

City of Albuquerque

Antibiotic Resistance Analysis of Contamination in Stormwater Final Report

June 2002









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Section 1 Overview

1.1 Project Background

The United States Environmental Protection Agency (EPA) has identified bacterial impacts to surface waters as a widespread issue. In arid and semi-arid climates, as in Albuquerque, elevated bacteria levels are common in stormwater and in receiving waters during and after storm events. There are numerous potential sources of bacteria in surface waters and groundwater. Among potential human-based sources of bacteria are septage from leaking or faulty septic drain fields or tanks and sanitary sewer integrity problems. Surface waters can also be impacted by nonhuman sources such as household pets or runoff from agricultural activities. Fecal material from wildlife in open space areas may also enter surface waters via runoff or direct contamination.

Bacterial contamination is a concern because of the potential risk to human health, whether via recreational contact or due to use of a water body as a source of potable water supply. Many pathogenic bacteria, viruses, and protozoa are passed from one host to the next by the "fecal-oral route" of transmission, and water can serve as the carrier for these organisms. Of particular concern among the bacteria pathogens are *Salmonella*, *Shigella*, diarrheagenic *Escherichia coli* and *Vibrio*. Many viruses can be transmitted by water including poliovirus, rotaviruses, Norwalk and other caliciviruses, and Hepatitis A and E viruses. Among the protozoan pathogens that can be transmitted by water are *Giardia* and *Cryptosporidium*.

Because bacterial contamination has the potential to result in increased human health risks it is crucial to identify the source of bacteria. This knowledge could aid in identifying watershed management practices to address bacteria levels and thus a reduction in the potential risk to human health. Moreover, identification of any non-human contributors to bacterial loads in receiving waters can help identify the risk-based need for, and potential feasibility of and mechanisms for, mitigation of those sources.

1.2 Regulatory Background

The water quality standards for the middle Rio Grande segment located within the study area are listed in the State of New Mexico Standards for Intrastate and Interstate Surface Waters section 20.6.4.105.B.2. The standards for the protection of recreational use state that "the monthly geometric mean of fecal coliform bacteria shall not exceed 1,000/100 mL; no single sample shall exceed 2,000/100 mL (see Subsection B of 20.6.4.13 NMAC)." This area includes the middle Rio Grande Basin, including the main stem of the Rio Grande from the headwaters of Elephant Butte Reservoir upstream to Alameda bridge (Corrales bridge), the Jemez River from the Jemez Pueblo boundary upstream to the Rio Guadalupe, and intermittent flow below the perennial reaches of the Rio Puerco and Jemez River that enter the main stem of the Rio Grande.



The standards make no mention of the source of fecal contamination. Nonhuman sources of fecal coliform may not pose significant risk to human health, and the standards do not provide any regulatory relief for situations in which the sources are demonstrated to be of nonhuman origin. Other states have begun to provide such relief in recent years.

For example, the Georgia Environmental Protection Division allows a relaxation of fecal coliform stream standards in cases in which natural or nonhuman sources are demonstrated to comprise the primary source of fecal coliform. The State of Florida's environmental department is required to evaluate the source of bacteriological contamination and verify that the impairment is due to chronic discharges of human-induced bacteriological pollutants before placing the water segment on Florida's 303(d) list for water quality-impaired waters. The department must then reevaluate the data, excluding any values that are elevated solely due to wildlife.

In urbanized areas across the country, regulators have increased their scrutiny of stormwater systems as potential contributors to degradation of bacteriological water quality. The New Mexico Environment Department (NMED) issued a draft Total Maximum Daily Load (TMDL) for fecal coliform in the middle Rio Grande in October 2001.

The City of Albuquerque (City) Municipal Stormwater Discharge Permit requires fecal coliform monitoring in portions of the City's stormwater system. Historically, fecal coliform has been used as the primary indicator organism for bacteriological water quality in the middle Rio Grande. However, only the magnitude of fecal coliforms is typically measured – with no indication of the source of the fecal coliforms or the potential risk to human health.

Although the final TMDL for the middle Rio Grande segment has yet to be developed, the City undertook this study to assess the sources of fecal contamination within their stormwater system. Characterizing the sources of bacteria in the watershed may help bring resolution to the risks to human health associated with fecal coliforms in the receiving water. Moreover, it has the potential to provide insight into the feasibility of implementing measures to mitigate sources of bacteria in the watershed. This Bacterial Source Tracking (BST) Study was commissioned by the City to identify potential sources of fecal coliform in selected stormwater drainage areas tributary to the Rio Grande in Albuquerque using the antibiotic resistance analysis (ARA) methodology.

1.3 Project Goals

This BST Study investigated potential sources of fecal coliform contributing to outfalls from the City's stormwater collection system to the Rio Grande. The subject stormwater systems and sampling locations are described in more detail in Section 4.

The primary goal of this study was to characterize potential fecal coliform contributors in the subject storm drainage areas. As part of the study, nine potentially significant contributing sources were identified that included human, dog, cat, horse, cow, rabbit,



rat, pigeon and bird sources (as explained in Section 3) with fecal coliform loading from these groups investigated at the each of the stormwater collection sites.

Characterizing the sources of fecal coliform will help the City assess potential "hotspots" within its stormwater system, focus and guide any necessary corrective actions, and assist the City in planning for potential stormwater permit conditions that could result from the final middle Rio Grande bacteria TMDL. Given that bacteriological quality represents the most critical element of risk to recreational users, source characterization is the first step in determining the feasibility for source control or best management practices (BMPs) and if feasible, developing a strategy for implementation.



Section 2 Antibiotic Resistance Analysis Methodology 2.1 Overview of ARA

ARA is a demonstrated technique for distinguishing between sources of bacteria in a watershed. Previous methods for bacteria source identification – such as fecal coliform/streptococci ratio, species-specific bacteriophages, DNA fingerprinting/ genetic profiling, tracers and fatty acid profiling – have proven to be too expensive, complicated, or laborious for routine use in a watershed. Recent advances in ribotyping and other emerging technologies show promise, but are costly relative to ARA. ARA has been utilized successfully in other watershed projects such as Fulton County, Georgia's Watershed and Stormwater Management Assessment, and the City and County of Denver, Colorado's stormwater system.

The concept behind ARA is that different groups of animals are exposed to different antibiotic types and concentrations. Examples of animal groupings are humans, domestic animals (e.g. dogs and cats), recreational/agricultural animals (e.g. horses and cows), and wild animals (e.g. rabbits and birds). The bacteria associated with each animal type develop resistance to the antibiotics to which they are exposed. These resistance patterns are characterized for each source group and subsequently used to identify sources of bacteria in stormwater or stream samples. Bacteria that are resistant to a certain antibiotic will replicate in the presence of that antibiotic. Those bacteria that are not resistant to an antibiotic will not replicate in its presence.

An ARA study is conducted in two steps that can be conducted in either order or simultaneously. One step is to establish a database with antibiotic resistance patterns (ARPs) of known or suspected potential sources within the watershed. The target source groups are identified based on knowledge of potential contributors within the watershed. Antibiotics are selected with the intent of maximizing the differentiation between groups. Fecal coliform isolates are prepared from fecal samples of "known" sources. A database (or "library") of the ARPs of each known source is developed. This database is validated by assessing its propensity to produce false positive or false negative results.

In the other step, ARA and the database of ARPs are used to characterize the sources in the water bodies of concern. Fecal coliform isolates are prepared from stream or stormwater samples (with bacteria originating from "unknown" sources). The "unknown" isolates are then tested to identify their ARPs and compared to the database developed in the first step. Statistical discriminant analysis is used to group the unknowns into the "known" categories. The technique assigns each isolate to a most probable source from one of the "known" categories. Therefore, if the true source animal group of the "unknown" coliform isolate is not included in the database, that isolate will be misclassified. This requires careful consideration of the potential significant sources of bacteria in the subject watershed.



2.2 Sample Preparation and Antibiotic Resistance Pattern Determination

Fecal, wastewater, and stormwater samples were collected in sterile containers. For ARP library development, feces from individual scats were placed in separate containers, and the source and date was recorded. Samples were shipped to the University of South Florida laboratory by overnight courier service, in coolers, on ice packs. Laboratory personnel were notified by email when samples were shipped to ensure timely processing upon receipt.

Water samples were processed by filtering several different volumes of water by membrane filtration, with the goal of obtaining at least 42 well-isolated colonies per sample. Wastewater samples (for human ARP development) were first diluted, and were subsequently subjected to membrane filtration. Fecal coliforms were isolated by standard membrane filtration methods (i.e. samples were filtered through a 0.45 mm filter, which was placed on mFC agar and incubated in a water bath at 44.5° C). Colonies were counted after 22 to 24 hours incubation to estimate the extent of contamination of the water. Colony counts are expressed in colony forming units per 100 milliliters (CFU/100 mL).

Fecal samples were processed by inoculating a sterile swab with fecal material. The swab was streaked onto a petri dish (100 mm diameter) containing mFC agar. The mFC plates were incubated for 22 to 24 hours, in a water bath at 44.5° C. Isolated colonies (no more than ten per sample) were chosen for further analysis. (Note: one fecal sample represents fecal material from one animal in this study.) In some cases (notably rabbits), fecal coliform isolates were not readily obtained from the fecal material. In such cases, several grams of fecal material were added to Escherichia coli (EC) broth, which was incubated at 44.5° C for 22 to 24 hours. Growth from the broth cultures was streaked to mFC agar, which was incubated at 44.5° C for 22 to 24 hours. Very few fecal coliform isolates (four) were obtained from rabbit feces despite repeated culture attempts from many samples, some of which were obtained from freshly trapped rabbits. These difficulties suggest that (1) there are relatively few fecal coliforms in the feces of these rabbits, and/or (2) the fecal mass dries out relatively quickly because of its small size, which renders the fecal coliforms in the feces dead or nonculturable.

ARA was accomplished in the same manner for all sample types (fecal coliform isolates from feces, wastewater, and stormwater). Well-isolated colonies from mFC agar were transferred with sterile toothpicks to individual wells of a microtitre plate containing EC broth + MUG (4-methylumbelliferyl-b-glucuronidase). Bacteria were cultured overnight at 37° C, and fluorescence under UV light was observed after 24 hours to differentiate MUG-positive (E. coli) fecal coliforms from MUG-negative fecal coliforms. MUG-positive fecal coliform (E. coli) fingerprints were used to form the libraries and were picked for analysis from stormwater samples. 20 mL of each culture was individually diluted in 450 mL PBS in plastic dilution tubes. The diluted cultures were used to inoculate plates for ARA (see below).



Each antibiotic was used at three concentrations, as well as a control (no antibiotic) plate for each series. Table 2-1 lists the antibiotics and concentrations used for this study. Isolates were transferred from EC-MUG broth to petri dishes (150 mm) containing Mueller-Hinton agar with a 96-prong replica-plating device. Each plate contained one concentration of one antibiotic. Antibiotic-containing plates were incubated for 24 hours at 37°C. For each antibiotic concentration, a bacterial isolate was scored positive if there was any discernible growth to the eye, and negative if there was no growth. For each isolate, the highest antibiotic concentration yielding growth was recorded for each antibiotic and entered into a *Microsoft Excel* spreadsheet.

Amoxicillin	4	20	128
Cephalothin	8	32	128
Chloramphenicol	0.8	8	32
Chlortetracycline	4	64	256
Doxycycline	4	64	128
Moxalactam	0.2	2	8
Oxytetracycline	25	100	200
Penicillin G	20	200	500
Polymixin B	0.1	1	10
Tetracycline	4	64	256
Trimethoprim	0.25	1	10
Trimethoprim/Sulfamethoxazole	0.5	5	50

Table 2-1 Antibiotics and concentrations used for ARA (µg/mL)



Section 3 Database Development 3.1 Source Sampling for Database Development

The primary database, or library, of fecal coliform isolates from known sources is comprised of 2,095 MUG-positive fecal coliform isolates obtained from the feces of birds, cattle, cats, dogs, horses, humans (wastewater), pigeons, and rabbits (Table 3-1). A summary of the total samples collected and corresponding isolates can be found in Appendix B.

Bird	Cat	Cattle	Dog	Horse	Human	Pigeon	Rabbit
160	329	233	285	281	458	345	4

Source

These specific known sources were chosen for the study because they were suspected contributors to fecal coliform loads in the Albuquerque area stormwater system. The fecal material for each source was collected during at least three separate sample events. Sewage was collected from points in the wastewater collection system where the dominant input was domestic, rather than hospital-associated or industrial waste. Because most of these wastewater isolates are from human sources, the term human is used interchangeably with wastewater in this report. General descriptions of the source groups evaluated in this study are provided below.

3.1.1 Humans

Human fecal matter is a potential concern in stormwater as discussed in Section 1.2, above. The goal for sampling to construct the ARP library for human sources was to collect wastewater that contained primarily domestic waste. The majority of Albuquerque's wastewater is generated from domestic sources. Industrial wastewater is concentrated in areas near the river in historically industrial areas. Therefore, two manholes were selected that have sources of primarily domestic and commercial origin for sampling of domestic wastewater for ARP library development.

The first manhole is located south of Lomas Boulevard, just west of Easterday Drive (manhole K2052). Flows to the manhole are from residential neighborhoods and commercial flows from Lomas Boulevard. Sampling occurred on two different occasions, both between 9 and 10 am on October 27, 2001 and February 5, 2002.

The second manhole is located west of Eubank Avenue, along the Bear Canyon Arroyo (manhole F20586). Flows to the manhole are from residential neighborhoods north of Bear Canyon and East along Spain. Samples were also collected on October 27, 2001 and February 5, 2002.



3.1.2 Dogs

Fecal matter of dogs was collected from locations throughout the watershed. Private homes, City open space, trailheads, the local Humane Society and City animal shelter, were sampled for this study. As dogs are a domesticated, captive animal, collection of samples was relatively straightforward, although issues associated with bacterial survival in arid climates were encountered. Further discussions on that topic are developed in the following sections.

3.1.3 Cats

As with dogs, samples of domestic cat feces were collected from private homes and private and city shelters.

3.1.4 Cattle

Two distinct types of cattle, beef and dairy, are present in the Albuquerque basin. For the purposes of this study, one ARP was composited from samples of both types. Collection of cattle samples was challenging, due to resistance by most owners to cooperate with government sponsored studies. Explanations were given that the background samples were generalized to the basin and would not directly identified with their livestock. However, frequently owners of livestock refused to allow sample collection from their animals. Additionally, the majority of cattle owners in the Albuquerque area (excluding dairies) have generally small herds. Traditionally many ranchers keep cattle in irrigated fields near the river, and as the population in the region grows, increasing demand for property near the river could further reduce the impact of cattle on the Rio Grande. Nevertheless, a number of cooperative cattle owners were found and an adequate number of both dairy and range cattle were sampled to form a database with 233 coliform isolates.

3.1.5 Horses

Horses were one of the more problematic sources from which to gather background samples. For efficient use of time, sampling was conducted at riding and boarding stables as well as breeding facilities. As with cattle, some owners/operators of these types of facilities were reluctant to participate. Cooperation from a number of both private owners and boarding stables did allow for collection of sufficient isolates. The majority of these facilities were very clean with a precise knowledge of the age of the manure in the stalls and fields. Most facilities had frequent (sometimes two or three times daily) collection of horse manure.

3.1.6 Pigeons

Pigeons inhabit a variety of areas within the Albuquerque basin. Primarily, they roost on roofs or ledges of tall buildings or in loft areas of barns. Collection points included a variety of sites in downtown Albuquerque, at the University of New Mexico (UNM), at one of the City stormwater lift stations, and in barns at a horse stable.



The size of the pigeon droppings led to two problems. One problem is the necessary sample volume required collection of 3 or 4 fresh droppings. The second is the small size makes the droppings very amenable to rapid desiccation. Frequently, locations with large volumes of droppings yielded no usable samples because of rapid desiccation. Practically, samples are desiccated within the first few hours after being deposited. This problem was a limitation with all of the small wildlife addressed in this study.

3.1.7 Birds

In addition to pigeons as a source group, the City expressed interest in looking at birds as an overall group. As with pigeons, sample collection is very difficult in Albuquerque's climate. The primary sources of bird droppings come from two species. Ducks and geese provide relatively accessible, large volume samples for collection. Attempts were made to collect droppings from smaller wild birds at the Rio Grande Nature Center, areas of the UNM campus, and various parks throughout Albuquerque. At the Nature Center, where small birds are attracted to multiple feeders, no viable samples could be collected. Except in areas where large flocks of ducks or geese congregate, which are generally near the river, birds are unlikely to generate a significant volume of fecal matter. However, areas in the bosque with large migratory populations could, theoretically, provide a volume of fresh (live bacteria) fecal matter to storm events.

3.1.8 Rabbits

Wild rabbits are populous in the basin as evidenced by the large volume of droppings in open space areas. This volume seemed to indicate rabbits may be a potentially significant contributor to bacteria in receiving waters. During storm events, rabbit fecal matter is flushed into arroyos and has been observed to accumulate in dam areas.

However, as discussed with the other small animals above, locating droppings fresh enough to contain viable bacteria was problematic. In order to obtain viable bacteria samples were collected early in the morning, when rabbits are most active, and samples were preserved with a buffer solution and placed on ice. CDM collected twenty samples, during multiple sampling events, none of which resulted in a single viable fecal coliform colony.

Finally, rabbit traps were placed in a variety of locations in an attempt to capture live rabbits and collect very fresh samples. However, this method was limited by the fact that traps could be checked only with a limited frequency. Therefore, droppings of captured animals could be as much as twelve hours old.

Contributing to the difficulties of modifying the study from fecal matter collection to animal trapping is overall condition of the rabbit population in the Albuquerque area. Albuquerque has been in drought conditions for the past two years and this generally has a strong adverse effect on small animal and rodent populations. The total precipitation for 2001 was 6.50 inches, which was 2.38 inches below normal. 2001 was the twelfth driest year on record since 1931 according to the National Weather Service Annual Summary for Albuquerque. Whereas the population of rabbits in the area is still likely very large, the challenge of coaxing a particular rabbit into a trap is not a small one.

3.1.9 Rats

Initially, the City requested rats be addressed as a possible source. The main concern was the location of warehouses and other industrial buildings, again which are more concentrated in the valley near the river, as being a location where rats could thrive. Subsequently, in discussion with City and County Environmental Health staff, Animal Control, and Vector Control personnel, no known locations of large concentrations of rats were evident. Rats are occasional nuisance problems in restaurants and warehouses, but the population appears to be small and disperse enough that they were excluded from this study.

3.2 Database (Library) Analysis

The accuracy of the primary library was assessed by using discriminant analysis (performed with SAS v. 8.2) to generate a source-by-source matrix of correct classification rates (Table 3-2, below). Discriminant analysis is a statistical tool used to determine which variables discriminate between two or more naturally occurring groups. The ARA process uses the antibiotic resistance patterns of isolates in the database as (1) the calibration data set or "standard", and (2) as the test subjects. This process allows an assessment of the internal consistency of the library. In tables in this report, columns (labeled in capital letters) indicate the source CATEGORY into which isolates were classified by discriminant analysis. The rows are labeled by the source of the isolates tested in the analysis. For each source the number of isolates classified into a given CATEGORY is given, and the percentage of isolates classified into that CATEGORY is listed directly below. The average rate of correct classification (ARCC) reflects the overall classification accuracy of the library (number of isolates correctly classified into each CATEGORY divided by total number of isolates), and is given at the bottom of each table.

As an illustration of how to interpret these tables, 160 fecal coliform isolates from bird feces were analyzed (Table 3, last column, bolded). Sixty-nine (43.1%) of the bird isolates were correctly classified as BIRD (bolded and underlined), while (reading horizontally along the row) 11 (6.9%) were incorrectly classified as cattle, 20 (12.5%) were classified as PIGEON isolates, and 21 (13.1%) were incorrectly classified as HUMAN isolates. The ARCC of this library is 44.5%. Although this ARCC may seem somewhat low compared with published values in the literature, it is important to note that there are a total of eight possible sources represented in this library, which is more than is used in most studies. The ARCC is far above what would be predicted if isolates were assigned to source by chance alone, which is 1/8 (12.5%).

Because there were so few fecal coliform isolates obtained from rabbit feces, these isolates do not comprise a representative sampling in the library. The isolates from rabbit feces were therefore omitted from the analysis when stormwater isolates were analyzed. The correct classification rates of the isolates in various categories did not significantly change when the rabbit isolates were deleted from the library (Appendix A, Table A-1).



SOURCE	BIRD	CAT	COW	DOG	HORSE	HUMAN	PIGEON	RABBIT	TOTAL
Bird	69	30	11	5	3	21	20	1	160
	<u>43.13%</u>	18.75%	6.88%	3.13%	1.88%	13.13%	12.50%	0.63%	100.00%
Cat	50	99	15	11	4	50	4	0	233
	21.46%	<u>42.49</u> %	6.44%	4.72%	1.72%	21.46%	1.72%	0.00%	100.00%
Cow	13	23	125	5	49	39	75	0	329
	3.95%	6.99%	<u>37.99</u> %	1.52%	14.89%	11.85%	22.80%	0.00%	100.00%
Dog	28	36	12	74	52	55	28	0	285
	9.82%	12.63%	4.21%	<u>25.96</u> %	18.25%	19.30%	9.82%	0.00%	100.00%
Horse	13	14	52	4	122	35	41	0	281
	4.63%	4.98%	18.51%	1.42%	<u>43.42</u> %	12.46%	14.59%	0.00%	100.00%
Human	81	84	5	21	33	187	46	1	458
	17.69%	18.34%	1.09%	4.59%	7.21%	<u>40.83</u> %	10.04%	0.22%	100.00%
Pigeon	23	13	20	2	24	10	253	0	345
	6.67%	3.77%	5.80%	0.58%	6.96%	2.90%	<u>73.33</u> %	0.00%	100.00%
Rabbit	0	0	0	0	0	0	0	4	4
	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	<u>100.00</u> %	100.00%
TOTAL	277	299	240	122	287	397	467	6	2095
	13.22%	14.27%	11.46%	5.82%	13.70%	18.95%	22.29%	0.29%	100.00%
ARCC = 933/2	2095 = 44.5%								

Table 3-2 Correct classification rates for known source isolates in the library

Pigeons and birds were combined into one source category (BIRD), resulting in an ARCC of 47.9% (Appendix A, Table A-2). Importantly, the correct classification rate for human isolates jumped from 40.8% to 55.2% (compare Appendix A Table A-1 to Table A-2). When categories were further consolidated, by pooling cattle and horse isolates into LIVESTOCK, the ARCC was 52.3% (Appendix A, Table A-3). Pooling dogs and cats into a PET category resulted in extensive misclassification of human isolates (Appendix A, Table A-4), and that grouping was therefore not used for analysis of isolates from water samples. Note that dog isolates misclassified frequently in all other animal and human categories, except cow. This problem has not been encountered in other ARA studies, and cannot be readily explained at this time. Future work should focus additional sampling and a variety of antibiotics to refine the differences between dogs and humans.

Discriminant analysis can be run with all isolates from animal sources pooled into one ANIMAL category. In this case (Table 3-3 below), it is evident that isolates from all animal sources do not misclassify very frequently as human isolates, but that almost half of human isolates misclassify as animal isolates. This finding is common in bacterial source tracking, whether ribotyping (Parveen et al., 1999), or ARA (Harwood et al., 2000) is used as the method. In terms of interpreting the results, these results suggest that an elevated percentage of human isolates in a water sample is a robust finding, since one is more likely to miss human contamination when it is present than to identify human contamination when it is absent.



SOURCE	Animal	Human	TOTAL			
Animal	1404	233	1637			
	85.77%	14.23	100.00			
Human	223	235	458			
	48.69	<u>51.31%</u>	100.00			
TOTAL	1627	468	2095			
	77.66	22.34	100.00			
ARCC =1177/20911 = 56.3%						

Table 3-3 Discriminant analysis.	ANIMAL vs. HUMAN.
Rabbit isolates included	

Further validation of the accuracy of the databases was measured by a challenge, known in statistical language as a "hold-out validation." A random sample of 209 isolates was obtained by sorting the library by isolate number. These isolates were removed from the library, and were used as "unknowns" in the analysis. The analysis of the smaller library minus holdout isolates (1,882 isolates) demonstrates that the ARCC of the library was not affected by holding the 209 isolates out, as the ARCC was 44.3% for the larger library, and 44.1% for the smaller library (Appendix A, Table A-5). The 209 hold-out isolates were correctly classified by the smaller library, in which they were not included, at a frequency of 41.6%, which is little different from the ARCC for the self-cross of the larger library (Appendix A, Table A-6). The rationale behind the hold-out analysis is that if the database is representative of the diversity of the fecal coliform population in the various fecal sources tested, the correct classification rate for the hold-out isolates will be approximately the same as that of the internal validation of the library.

Section 4 Analysis of Water Samples

Fecal coliforms were isolated from water samples obtained at designated sites along the stormwater drainage system during runoff events. No baseflow sampling was performed as a part of this study. Due to the ephemeral nature of runoff at these sites, only one stormwater sample was obtained from some sites. Due to localized storm patterns, many of the sites experience few (if any) runoff events during the monsoon season.

4.1 Storm Events

The Albuquerque basin receives more than half its annual precipitation during what is known as the "monsoon" season. The monsoon season in Albuquerque is generally from late July to early September. Additionally, precipitation is typically concentrated in small, intense, fast moving storms, which frequently impact only portions of the watershed. These thunderstorms usually move through the area from the evening until early morning. As a result, often times the official precipitation gauging station for Albuquerque, located at the Albuquerque International Sunport, recorded trace or no precipitation when sampling sites further north had significant flow.

4.2 Stormwater Sampling Sites

Sixteen locations were chosen for stormwater sampling throughout the Albuquerque area. The goal was collection of samples from two storm events at each location. Two of the sixteen locations have a small amount of baseflow, the remainder do not. Baseflow sampling was not included in this study. Sampling was performed in conjunction with the existing stormwater sampling performed for the City and the Albuquerque Metropolitan Arroyo and Flood Control Authority (AMAFCA) by the United States Geological Survey (USGS). USGS personnel currently perform sampling from a variety of points in the Albuquerque area during large storm events.

The sites were chosen to be well distributed over the watershed and encompass a variety of potential sources and land uses. A number of sites are paired along a given sub-basin, with one sampling location high in the basin and the other further toward the river. Sites were included on both the east and west sides of the Rio Grande, with the majority on the east side. A brief description and map of each arroyo is included for each site below, a map of the City with drainage structures is included as Appendix C, and site photos are included as Appendix D.

4.2.1 North Domingo Baca Dam Area

The North Domingo Baca Dam (North Domingo) is located just east of Eubank and north of Paseo del Norte in the North Albuquerque Acres Area (see Figure 1). North of the dam area is the Greiner soccer field, a county park facility. The North Domingo Baca Arroyo, which flows into the dam area, is an unlined channel for most of its length. Just east of the soccer field the arroyo is concrete lined for approximately 200 yards. The drainage area for this sampling site is entirely within North Albuquerque Acres. Additionally, due



to the high volume of rabbit droppings this location was also one of the sites where rabbit traps were placed. Two samples were collected from the North Domingo Baca Site. This site was sampled twice, on 8/2/01 and 8/15/01. Because only one isolate was recovered for analysis from the 8/2/01 sample due to excessive crowding on the plates, the two sample dates were not analyzed separately. The strongest influences at this site were dogs and pigeons.

4.2.2 North Diversion Channel

The North Diversion Channel (North Diversion) captures all flows from the northeast heights and discharges to the river just south of the Sandia Pueblo north of 4th Street (see Figure 2). The USGS has a gauge and sampling equipment just south of where Edith crosses the channel. Due to the large size of the channel, the City agreed that the USGS would be responsible for collection of the North Diversion Channel sample. However, CDM collected one sample from the bridge at Edith (North Diversion – Edith) and one at the bridge at Paseo del Norte (North Diversion - Paseo) during one storm event. These two sites are very close to one another and are located in the same channel. Both were sampled 11/16/01, therefore they are virtually replicate samples. This relationship is reflected in the strong agreement on sources between the two samples; human and livestock dominate both. Cattle were identified as the dominant livestock source at NDE, while horses were the dominant source at NDP. This difference may be due to true variations in the source of isolates, and would reflect sample-to-sample variability. Alternatively, cattle isolates most frequently misclassify as horse, and vice versa, therefore the difference may be due to cross-classification of livestock isolates. Because these sites were sampled only twice, such questions in the data were expected, and can be resolved with (1) further sampling of the sites and/or (2) more intensive sampling of horses and cattle in the area.

4.2.3 Pino Dam

The Pino Dam (Upper Pino) is north of Academy at Tramway and captures flow from the Pino Arroyo entering from the east and the Bear Tributary Diversion from the south (see Figure 3). This area has a persistent pond during the summer and fall. Samples were collected where the Pino Arroyo enters the dam area, at a concrete drop structure at the east side. Samples from two storm events were collected at Pino Dam. This site was sampled on 8/15/01 and 8/31/01. Dog isolates dominated this site, particularly on 8/15/01. Significant bird contributions were detected at this site on 8/31/01 only.

4.2.4 Pino Arroyo at Washington

The Pino Arroyo (Lower Pino) travels from the Pino Dam and enters the North Diversion Channel west of Jefferson (see Figure 3). Approximately 200 yards west of Jefferson, there is a high flow bypass structure. All low flows enter an AMAFCA pilot wetlands project. Just prior to the structure was the Pino Arroyo at Washington sampling point. Pigeon isolates were extremely dominant at this site on both sampling dates (8/2/01 and 8/9/01).



4.2.5 Bear Canyon at Juan Tabo

Bear Canyon at Juan Tabo, behind the John Robert Dam, (Upper Bear) is the uppermost sampling point on the Bear Canyon Arroyo (see Figure 4). The samples were collected at the structure that runs through the John Robert Dam. The dam area above the spillway is a sand bottom channel. During a number of storm events, flow could be seen up the channel, but it wouldn't come within a hundred yards of the dam. Only one sample was collected, on 7/27/01, and was dominated by Bird and Human sources.

4.2.6 Bear Canyon at San Mateo

Bear Canyon at San Mateo (Lower Bear) is near the bottom of the Bear Canyon Arroyo, just east of the discharge to the North Diversion Channel (see Figure 4). Although the arroyo is a sand channel at this point, the majority of upstream flow is contributed by runoff from urban areas. Two samples were collected, one on 7/27/01 and one on 8/2/01. Human and dog isolates were the dominant sources when sample dates were combined (Appendix A Tables A-9 and A-10). When samples taken on different dates were analyzed separately (Appendix A Table A-11), it is clear that the dog isolates were present on one date (7/27/01), human isolates were present on both dates, and that the bird source contributed a significant percentage of isolates on 8/2/01.

4.2.7 Embudo at Monte Largo

The Embudo Arroyo (Upper Embudo) drains from the foothills of the Sandias and runs under Monte Largo Street, where the channel is concrete lined and begins receiving flows from urban areas (see Figure 5). The USGS maintains a gauge, which records peaks, however, due to the short duration of runoff the USGS has never collected samples at the gauge. During sampling, runoff typically lasted between fifteen and thirty minutes for measurable flow. ML was sampled on 7/27/01 and 8/9/01. Bird sources also dominated this site, however, on 8/15/01 only, there was a strong human influence detected.

4.2.8 Embudo at Snow Heights

Embudo Arroyo passes through Snow Heights Park (Lower Embudo), between Eubank and Wyoming on Indian School (see Figure 5). At this point, Embudo has received the flows of both Embudito and Piedra Lisa Arroyos. From Snow Heights, Embudo travels southwest and empties into the I-40 Channel. Lower Embudo was sampled twice, on 7/27/01 and 8/2/01. When all isolates from Lower Embudo were combined, pigeon, cat and human sources were identified. On both dates, bird isolates were an important source, but human isolates were identified only on 7/27/01. Cat isolates were codominant with bird isolates on 8/2/01, but these results must be interpreted cautiously because only four isolates could be obtained for analysis.

4.2.9 Tijeras at Four Hills

Samples for this site were collected below the overpass where Four Hills Blvd crosses the Tijeras Arroyo (Upper Tijeras) (see Figure 6). Flows come from rainfall in Tijeras Canyon, from the communities of Carnuel and Tijeras. Upper Tijeras was sampled 7/27/01 and 8/3/01. No fecal coliforms were isolated from the 8/3/01 sample due to smeared



colonies on the membrane filters. Birds (other than pigeon) and human sources dominated this site on 7/27/01.

4.2.10 Tijeras at Broadway

The USGS gauge where the sample is collected is west of Broadway (Lower Tijeras) and the closest urban stormwater source upstream from this site are outfalls from the Albuquerque International Sunport and Kirtland Air Force Base (see Figure 8). The channel itself is wide and sandy and the majority of the land immediately upstream is undeveloped. Flow was only noted to reach this location during one event (9/13/01) and the sample could not be transported to the lab due to the events of September 11th.

4.2.11 South Diversion Channel at Avenida de Cesar Chavez

This sample location is in the South Diversion Channel, immediately behind the Motel 6 on the south side of Avenida de Cesar Chavez (Upper South Diversion) (see Figure 6). The South Diversion Channel collects flow from largely urban areas and is one of the few locations that have a relatively constant supply of water. As a result, the channel is filled with cattails and other vegetation. The flow in the channel was observed during base flow and stormwater samples were collected during events with significantly elevated flow. This site was sampled twice, on 8/2/01 and 8/9/01. Most of the isolates were identified as bird and pigeon source on both sample dates.

4.2.12 South Diversion Channel at Tijeras Arroyo

The South Diversion Channel at the junction with the Tijeras Arroyo (Lower South Diversion) collects flow from approximately south of Central Avenue and east from the Ridgecrest neighborhood and areas south of downtown (see Figure 8). The drained area is much less than that which is channeled to the North Diversion Channel. Only one sample was taken at this site. Dog isolates dominated the source assignments.

4.2.13 Barelas Pump Station

The Barelas Pump Station (Barelas) operates to collect runoff from areas east of the river and west of the South Diversion Channel and provide lift to drain the water to the Rio Grande (see Figure 8). The flows come primarily from the downtown Albuquerque and areas south. In addition to having older sewer systems, these areas have relatively large homeless populations. One sample was collected on 7/27/01, and was dominated by dog and human isolates.

4.2.14 San Jose at Woodward

This location (San Jose) drains the remainder of the southern portion of downtown not serviced by Barelas (see Figure 7). This site was sampled 8/2/01 and 8/3/01. Dog and human isolates were most important at San Jose. This site is one where the results are relatively difficult to interpret, as the percentage of isolated identified from various sources were relatively evenly distributed. Interestingly, the fecal coliform counts were over tenfold higher on 8/3/01 compared to 8/2/01 (Appendix A, Table A-8), and the relative contribution from dog isolates increased considerably, from 5.1% on 8/2/01 to 38.7% on 8/3/01.



4.2.15 Calabacillas at Coors

The Calabacillas Arroyo (Calabacillas) a large arroyo and gathers flow from much of northwest Albuquerque (see Figure 9). Flows come from north and south of the channel, including contributions from Rio Rancho by way of the Seven Bar Channel. This site was sampled twice, on 8/3/01 and 8/15/01. Dog and cat isolates dominated this site on both days. On 8/15/01 only, a significant percentage of isolates from livestock were detected. When the analysis was run using a six-way classification (birds, cats, cattle, dogs, horses and humans), equal numbers of isolates (16.1% each) were assigned to cattle and horse sources.

4.2.16 San Antonio at USGS gauge

The San Antonio Arroyo (San Antonio) is south of the Calabacitas and has inflows from the Ladera Detention Basin to the south and the Mariposa Detention Basin to the north (see Figure 10). The channel itself begins near the western edge of development in the City at Unser and Montano. This site was sampled 8/15/01 from a composite collected by USGS personnel. Dog isolates were identified as the dominant source of contamination.

4.3 Summary of Water Sampling

A summary of all stormwater samples collected, with the dates the samples were received, is included as Appendix B.

The antibiotic resistance data for each site were analyzed in several ways. Rabbit isolates were omitted from the analysis because very few bacteria were isolated from rabbit feces (4), all of which came from the same animal, which does not constitute a representative sample. A key for sample site abbreviations is included in Appendix A, Table A-7.

- The library (calibration data set) was divided into seven categories: bird, cat, cow, dog, horse, human, and pigeon (Appendix A, Table A-9).
- The library was divided into five categories: bird (pigeon and other birds combined), cat, dog, human, and livestock (cattle and horses combined) (Appendix A, Table A-10).
- Samples from the same sites collected on different dates were analyzed separately using the 5-category arrangement of the library (Appendix A, Table A-11).

The impact of each source at each sampling location is also outlined in Section 4.2. Approximate fecal coliform counts for samples were obtained by membrane filtration (Appendix A, Table A-8), and the isolates were subjected to ARA as above (Appendix A, Tables A-9 through A-11).

A summary table of the stormwater results is included in Section 5 (Table 5-1). This table indicates that, overall, the major sources of contamination to the stormwater system are dogs and humans (8 of 16 sites), ducks and geese (6 of 16 sites), and pigeons (4 of 16). Cats, cattle, and horses appear to be responsible for far less contamination (2 of 16 sites each). These values are also reflected in the total percent of isolates from each source in all samples (Appendix A, Table A-11, bottom row), which is 25.2% for birds and pigeons combined, 24.4% for dogs, and 25.5% for humans.

Section 5 Results and Recommendations 5.1 Results

The ARP library used as the calibration data set for water samples obtained from selected stormwater locations tributary to the Rio Grande, was composed of 2,091 MUG-positive fecal coliform isolates from bird (duck and goose), cat, cattle, dog, horse, human (wastewater), and pigeon feces. The average rate of correct classification (ARCC) for this library was 44.3% when these seven source categories were used for analysis. The ARCC increased to 52.3% when pigeons and other birds were pooled into one category, and cattle and horses were pooled into another to yield a total of five categories.

A total of 1,027 bacteria from 16 sites were classified into source categories by discriminant analysis. As indicated by impact at eight of the sixteen sites, dog and human isolates dominated the watershed overall, followed by bird, which impacted six of the sixteen sites. Identification of contamination from cat and livestock feces was rare, and the livestock hits occurred at outlying points in the stormwater system that might be most likely to be influenced by horses and cattle. The dominant sources at each site are shown in Table 5-1, below.

SOURCE	DATE	BIRD	CAT	DOG	HUMAN	LIVESTOCK	Fecal Coliform Count ¹
North Domingo	08/15/01	34%	4%	42%	4%	16%	770
North Diversion- Edith	11/16/01	11%	2%	6%	25%	56%	9,200
North Diversion- Paseo	11/16/01	20%	10%	5%	31%	35%	17,000
Upper Pino	08/15/01	6%	12%	71%	0%	12%	2,300
Upper Pino	08/31/01	35%	2%	31%	11%	21%	14,800
Lower Pino	08/02/01	86%	0%	0%	14%	0%	2,500
Lower Pino	08/09/01	80%	0%	3%	18%	0%	NA
Upper Bear	07/27/01	40%	0%	9%	47%	4%	83,000
Lower Bear	07/27/01	0%	5%	31%	60%	3%	93,000

TABLE 5-1 SUMMARY OF SOURCES AT EACH SAMPLE AT EACH SITE

SOURCE	DATE	BIRD	САТ	DOG	HUMAN	LIVESTOCK	Fecal Coliform Count ¹
Lower Bear	08/02/01	42%	0%	9%	45%	3%	5,530
Upper Embudo	07/27/01	67%	17%	8%	8%	0%	17,000
Upper Embudo	08/09/01	25%	0%	0%	50%	25%	1,250
Lower Embudo	07/27/01	38%	17%	7%	31%	7%	42,700
Lower Embudo	08/02/01	50%	50%	0%	0%	0%	200
Upper Tijeras	07/27/01	22%	17%	0%	61%	0%	17,300
Upper South Diversion	08/02/01	47%	26%	0%	16%	11%	1,570
Upper South Diversion	08/09/01	63%	2%	10%	24%	0%	62,500
Lower South Diversion	08/18/01	11%	7%	68%	4%	11%	16,300
Barelas	07/27/01	10%	3%	33%	53%	0%	12,300
San Jose	08/02/01	33%	13%	5%	38%	10%	7,900
San Jose	08/03/01	15%	13%	39%	24%	9%	131,000
Calabacillas	08/03/01	1%	30%	46%	22%	1%	16,900
Calabacillas	08/15/01	11%	27%	20%	11%	32%	36,300
San Antonio	08/15/01	17%	2%	58%	11%	14%	17,300

NA = Not Available. Colonies on filters were uncountable because they were either too numerous to count or smeared.
¹ = Fecal Coliform Count expressed in Colony Forming Units (CFU) per 100 milliliters (CFU/100mL).
Percentages are rounded and therefore may total greater or less than 100%.

All of the sites that were sampled twice displayed at least one dominant source that was common to both samples, suggesting consistency in sources for each location (see Table 5-1). A dominant source, for purposes of this analysis, is considered to be the top two sources identified in a given sample. There was temporal variation in some sources, which is to be expected in an open stormwater system that can receive inputs from many sources.

5.2 Recommendations

The limited time period over which the stormwater samples were collected provides a "snapshot" of conditions and bacteria sources in the stormwater system. Ideally, the sites should be sampled over a longer time period (at least a year) to capture the range of conditions, and potentially, range of sources. Therefore, continued sampling of stormwater events will allow for better refinement of specific sources and eventually could be used to better assess the potential effectiveness of BMPs in a given sub-basin.

One of the great advantages of ARA over other bacterial source tracking techniques is the fact that the methodology is relatively "low tech," and can be transferred to local laboratories. To continue source tracking in the Albuquerque area, future work would be greatly assisted by having the laboratory work conducted by a local laboratory.

Dr. Harwood's laboratory (Harwood lab) at USF has developed a limited library of *E. coli* isolates from known sources including human (wastewater), dog, livestock, etc. This library should be expanded with additional isolates to improve its usefulness for bacterial source tracking (BST). If this task is to be accomplished by personnel in Albuquerque, the following minimal specifications should be met:

- A laboratory with at least ten linear feet of free counter space, equipped with an autoclave for sterilizing media and biohazard waste
- Access to appropriate biohazard waste disposal (varies depending upon State)
- Analytical balance for weighing out small quantities of antibiotics
- A nanopure (i.e. Millipore-filtered) water source
- A vacuum pump or in-house vacuum system
- Gas for Bunsen burner or other appropriate device for flame
- Appropriate glassware and measurement devices, i.e. membrane filtration funnels and filter holders, micropipettes, replica plating device, etc.
- -80° C freezer for preserving bacterial isolates
- -20° C freezer for storing chemicals and antibiotic solutions
- Refrigerator
- Computer with a writeable CD drive for data storage and statistical analysis, with SAS 9.0 (SAS Institute, Cary, NC) installed.



Personnel involved in the project should be supervised by a microbiologist with a Master's degree or equivalent. The point person on the project should have at least a Bachelor's degree in Microbiology, Biology, or a related field. A City, State, or University program should provide annual training on safety issues associated with infectious agents. Table 5-2, below, outlines the timeline and procedure for implementation of a local laboratory.

Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Set up lab Order supplies				Install SAS (Albuquerque computer techs)	
	Site visit 1 Inspect lab Run control strains	Run control strains once/week; record consistency		Site visit 2 Train personnel on discriminant analysis	
	Collect fecal samples from known sources; save isolates	Carry out ARA on known source isolates	Carry out ARA on known source isolates	Carry out ARA on known source isolates	Analyze library & water isolates
Collect Isolates from Water	Collect Isolates from Water	Collect Isolates from Water	Collect Isolates from Water	Collect Isolates from Water	Collect Isolates from Water ¹

Table 5-2 Timeline for Implementing Technology Transfer

¹Isolates can be collected from water through the course of the study and frozen for later analysis

Additionally, application of a wider variety of antibiotics will assist in refining the differences between canine and human sources. Along with this source refinement, a program of evaluating additional sources not considered in the first phase of the study could be undertaken. Specifically, as wild birds are the only form of wildlife represented, additional potential sources should be identified and evaluated.

The BST data obtained via this study and future efforts will be instrumental in evaluating potential management practices associated with source reduction. These data provide key technical bases that the City can subsequently use to:

- negotiate the conditions of the TMDL for the middle Rio Grande
- define the maximum extent practicable (MEP) for stormwater controls
- evaluate the appropriateness of the recreational water quality standards during wet weather events and associated risk to human health.

In addition, the City may benefit from an evaluation of NMED's work plan for its forthcoming BST study, coordination with the findings of that study towards a regional resolution to the TMDL, and development of an enhanced monitoring plan towards ongoing understanding of the impacts of bacteria on receiving waters and options for effective receiving water quality management.