Division of Science, Research and Technology

Research Project Summary

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Microbial Source Tracking in the Manasquan River Estuary

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Abstract

Multiple Antibiotic Resistance (MAR) analysis, one of several new tests developed within the past decade, was used in an attempt to identify the sources of fecal pollution in the Manasquan River estuary. The estuary is vital for recreational activities and as a shellfish resource. The test identifies fecal-derived, human- and animal-specific *Escherichia coli (E. coli)* bacteria through host-influenced differences in antibiotic resistance. The estuary contains multiple sources of fecal pollution. At many sites there appears to be an abundance of inputs from wild animals. This is an ongoing study. The work to date is best viewed as a "pilot project," providing a tool to begin to identify sources of *E. coli* in the estuary. Due to the current limitations of the MAR technology and the lack of unambiguous source apportionment at most sites, the best use of the data may be to rule out potential sources of pollution.

Introduction

The Manasquan River estuary is an important shellfish resource in New Jersey. Since 1996 the waters in the estuary, west of the Route 70 bridge (see Figure 1), have been closed to harvesting due to high levels of coliform bacteria (an indicator of fecal contamination). The waters east of Route 70 are only slightly better, listed as *Special Restricted* (waters condemned except for harvesting for further processing under special permit). The estuary is also an important resource for primary contact recreational activities such as swimming and water-skiing.

Human illness can occur if water that contains fecal wastes from humans or animals is ingested. Illness can also occur if shellfish harvested from such waters are consumed. Shellfish are filter-feeders and they concentrate waterborne microbes from the surrounding waters. Fecal pollution sources may include agricultural runoff, leachate from landfills and hazardous waste sites, runoff from roads and other developed areas, marinas, boating activity, siltation from streambank modification and erosion, and natural sources such as waterfowl and other bird species.

In addition to cross-species pathogens, human fecal waste may contain pathogens that are human-specific and human-adapted (*e.g., Vibrio cholera, Shigella* spp., and many viral pathogens). Thus, human fecal pollution in water is more hazardous to humans than fecal pollution from animals (AWWA, 1999). In recent years there have also been many documented human disease outbreaks due to pathogens from domestic animals, but far fewer outbreaks due to pathogens from indigenous (wild) animals (Craun *et al.* 2004). Hence, it is generally accepted that the comparative human health risk of these fecal sources is (high to low): human > domestic animal > wild animal. Therefore it is important to detect and quantify fecal waste contamination if present and, if possible, determine the specific source(s).

Over the last decade new methods collectively called microbial source tracking (MST) tests, also known as bacterial source tracking (BST) tests, have been developed. These tests have demonstrated value for discriminating sources of fecal bacteria in waterbodies. These tests rely on the premise that humans and animals are hosts to some host-specific or host-adapted strains of *Escherichia coli* (*E. coli* or EC) or other target bacteria. This project employed a MST technology, called multiple antibiotic resistance (MAR) testing to try to determine the sources of EC in the Manasquan River estuary.

Design and Methods

Many EC were isolated from multiple samples of feces from humans (sewage influent samples) and various animals. Each isolated EC was grown, individually, in the presence of 12 different antibiotics. A growth or no-growth pattern or antibiotic resistance (AR) profile was obtained for each EC as shown in Table 1. A

Table 1. Multiple Antibiotic Resistance (MAR) patterns of *E. coli* bacteria isolated from feces of the indicated animals.

MAR Pattern	% Total	MAR Index
Domestic Cats		
Pen-Van	71.2	0.17
Pen-Str-Van	28.8	0.25
Total isolates = 163 Different isolates = 2 Avg. MAR Index = 0.19		
Humans		
Amp-Amx-Nal-Otc-Pen-Sul-Van	41.7	0.583
Amp-Amx-Nal-Otc-Pen-Str-Sul-Van	12.5	0.667
Amp-Amx-Kan-Nal-Otc-Pen-Sul-Van	4.17	0.667
Amp-Amx-Kan-Nal-Otc-Pen-Sul-Tet-Van	1.39	0.75
Amp-Amx-Ctc-Nal-Otc-Pen-Str-Sul-Van	37.5	0.75
Amp-Amx-Ctc-Nal-Otc-Pen-Str-Sul-Tet-Van	1.39	0.833
Amp-Amx-Ctc-Kan-Nal-Otc-Pen-Str-Sul-Van	1.39	0.833
Amp-Amx-Ctc-Kan-Nal-Neo-Otc-Pen-Str-Sul-Tet-Van	1.39	1
Total isolates = 72 Different isolates = 8 Avg. MAR Index =0.67 Total antibiotics tested = 12.		

Table 1. Antibiotic resistance profiles of E. coli isolates from domestic cats and humans in the Manasquan River estuary watershed (from Palladino and Tiedemann, 2004). Resistance to an antibiotic is shown by a three-letter abbreviation for that antibiotic. Amp = ampicillin (40 µg/ml), Amx = amoxicillin (15 µg/ml), Ctc = chlortetracycline (25 µg/ml), Kan = kanamycin (25 µg/ml), Nal = naladixic acid (25 µg/ml), Neo = neomycin (50 µg/ml). Otc = oxytetracycline (25 µg/ml), Pen = penicillin (75 U/ml), Str = streptomycin sulfate (15 µg/ml), Sul = sulfathiazole (750 µg/ml), Tet = tetracycline (25 µg/ml), Van = vancomycin (10 µg/ml). MAR Index = number of antibiotics resistant / the number of antibiotics tested.

database or "library" of AR profiles was compiled from 4,279 EC isolated from feces of humans and 16 other animal sources. For several reasons fecal sampling from some types of animals was limited. Therefore, the AR profiles in the fecal source library were grouped into 5 categories; humans (15 sewage samples; 475 EC isolates), pets (house cats [6] and dogs [12]; 722 EC isolates), domestic animals (horses [12], cattle [7], pigs [2], and chickens [5]; 1215 EC isolates), avian wild animals (Canada geese [13], gulls [12], Mallard ducks [5], Black ducks [5], and pigeons [3]; 1331 EC isolates), and non-avian wild animals (stray cats [5], raccoons [3], opossum [2], rat [1], and deer [5]; 536 EC isolates).

The AR profile of each isolate in the fecal source library was tested as an "unknown" against the entire database to determine an "average rate of correct classification" (ARCC) for each of the 5 groups. If each isolate was correctly assigned to its source group by random chance, the ARCC for each group would be 20%. The ARCC for the 5 groups were: human, 93%; pets, 65%; farm animals, 81%; avian wild animals, 74%; and non-avian wild animals, 79%. The ARCCs are less than 100% due to AR profile overlaps among the 5 groups. The high ARCC values demonstrate a significant degree of EC source specificity. However, the relevance of library ARCCs with respect to the environmental EC population is unclear (Gordon, 2002; McLellan, 2004).

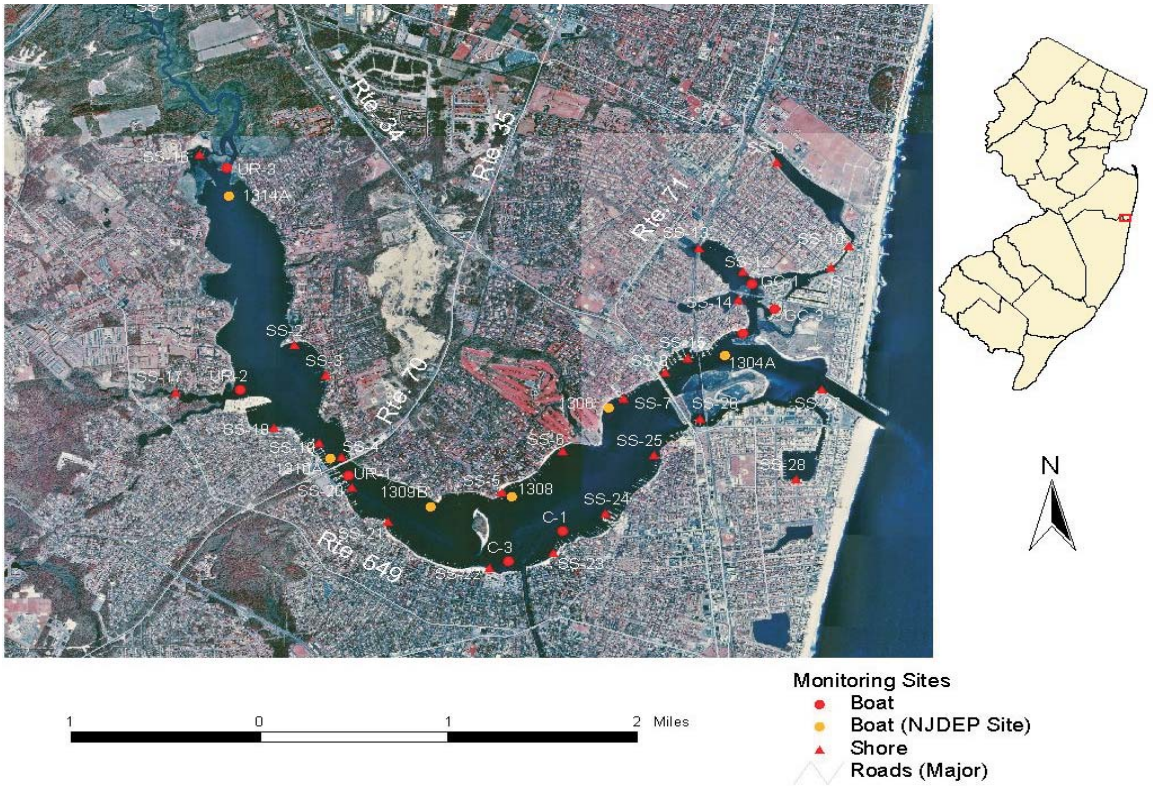

Figure 1. The Manasquan River estuary showing the sampling locations.

Between May 2002 and December 2003, 14 in-water (boat) sites and 28 shoreline sites shown in Figure 1 were alternately sampled on a once-per-month basis. EC were isolated from 50-ml water samples and the AR profile of each established. The AR profiles were compared to the AR profiles in the fecal source library using discriminant analysis (Wiggins *et al.* 2003). The pattern-matching algorithm assigned each environmental EC isolate to its probable source group based on AR profile matches, as illustrated in the cartoon in Figure 2. Some AR profiles were common to several fecal sources, so they were assigned to more than one group. Other AR profiles did not match any AR profile in the source library, so the source of these strains is unknown. The relative contribution of each source group to the total EC population at each site was then calculated.

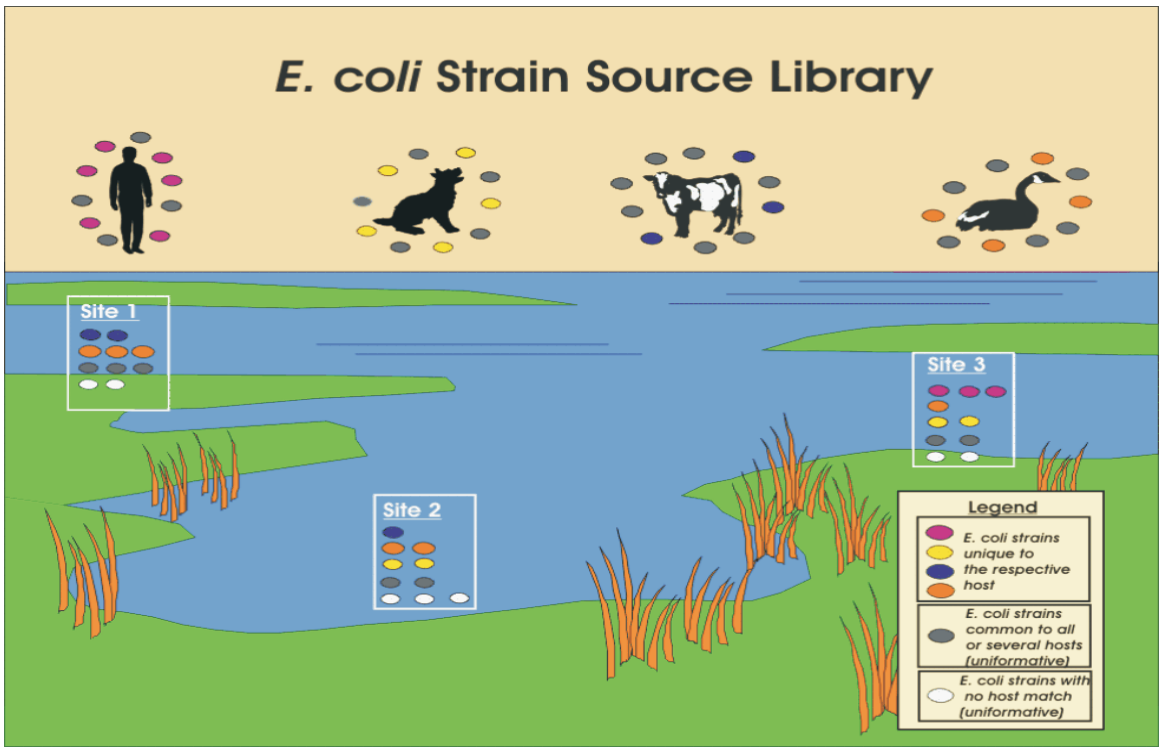

Figure 2. Cartoon illustrating the microbial source tracking method. Antibiotic resistance (AR) profiles of *E. coli* (ovals) isolated from environmental sites (#1, #2, and #3) were compared to the AR profiles of *E. coli* isolated from the feces of targeted animals to determine their likely host of origin. Some AR profiles are unique to certain hosts (colors) while others (grey) are common to multiple hosts (assigned to more than one host group). Some *E. coli* from the environment have profiles (white) that do not match any in the "fecal source library" (their host group origin is unknown).

Results

Aggregated data is shown in Figure 3. Fewer than 10 EC were isolated from 7 of the 42 sites (data not shown). This made inter-site source comparisons difficult or impossible in some cases. Also, the number of EC strains from a given site with an AR profile that matched a profile in multiple source groups was high. This large "profile overlap" confounded efforts to apportion sources at most of the sites. An extreme example of these 2 problems was observed at site "SS-7" where just 10 EC were isolated (Figure 3C). At SS-7, the AR profiles of 8 of the 10 EC matched profiles in the human source library. However, 8 of the 10 profiles also matched profiles in the non-avian wild animal group, and all 10 matched profiles in the avian wild animal group. Therefore, the amount of fecal pollution at SS-7 from each of the sources is not known. Removing EC isolates with overlapping AR profiles from the analysis resulted in an unsatisfactory increase in the number of unclassifiable EC. Even when overlapping profiles are included in the analysis, high rates of unclassifiable EC were observed at a few sites (Figure 3E, SS-1 for example). This indicates that either the library (source coverage) is not adequate or that there are a high percentage of non-fecal EC at those sites (McLellan, 2004).

Most sites show a combination of possible sources. At many of the sites there appears to be an abundance of inputs from wild animals.

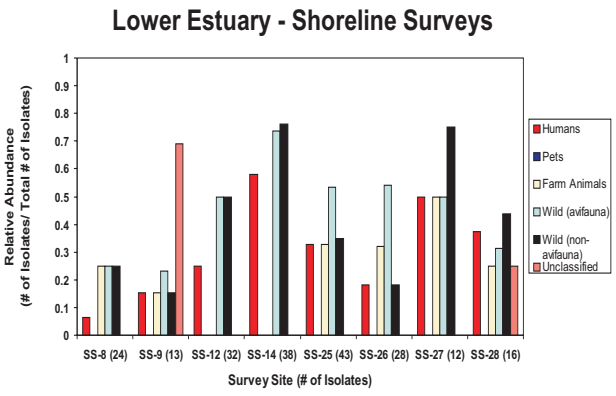

Figure 3A.

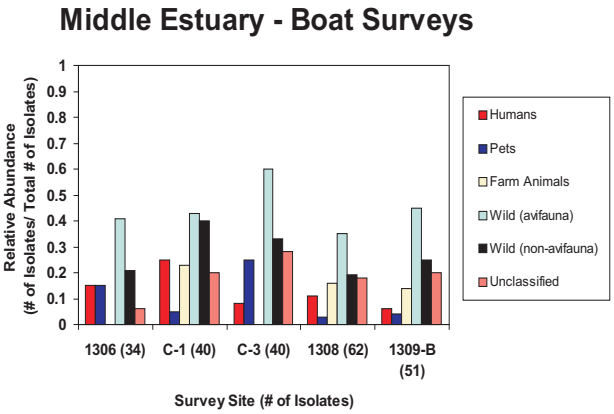

Figure 3D.

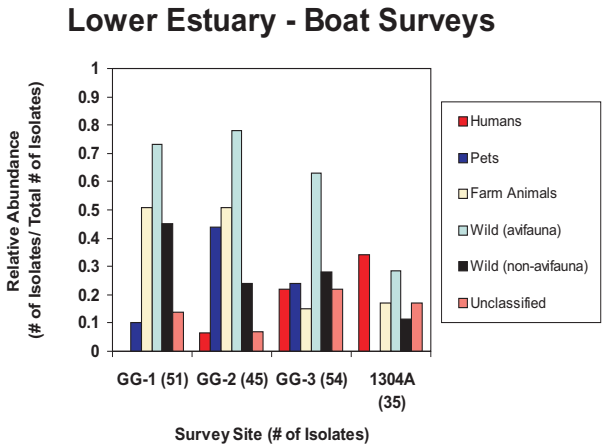

Figure 3B.

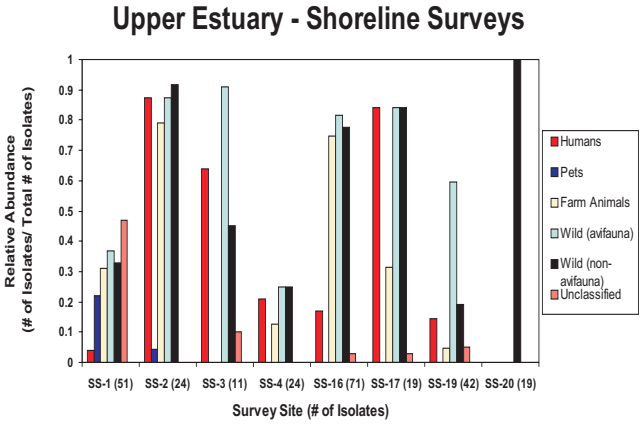

Figure 3E.

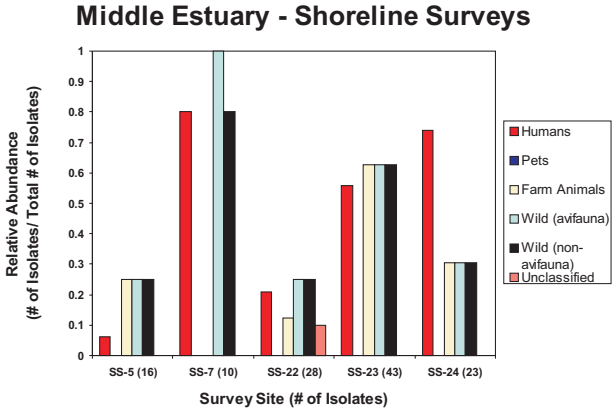

Figure 3C.

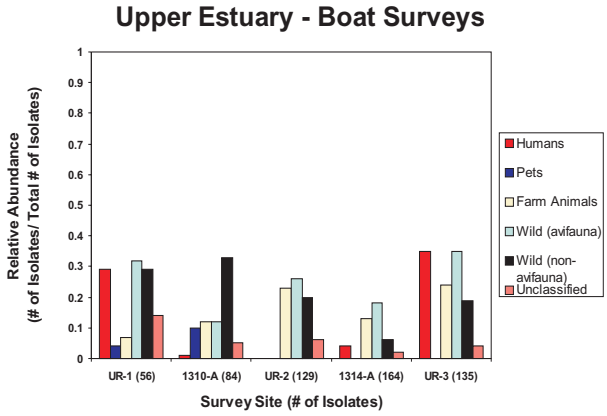

Figure 3F.

Discussion

Despite a number of successful applications, MST techniques are still under development, so the results of any MST project need to be analyzed carefully. For a summary discussion of the limitations of current library-based MST methods, see Atherholt (2004).

This study is best viewed as a "pilot project," providing a tool to begin to identify sources of EC in the estuary. Several methodological problems were encountered. Some of these problems have since been overcome. For example, a more discriminating EC growth medium (mTEC) is now being used and EC are now being recovered from sediment samples (no EC could be recovered from sediment samples in this study).

One significant limitation is that almost all MST methods and MAR methods, in particular, are subject to a significant amount of "false positives." That is, they identify pollution sources that are not present. Also, these tests have "false-negative" results in that they do not always identify sources when present. When sources are apportioned, there are no error bars or confidence intervals provided to assist the reader in determining the uncertainty in the values. The uncertainty can be considerable. A source may have to contribute more than 25% of the total amount to be considered a real source and not a possible misclassification error (Wiggins *et al.* 2003). The financial implications of incorrectly identifying the presence of fecal sources and taking management actions in response can be serious.

For this and other reasons the best use of the data generated in this study may be to rule out potential sources of pollution.

When the Manasquan River estuary project work is completed a peer-reviewed publication is anticipated.

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References

Atherholt, T.B. 2004. Microbial Source Tracking: Library-based Methods. A Critique. Division of Science, Research, and Technology, NJ Department of Environmental Protection, Trenton, NJ. www.state.nj.us/dep/dsr

AWWA. 1999. Waterborne Pathogens; Manual M48. American Water Works Association, Denver, CO.

Craun, G.F. *et al.* 2004. Waterborne outbreaks caused by zoonotic pathogens in the USA. p. 120-135. In J.A. Cotruvo, A. Dufour, G. Rees, J. Bartrum, R. Carr, D.O. Cliver, G.F. Craun, R. Fayer, and V.P.J. Gannon (eds.), Waterborne zoonoses: identification, causes and control. World Health Organization, IWA Publishing, London, UK.

Gordon, D.M. *et al.* 2002. The genetic structure of *Escherichia coli* populations in primary and secondary habitats. Microbiology 148: 1513-1522.

McLellan, S.L. 2004. Genetic diversity of *Escherichia coli* isolated from urban rivers and beach water. Appl Environ Microbiol 70: 4658-4665.

Palladino, M.A., and J.A. Tiedemann. June 23, 2004. Differential identification of *Escherichia coli* (*E. coli*) in the Manasquan River estuary by multiple antibiotic resistance testing and DNA fingerprinting analysis. Final Report, Contract SR02-027, Division of Science, Research and Technology, NJ Department of Environmental Protection, Trenton, NJ.

Wiggins, B.A. *et al.* 2003. Use of antibiotic resistance analysis for representativeness testing of multiwatershed libraries. Appl Environ Microbiol 69: 3399-3405.

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