

Identifying Fecal Pollution Sources through Antibiotic Resistance Analysis (ARA): Sand River, Aiken County, SC

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Abstract

The Horse Creek watershed in Aiken County, SC, is known for its history of high coliform pollution. Preliminary data have identified one particular tributary, Sand River, as being a high bacterial contributor to the upper portions of the watershed, but the source remains unknown. Because the contaminated stream flows into Langley Pond, a local reservoir providing numerous recreational opportunities for many low-income and minority citizens, the presence of fecal pollution in this stream and reservoir is a children's health risk issue, as well as an Environmental Justice issue. We have applied a phenotypic method of antibiotic resistance profiles for the identification of source species in a polluted environment. The process involves developing an antibiotic resistance profile library of fecal coliforms isolated from the feces of known species that are present within the watershed. Potential source species that were selected for the coliform library included horses, waterfowl, canines, and untreated sewage. An array of antibiotics (Kanamycin, Streptomycin, Tetracycline, Apramycin, Trimethoprim, Rifampicin), in a range of differing concentrations, was used to determine the antibiotic resistance profiles for known sources. Once the profiles were established, fecal coliforms isolated from Sand River were tested using the same antibiotics and the range of resistance. A discriminant function analysis was used to statistically compare each unknown fecal isolate to the library of known species in order to determine its probable source. The majority (>70%) of fecal coliform isolates statistically grouped with untreated sewage, with the remainder grouping with waterfowl or horses.

Study Area



Figure 1. Coliform sampling points within the Horse Creek Watershed, specifically the Sand River.

Sand River, a major tributary within the Horse Creek watershed, has its headwaters within the highly urbanized downtown area of Aiken, SC, where it flows through Hitchcock Woods, a 2100-acre urban forest, before joining with Horse Creek immediately upstream of Langley Pond. The major recreational activities within Hitchcock Woods are equestrian in nature. This urban forest is also surrounded by older housing developments, some of which are still dependent upon aging septic systems. Over the years, there have been indicators that this watershed is receiving additional nonpoint source pollutants.

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Project Objectives

1. To create a library of antibiotic resistance profiles of coliform bacteria isolated from the feces of 4 species found in the Horse Creek watershed: horse, waterfowl, canine, and sewage.
2. To compare the antibiotic resistance of fecal coliform isolated from Sand River to the library of known sources in order to identify the source of fecal pollution within this particular watershed.

Methods

Sample Collection

Feces from known species were collected within the Horse Creek watershed. These included five samples from horses, six samples from waterfowl, four samples from canines, and one sample of mixed sewage inflow from the Horse Creek Wastewater Treatment Plant. Fecal samples were homogenized under sterile conditions in the laboratory, and approximately 1 gram of homogenate was suspended in 10 mL of sterile Nanopure water. Samples were then diluted, and fecal coliform bacteria were isolated as shown below.

Samples from unknown sources were collected from two locations in the Sand River (Figures 1 and 2) during summer 2012.



Figure 2. Collection pole and sterile tube used to collect samples with bacteria from Sand River.

Fecal Coliform Isolation and Antibiotic Resistance

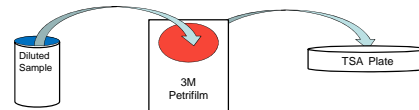


Figure 3. 1mL of each sample was pipetted onto a 3M Petrifilm and incubated overnight at 44°C. Gas-producing colonies were transferred to Tryptic Soy Agar (TSA) Petri plates and incubated at 37°C for 24 hours.

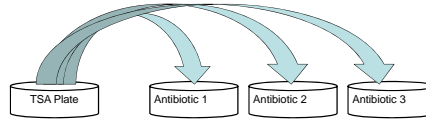


Figure 4. Fecal coliform isolates were transferred to TSA plates containing antibiotics to test for resistance. An isolate was considered to be resistant to a particular antibiotic concentration if growth occurred after 24 hours of incubation at 37°C. The specific antibiotics and the concentrations used are listed in Table 1.

Table 1. Antibiotics used to build the ARA Library for Horse Creek isolates.

Antibiotic	Concentration (µg/mL)
Streptomycin (Strep)	10, 20, and 40
Tetracycline (Tet)	5, 10, and 20
Kanamycin (Kan)	10, 25, and 50
Apramycin (Apram)	15, 50, and 100
Trimethoprim (Trimeth)	10, 30, and 60
Rifampicin (Rif)	12.5, 25, and 50

Results

A total of 566 isolates were obtained from known sources, and their patterns of antibiotic resistance were quantified and analyzed for the ARA library (Table 2). This library then served as a point of reference for the identification of unknown fecal pollutant sources shown in Table 3.

Table 2. Patterns of antibiotic resistance expressed as the percentage resistant isolates. Discriminant function analysis indicated that 89.2% of isolates from known sources were correctly classified.

Antibiotic	Isolates from known fecal sources				Isolates from Sand River	
	Horse (n=140)	Waterfowl (n=140)	Canine (n=143)	Untreated Sewage (n=143)	Dibble Rd. Sampling Location (n=121)	Greg Park Sampling Location (n=111)
Strep 10	65.0%	69.3%	99.3%	54.5%	13.1%	20.7%
Strep 20	11.4%	70.0%	5.6%	25.9%	3.3%	6.3%
Strep 40	5.7%	69.3%	0.0%	8.4%	1.8%	5.4%
Tet 5	98.6%	100.0%	98.6%	100.0%	94.3%	100.0%
Tet 10	37.1%	78.6%	98.6%	34.3%	45.9%	54.1%
Tet 20	7.9%	69.3%	0.0%	16.1%	4.1%	7.2%
Kan 10	94.3%	50.0%	97.9%	28.7%	13.9%	9.0%
Kan 25	10.0%	0.7%	2.1%	3.5%	0.0%	0.9%
Kan 50	0.0%	0.0%	0.0%	2.1%	0.0%	0.0%
Apram 15	85.0%	89.3%	99.3%	69.2%	32.0%	35.1%
Apram 50	5.0%	0.0%	5.6%	26.6%	0.8%	9.0%
Apram 100	0.0%	0.0%	0.0%	2.1%	0.0%	0.0%
Trimeth 10	23.6%	32.9%	11.9%	72.0%	29.5%	32.4%
Trimeth 30	23.6%	3.6%	2.1%	64.3%	26.2%	30.6%
Trimeth 60	23.6%	0.0%	2.1%	58.0%	15.6%	27.0%
Rif 12.5	100.0%	97.1%	19.6%	100.0%	99.2%	99.1%
Rif 25	91.4%	17.1%	3.5%	88.1%	74.6%	84.7%
Rif 50	15.0%	1.4%	3.5%	55.2%	48.4%	64.9%

ARA Comparison

A total of 121 and 111 isolates were obtained over four sampling periods in summer 2012 from the Dibble Road and Greg Park sampling stations, respectively. When these antibiotic resistance profiles were compared to the ARA library using discriminant function analysis on SPSS statistical software, greater than 70% of the isolates were classified as sewage (Table 3).

Table 3. Classification of the fecal coliform isolates from Sand River based on comparison to the ARA library from known sources.

Sampling Location	Horse	Waterfowl	Canine	Untreated Sewage
Dibble Road (n=121)	7.4%	16.5%	5%	71.1%
Greg Park (n=111)	9.9%	10.8%	0.9%	78.4%

Conclusions

- Our analysis shows that the antibiotic profiles of each library are distinct. At least 89.2% of the isolates in each library were correctly grouped.
- The largest component of the fecal coliforms in Sand River is from sewage. More than 70% of the isolates from each sampling station grouped with sewage. At this time we cannot explain why the number is higher at the Greg Park location.
- Based on these results, we can conclude that the majority of the fecal coliform pollution load in this stream is originating from failing sewer and/or septic systems in the Aiken area.