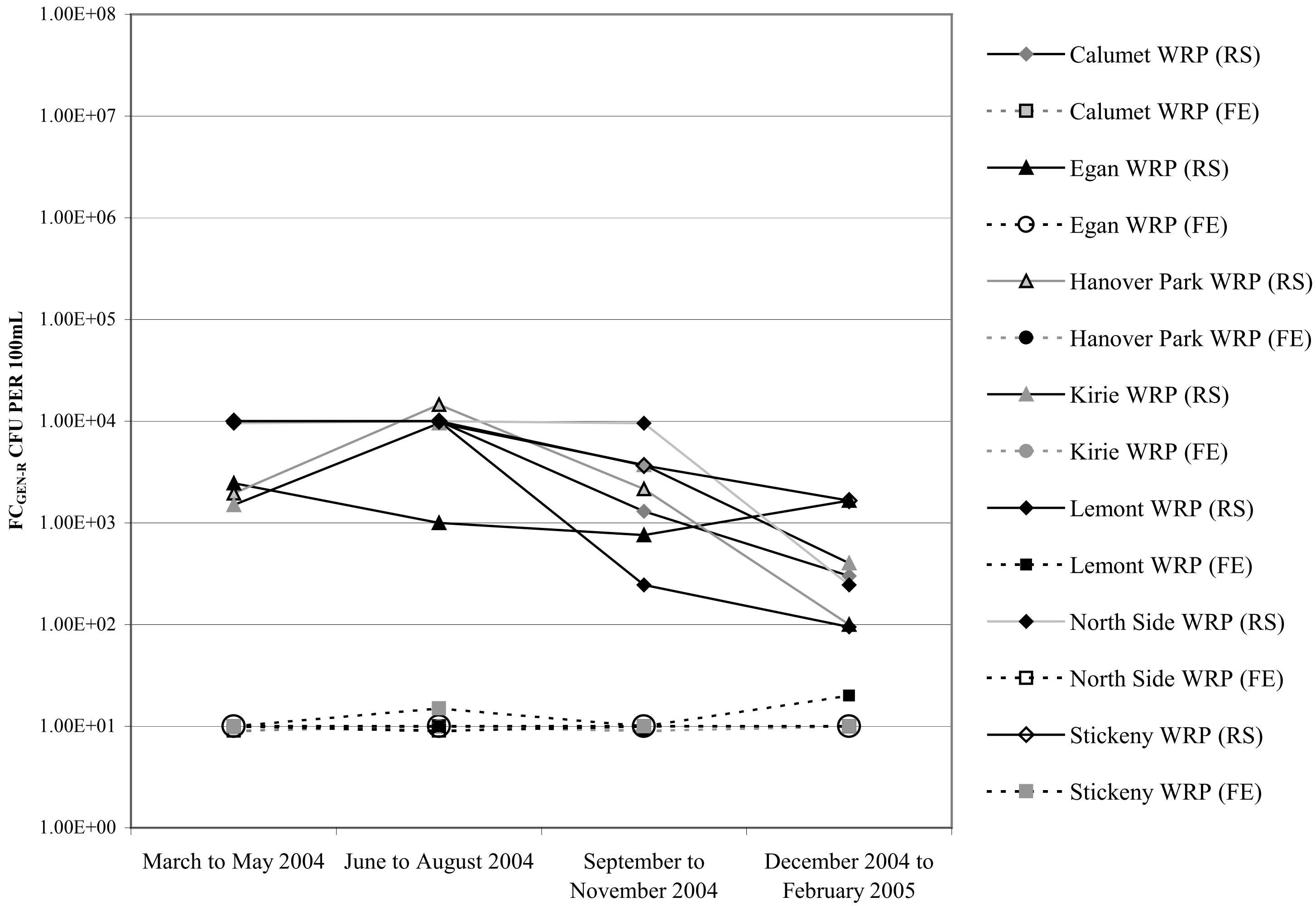

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FIGURE 5

NUMBER OF $FC_{AMP/TET/GEN\text{-}R}$ PER 100 mL OF RAW SEWAGE (RS) AND FINAL EFFLUENT (FE) FROM THE DISTRICT'S SEVEN WRPs

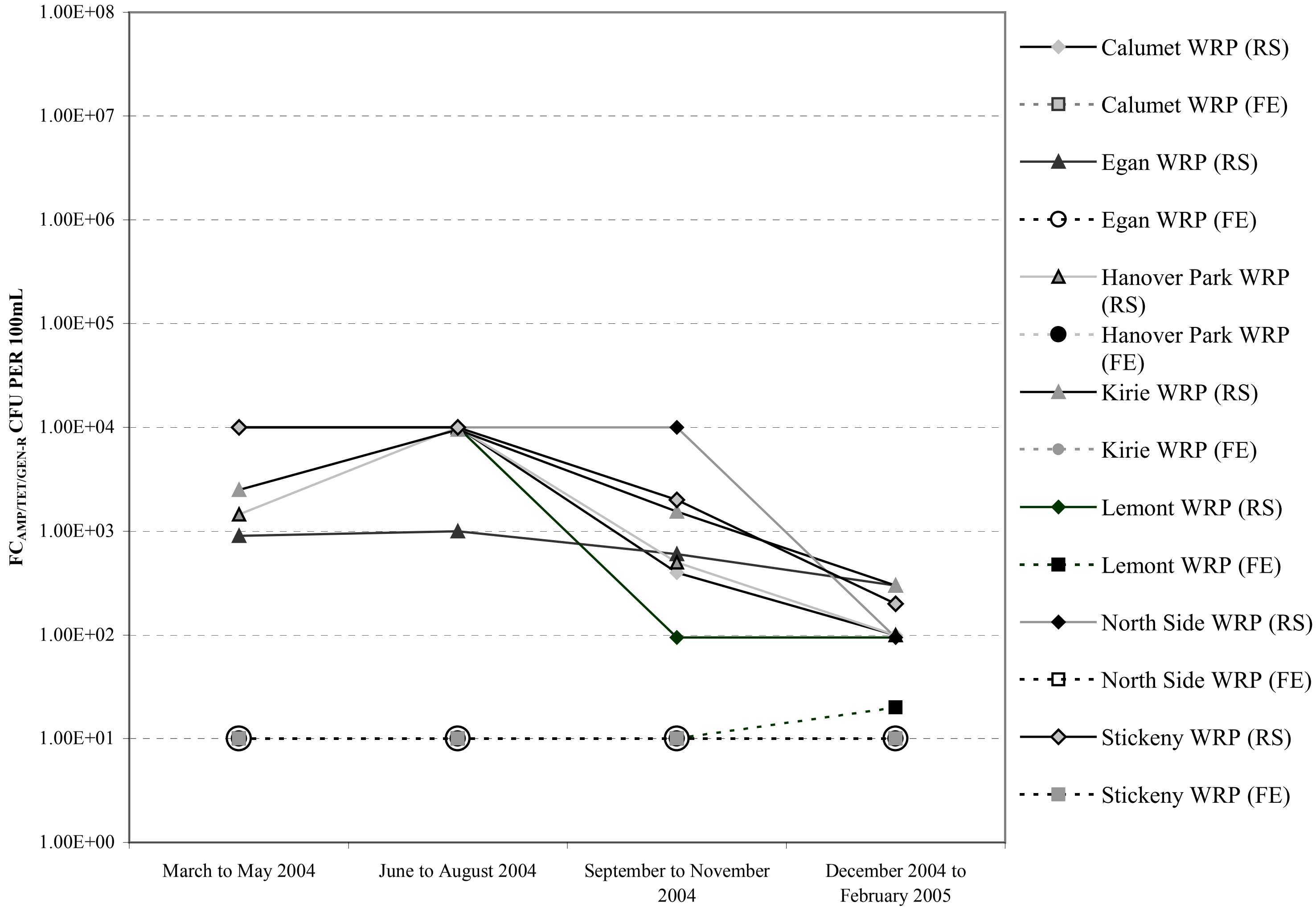

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TABLE 16

IDENTITIES OF FC_{AMP-R}, FC_{TET-R}, FC_{GEN-R} AND $FC_{AMP/TET/GEN-R}$ ISOLATED FROM RAW SEWAGE (RS) AND FINAL EFFLUENT (FE) IN THE DISTRICT

Source	FC_{AMP-R}	FC_{TET-R}	FC_{GEN-R}	$FC_{AMP/TET/GEN-R}$
RS	*E. coli*[1] (5)[2]	*E. coli* (4)	*E. coli* (6)	*E. coli* (9)
RS		*K. oxytoca*[3] (1)		
FE	*E. coli* (4)	*E. coli* (5)	*E. coli* (4)	*E. coli* (1)[4]
FE	*K. pneumoniae*[5] (1)	Unidentified (1) [6]		

[1]*Escherichia coli.*
[2]Number of isolates.
[3]*Klebsiella oxytoca*.
[4]Only one colony of $FC_{AMP/TET/GEN}$ was isolated from FE.
[5]*Klebsiella pneumoniae*.
[6]The biochemical profile of this organism is not in the Crystal™ ID System database.

pneumoniae and the biochemical profile of the other was not in the Crystal™ MIND Software database. The complete data are shown in Table BI-1.

Public health officials are concerned about all antibiotic resistant bacteria in the environment (the entire gene pool) because antibiotic resistance genes in normally harmless bacteria may be transferred to bacteria, which are human pathogens. Multiple-antibiotic-resistant bacteria in the environment represent the greatest concern for obvious reasons. Morozzi et al. (1988) and Andersen (1993) reported finding higher percentages of multiple-antibiotic-resistant bacteria including FC in treated sewage compared with raw sewage (as referenced by Guardabassi and Dalsgaard, 2002). These results suggested that the environments in sewage treatment plants might actually be conducive to the propagation of multiple-antibiotic-resistant bacteria. The results of the study conducted by the District reported here, as discussed above, indicate that conditions within the District's seven WRPs do not enhance the propagation of antibiotic resistant bacteria including multiple-drug-resistant FC bacteria. In this respect the ranges of $FC_{AMP\text{-}R}$, $FC_{TET\text{-}R}$, $FC_{GEN\text{-}R}$, and $FC_{AMP/TET/GEN\text{-}R}$ in RS and FE reported in this study are in general agreement with the findings of Guardabassi and Dalsgaard (2002) who studied antibiotic resistant total coliforms (TC) and acinetobacters. These authors reported two to three log reductions of antibiotic resistant TC in RS by tertiary sewage treatment at two sewage treatment plants in Denmark serving a combined population of approximately 740,000 people. The actual numbers of $TC_{AMP\text{-}R}$, $TC_{TET\text{-}R}$, $TC_{GEN\text{-}R}$, and $TC_{AMP/TET/GEN\text{-}R}$ reported by these authors, shown in Table 17, are not strictly comparable, of course, to the levels of $FC_{AMP\text{-}R}$, $FC_{TET\text{-}R}$, $FC_{GEN\text{-}R}$, and $FC_{AMP/TET/GEN\text{-}R}$ reported here. However, in the District, FC present in RS and FE represent approximately five to fifteen percent of the TC present in these matrices, and there is no reason to assume this ratio would be radically different for the RS and FE in Denmark.

TABLE 17

NUMBERS OF TC_{AMP-R}, TC_{TET-R}, TC_{GEN-R} and $TC_{AMP/TET/GEN-R}$ IN RAW SEWAGE (RS) AND TREATED SEWAGE (TS) FROM TWO LARGE-SCALE SEWAGE TREATMENT PLANTS IN DENMARK

Source	COLONY FORMING UNITS PER 100 ML[1]			
	TC_{AMP-R}	TC_{TET-R}	TC_{GEN-R}	$TC_{AMP/TET/GEN-R}$
RS Plant 1	$3.0 \times 10^7 – 2.0 \times 10^8$	$9.0 \times 10^5 – 5.0 \times 10^6$	$8.0 \times 10^5 – 5.0 \times 10^6$	$7.0 \times 10^4 – 1.0 \times 10^5$
TS Plant 1	$1.0 \times 10^5 – 3.5 \times 10^5$	$2.0 \times 10^3 – 2.0 \times 10^4$	$1.0 \times 10^3 – 2.0 \times 10^4$	No Data
RS Plant 2	$2.0 \times 10^6 – 9.0 \times 10^7$	$9.0 \times 10^5 – 4.0 \times 10^6$	$8.0 \times 10^4 – 6.0 \times 10^6$	$2.0 \times 10^4 – 2.0 \times 10^5$
TS Plant 2	$2.0 \times 10^4 – 2.0 \times 10^7$	$3.0 \times 10^3 – 5.0 \times 10^5$	$1.0 \times 10^3 – 5.0 \times 10^5$	$1.0 \times 10^3 – 1.0 \times 10^4$

[1]Estimated from graphs shown in Figure 4.1 in Guardabassi and Dalsgaard (2002).

If this assumption, i.e., FC = approximately 5 to 15 percent of TC, is accepted, a rough comparison of the data reported here and the data collected by Guardabassi and Dalsgaard (2002) can be made, albeit cautiously. This comparison indicates the following.

1. The $FC_{AMP\text{-}R}$ and $FC_{TET\text{-}R}$ levels in RS reported here are in the range expected based upon the $TC_{AMP\text{-}R}$ and $TC_{TET\text{-}R}$ levels in RS reported by Guardabassi and Dalsgaard (2002).
2. Levels of $FC_{GEN\text{-}R}$ and $FC_{AMP/TET/GEN\text{-}R}$ reported in this study are lower than would be expected based upon the $TC_{GEN\text{-}R}$, and $TC_{AMP/TET/GEN\text{-}R}$ reported by Guardabassi and Dalsgaard (2002).
3. The numbers of $FC_{AMP\text{-}R}$, $FC_{TET\text{-}R}$, $FC_{GEN\text{-}R}$, and $FC_{AMP/TET/GEN\text{-}R}$ in FE reported here are all less than expected based upon the $TC_{AMP\text{-}R}$, $TC_{TET\text{-}R}$, $TC_{GEN\text{-}R}$, and $TC_{AMP/TET/GEN\text{-}R}$ levels reported by Guardabassi and Dalsgaard (2002) for the sewage treatment plants which they studied.
4. As mentioned above, $FC_{AMP/TET/GEN\text{-}R}$ was virtually eliminated by secondary sewage treatment in the District.

These data indicate that secondary sewage treatment in the District may be more effective in reducing the numbers of $FC_{AMP\text{-}R}$, $FC_{TET\text{-}R}$, $FC_{GEN\text{-}R}$, and $FC_{AMP/TET/GEN\text{-}R}$ in RS than the tertiary treatment at the two sewage treatment plants in Denmark studied by Guardabassi and Dalsgaard (2002). Although the data collected support this hypothesis, it must be considered speculative.

Guardabassi and Dalsgaard (2002) reported the following average percentages of antibiotic resistant coliforms in RS at two treatment plants: $TC_{AMP\text{-}R}$ (51.4 and 47.7); $TC_{TET\text{-}R}$ (2.0 and 4.9); $TC_{GEN\text{-}R}$ (1.4 and 3.3), and $TC_{AMP/TET/GEN\text{-}R}$ (0.1 and 0.2). Guardabassi and Dalsgaard

(2002) reported the following average percentages of antibiotic resistant coliforms in TS at these two treatment plants: TC_{AMP-R} (60.3 and 50.5); TC_{TET-R} (2.2 and 2.2), TC_{GEN-R} (1.8 and 3.1) and $TC_{AMP/TET/GEN-R}$ (not detected and 0.3). (This trend is in agreement with the trend observed in the District for antibiotic resistant FC in RS and FE.) No significant differences in the percentages of these organisms in RS and TS were observed. Guardabassi and Dalsgaard (2002) reported that the relative numbers of antibiotic resistant TC were not significantly increased by sewage treatment.

The numbers of HB_{AMP-R}, HB_{TET-R}, HB_{GEN-R}, and $HB_{AMP/TET/GEN-R}$ observed in RS and FE in the District are shown in Tables CI-1 and CII-1, respectively. These data are limited but do indicate that FC_{AMP-R}, FC_{TET-R}, FC_{GEN-R}, and $FC_{AMP/TET/GEN-R}$ represent only a small percentage of the HB_{AMP-R}, HB_{TET-R}, HB_{GEN-R}, and $HB_{AMP/TET/GEN-R}$ observed in both RS and FE. The highest levels of FC_{AMP-R}, FC_{TET-R}, FC_{GEN-R}, $FC_{AMP/TET/GEN-R}$ observed in Stickney and Lemont WRP RS (Tables 2 through 5) represent 1 to 3, 7 to 13, <1, and <1 percent of the respective HB_{AMP-R}, HB_{TET-R}, HB_{GEN-R}, and $HB_{AMP/TET/GEN-R}$ levels shown in Table CI-1. The highest levels of FC_{AMP-R}, FC_{TET-R}, FC_{GEN-R}, $FC_{AMP/TET/GEN-R}$ observed in Stickney and Lemont WRP FE (Tables 6 through 9) represent <1 to 1, 2 to 8, <1, and <1 percent of the respective HB_{AMP-R}, HB_{TET-R}, HB_{GEN-R}, and $HB_{AMP/TET/GEN-R}$ levels shown in Table CII-1. Antibiotic resistant HB data were collected merely as an attempt to put the antibiotic resistant FC data into perspective. Collection of antibiotic resistant HB data was not a planned part of this study.

Although antibiotic resistant FC in sewage and environmental waters have been studied by other investigators, it is difficult to compare data previously collected with the data published in this report. There is no standard method for monitoring antibiotic resistant bacteria in environmental samples. Most published studies report on antibiotic resistance tests conducted on isolated bacterial species of interest in contrast to the method of Guardabassi and Dalsgaard (2002), i.e., incorporating antibiotics in the agar plates used for isolating the bacteria, as employed in this study. Furthermore, the concentrations of antibiotics used by different investigators also vary, and antibiotic susceptibility testing is often done using adaptations of the Kirby-Bauer disc method (Bauer et al., 1966). In order for any meaningful comparison of data quantifying antibiotic resistant bacteria in different locations to be made, a standard method must be used. The data collected for this study indicate that the method of Guardabassi and Dalsgaard (2002) as modified here would be a good standard method for monitoring antibiotic resistant FC bacteria in the environment.

The effluents from the Stickney, Calumet, and North Side WRPs are the dominant source of flow during low-flow periods in the deep-draft portions of the Calumet and Chicago River Systems and in the Lower Des Plaines River from Lockport to the confluence with the Kankakee River. Ash et al. (2002) reported levels of gram negative bacteria resistant to AMP (150 µg/mL in Luria-Bertani plates incubated at 30°C to 32°C) in 22 U.S. rivers ranging from 2.1×10^4 to 6.3×10^6 cfu/100mL including one value of 1.6×10^6 cfu/100mL for a sample from the Chicago Waterway System. It would be difficult to compare the District's FE FC_{AMP-R} data collected for this study with those of Ash because the FC_{AMP-R} were isolated by the District on the more restrictive mFC medium containing only 16 µg AMP/mL (an order of magnitude lower than the concentration used by Ash) at the restrictive temperature of 44.5 ± 0.2°C. The range of percentages of AMP resistant gram negative bacteria in U.S. rivers reported by Ash (3.9 to 53.0) is larger than the range of percentages of FC_{AMP-R} in FE reported here (9.0 to 28.4). The District is currently conducting a study to determine levels of FC_{AMP-R}, FC_{TET-R}, FC_{GEN-R}, and $FC_{AMP/TET/GEN-R}$ in the Chicago Waterway System and will compare the data collected for that study with the data reported here.

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