Where's it coming from? Faecal source tracking - the current state of play

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The use of enteric microorganisms to indicate faecal contamination is a well established practice in water and wastewater management. This broad identification of faecal contamination is a vital first step in water quality management and the protection of public health. However, management and mitigation of faecal pollution would be more cost-effective if the correct contaminant sources could be identified and apportioned, both between human and animals, and between different animals. Unfortunately, the commonly-used indicators do not readily identify contaminant sources; other tools are needed to achieve this result.

New acronyms — FST and MST

Since we last reviewed this topic around ten years ago (Sinton et al. 1998) the faecal source discrimination field has grown dramatically. While some approaches have fallen by the wayside, a number of new tools have become available, particularly those based on the characterisation of microbial DNA.

New terminologies and acronyms have followed the development of these new methods. Faecal source identification has become "faecal source tracking" (FST), and the subset of microbial identification techniques based on PCR markers and other DNA methods is now called "microbial source tracking" (MST).

The greater choice of tools for identifying faecal contamination sources has led to a need for clearer guidance on the selection and use of these tools, and the interpretation of the results they generate. Here, we look at the microbial and chemical methods available for FST, and discuss the attributes and limitations of each approach. The principal approaches are discussed below; a more complete list is presented in Table 1.

Library based methods	Typing method	Reference*
Multiple Antibiotic Resistance analysis	Phenotypic	Scott et al. (2002)
Carbon source utilisation	Phenotypic	Cimenti et al. (2007)
Ribotyping	Genotypic	Scott et al. (2002)
PFGE	Genotypic	Scott et al. (2002)
Repetitive element PCR	Genotypic	Johnson et al. (2004)
Non-library based methods		
Culture and/or PCR methods	**Host Specificity**	**Reference***
Bifidobacterium adolescentis	Human	Scott et al. (2002)
Bacteroides-Prevotella markers	Human, ruminant, dog, horse, pig	Field and Samaadpour (2007)
Enteric viruses	Human	Jiang (2006)
F-specific RNA bacteriophages	Human or animal	Sinton et al. (1998)
Bacteroides fragilis HSP40	Human	Sinton et al. (1998)
Human polyomavirus	Human	McQuaig et al. (2006)
Rhodococcus coprophilus	Cattle	Savill et al. (2001)
Ruminococcus flaveciens	Ruminant	Tajima et al. (2001)
Desulfovibrio-like	Duck	Devane et al. (2007)
Chemical methods		
Faecal sterols	Human or animal	Gilpin et al. (2002)
Fluorescent whitening agents	Human	Gilpin et al. (2002)
Bile salts	Human	Chaler et al. (2001)
Caffeine	Human	Scott et al. (2002)
Sodium tripolyphosphate	Human	Sinton et al. (1998)
Long-chain alkylbenzenes	Human	Sinton et al. (1998)

*Where there are many references on a topic, a relevant review is cited.

Table 1 - Summary of principal methods investigated for faecal source tracking.

Microbial Methods

Library-based methods

In this approach, bacterial colonies, usually of *E. coli* or enterococci, are isolated from water samples, and also from potential sources of faecal contamination. These isolates are then fingerprinted using either DNA-based or phenotypic/biochemical based-methods.
DNA-based methods, such as pulsed field gel electrophoresis or Rep-PCR, generate bar code-like DNA fingerprints that differ according to the DNA of individual isolates. Biochemical or phenotypic methods generate a profile of each isolate based on characteristics such as resistance to a range of antibiotics, or utilisation of different carbon sources.

Once a library of known sources has been constructed, it can be used as a reference database with which to compare isolates from unknown sources.

The key attraction of library methods is that the results relate directly to the *E. coli* or enterococci which have prompted the investigation. However these indicator organisms do not appear to be particularly host-specific, and the same fingerprint types are often found in multiple sources. This can be partially overcome by the application of statistical techniques, such as discriminate analysis.

This approach also relies on the library containing all possible sources of the selected indicator, and the usefulness of libraries tends to be limited to particular geographical areas and time periods. In addition, creating and maintaining libraries large enough to provide source discrimination is expensive (e.g., the largest ribotyping database in the USA has over 250,000 isolates).

Non library-based Methods

PCR-based methods

One of the most promising set of tools to emerge from the MST toolbox has been a suite of assays based on the polymerase chain reaction (PCR). PCR assays detect and amplify specific DNA sequences, producing banding patterns and other signals which can be detected and quantified by various means.

PCR-based methods distinguish between species of bacteria that can be almost impossible to tell apart using phenotypic tests. More importantly, PCR enables the detection of microbes that cannot be grown in the laboratory. If these species of enteric microbes are highly host-specific, then a PCR assay can be a very useful MST tool. The following is a brief discussion of some of the most widely used assays.

The *Bacteroides-Prevotella* group
The *Bacteroides-Prevotella* group of bacteria resides exclusively in the gut of warm-blooded animals. They are strict anaerobes, which mean they are highly unlikely to replicate in the environment and most species cannot be cultured in the laboratory. The value of the group lies in the existence of different "genetic markers" — segments of DNA — that appear to be specific to *Bacteroides-Prevotella* bacteria present in the faeces of humans and various animals.

At ESR, we have screened faeces from 12 different types of animals using a human specific Bacteroides-Prevotella assay. All but one animal produced a negative result. The exception was the possum, which contained the same genetic marker as humans. We have since developed, and are now testing, a Bacteroides-Prevotella assay specific for possums, which suggests that the group holds promise for distinguishing human from animal sources. We are also working on *Bacteroides-Prevotella* markers specific for ruminants (sheep and cattle), horses and dogs.

Bifidobacteria
Bifidobacteria are anaerobic bacteria that are present in the faeces of humans and animals at levels typically 10-100-times greater than coliform bacteria. A PCR assay has been developed to detect *B. adolescentis* which is associated with human faeces. This test can be a useful adjunct to the human specific *Bacteroides-Prevotella* PCR assay. However, *B. adolescentis,* appears to be short-lived in the environment, and we have detected this species only when relatively recent and high levels of human pollution are present.

Rhodococcus coprophilus
Rhodococcus coprophilus is an actinomycete bacterium that grows in the dung of herbivores. A PCR assay developed at ESR targets this organism (Savill et al., 2001), and provides useful confirmation for the ruminant *Bacteroides-Prevotella* PCR assay.

***Desulfovibrio*-like bacteria**
Researchers have found it difficult to develop bird-specific assays. However, we have recently developed a PCR assay targeting a *Desulfovibrio*-like bacterium isolated from the faeces of mallard ducks (Devane et al., 2007). The assay produced negative results when tested against faecal samples from humans, dogs, cattle, sheep and possums, but positive results for 76% of ducks and smaller percentages for other wildfowl.

F-RNA bacteriophages

Bacteria can be infected by viruses called bacteriophages. One group, the F-RNA coliphages, has been investigated for MST on the basis of the predominance of four different subgroups in human and animal faeces.

However, uncertainties remain over the degree of specificity of the different subgroups to human and animal sources. It has also been suggested that some subgroups may survive longer in lake water than others. Natural waters contaminated by animals often contain very low concentrations of F-RNA phages, which may limit their usefulness to identifying contamination sources in shellfish, which tend to concentrate enteric microbes, including phages.

A promising approach using bacteriophages in MST, at least for the identification of human sources, has been described by Ebdon et al. (2007). In comparison to their hosts, the phages infecting *Bacteroides* survive for longer periods and in higher numbers in waterways, suggesting they may be useful indicators of human contamination.

Chemical methods

In addition to microbes, faecal material can contain, or become associated with, a range of chemicals useful in FST investigations.

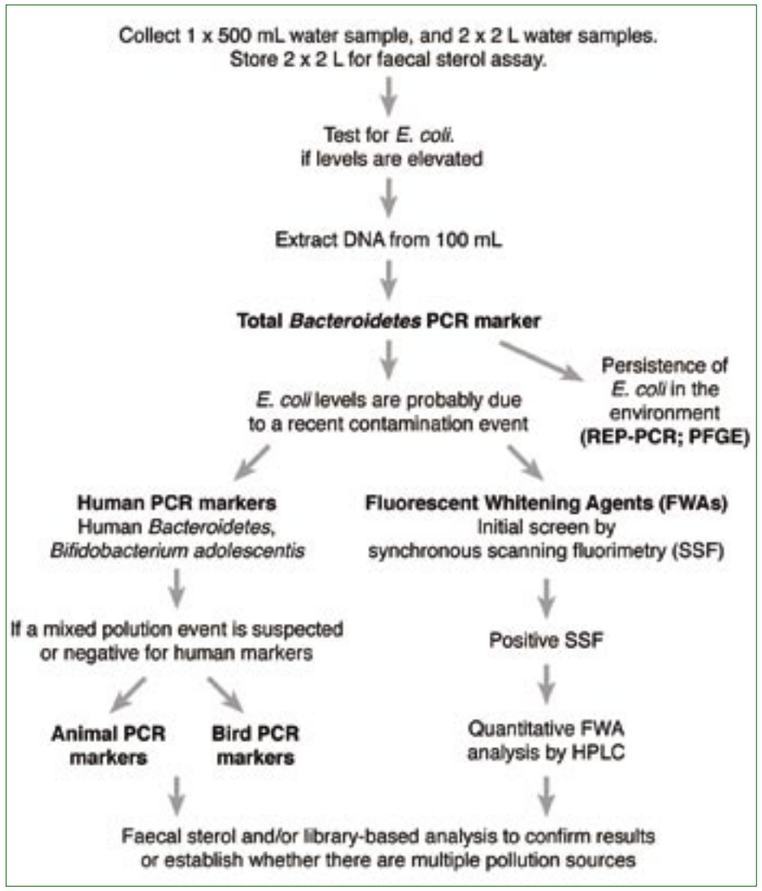

Figure 1 - A provisional FST Decision Tree System

The two groups of chemicals most commonly investigated for FST are listed below.

Fluorescent whitening agents

Advertisements for washing powders that will make your pillow cases "sunshine bright" have a grain of truth in them. Washing powder manufacturers regularly add fluorescent whitening agents (FWAs) that adhere to woven fabrics and absorb the short (ultraviolet) wavelengths from sunlight, and emit most of the absorbed energy as higher wavelength blue light, making clothing appear brighter and cleaner.

Most household plumbing mixes toilet effluent with "grey water" from washing machines and, consequently, FWAs are usually associated with human faecal contamination from both individual septic tank systems and community wastewater schemes.

Although a range of FWAs is available, only one is used in New Zealand, which simplifies their analysis. Samples are analysed by High Pressure Liquid Chromatography (HPLC). Levels of FWA > 0.1 µg/L suggest the presence of human sewage, with levels > 0.2 µg/L a strong indication of human sewage.

Faecal sterols

Sterols are lipids that have important biological functions in plants and animals. The subgroup of "faecal" sterols is found mainly in animal faeces, and the sterol "fingerprint" can be quite distinctive between species.

Although individual sterols are not unique to a particular animal, examination of the entire sterol profile in a faecal sample can be useful in determining the likely pollution source. Coprostanol is of particular interest in the detection of human faecal pollution, as it comprises approximately 60% of the human sterol concentration.

FST by sterol profile analysis in waterways relies on sterol ratios rather

Gas chromatograph-mass spectrometer for faecal sterol measurements

than absolute concentrations. First, the ratio of coprostanol: cholestanol needs to be established to indicate whether the coprostanol present is of faecal origin. Cholestanol is an isomer of coprostanol and commonly occurs in pristine environments. Coprostanol is produced in faeces when cholesterol is reduced by gut microbiota. A coprostanol:cholestanol ratio > 0.5 in a water sample suggests faecal contamination.

Second, sterol ratios can help to differentiate human from herbivore pollution. Herbivores produce 24-ethylcoprostanol from plant sterols at higher rates than in generally omnivorous humans. Conversely, human faecal pollution typically has a ratio of > 1 for coprostanol: 24-ethylcoprostanol. Other sterol ratios point to particular animals and birds.

At ESR, we can analyse water samples for 10 different sterols, and ratio analysis of the results can identify a wide range of animals and birds. In addition, this method may estimate the relative contributions of faecal contamination where a mixed human/herbivore pollution event is suspected. However, faecal sterol analysis requires the collection of at least two litres of water for analysis, and is the most time consuming and expensive of the faecal discrimination tools offered by our laboratory.

The right set of tools

How do water managers know which tools are best for their situation? It is important to recognise that there are no "magic bullets" available for FST — all of the tools reviewed here have advantages and limitations and none are particularly effective when used in isolation.

To help water managers select the most cost-effective set of tools for the job, ESR is developing a "decision tree" system as shown in Figure 1. The process starts by examining the water sample for traditional faecal indicators. Faecal contamination is frequently intermittent and the water source may require resampling. When high levels of indicators are recorded, then a detailed site inspection is undertaken to identify likely contaminant sources. Analysis generally begins with the less costly, faster tools such as human PCR markers and FWA analysis. If the site inspection suggests non-human contamination, then additional PCR analyses, specific to various animals and birds, can be performed. If the cost is warranted, the existence of mixed pollution sources can be confirmed by faecal sterol analysis.

Summary

FST, and its offspring, MST, are expanding research areas requiring the regular trial of new methods for addition to the FST toolbox. A wide range of chemical and microbial approaches to FST has been investigated and applied but, to date, no single method has been shown to reliably identify faecal sources. Thus, ESR is developing a step-wise process, around the format of a "decision tree", which applies a range of tools appropriate to the site and problem. The process is flexible, and can be modified in the light of experience and new information generated by ESR's research programme. If a new tool can contribute to FST, it can be added to the toolbox underpinning the decision tree system. The system has already helped several water managers to identify contamination sources. With further development we expect the system to become a valuable resource in the water managers' arsenal of strategies for the management of waterways.

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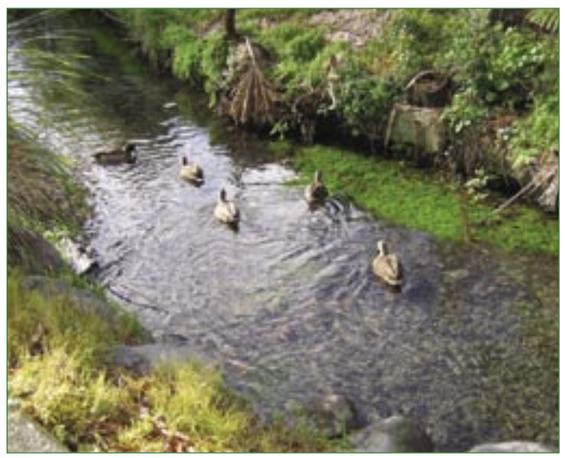

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References

Chaler, R., Simoneit, B.R., Grimalt, J.O., 2001. Bile acids and sterols in urban sewage treatment plants. J. Chromatogr. A. 927, 155-160.

Cimenti, M., Hubberstey, A., Bewtra, J.K., Biswas, N., 2007. Alternative methods in tracking sources of microbial contamination in waters. Water SA. 33:1, 183-194.

Devane, M.L., Robson, B., Nourozi, F., Scholes, P., Gilpin, B.J., 2007. A PCR marker for detection in surface waters of faecal pollution derived from ducks. Water Res. 41, 3553-3560.

Ebdon, J., Muniesa, M., Taylor, H., 2007. The application of a recently isolated strain of Bacteroides (GB-124) to identify human sources of faecal pollution in a temperate river catchment. Water Res. 41, 3683-3690.

Field, K.G., Samadpour, M., 2007. Fecal source tracking, the indicator paradigm, and managing water quality. Water Res. 41, 3517-3538.

Gilpin, B.J., Gregor, J.E., Savill, M.G., 2002. Identification of the source of faecal pollution in contaminated rivers. Water Sci. Technol. 46, 9-15.

Jiang, S.C., 2006. Human adenoviruses in water: occurrence and health implications: a critical review. Environ. Sci. Technol. 40, 7132-7140.

Johnson, L.K., Brown, M.B., Carruthers, E.A., Ferguson, J.A., Dombek, P.E., Sadowsky, M.J., 2004. Sample Size, Library Composition, and Genotypic Diversity among Natural Populations of Escherichia coli from Different Animals Influence Accuracy of Determining Sources of Fecal Pollution. Appl. Environ. Microbiol. 70, 4478-4485.

McQuaig, S.M., Scott, T.M., Harwood, V.J., Farrah, S.R., Lukasik, J.O., 2006. Detection of human-derived fecal pollution in environmental waters by use of a PCR-based human polyomavirus assay. Appl. Environ. Microbiol. 72, 7567-7574.

Savill, M.G., Murray, S.R., Scholes, P., Maas, E.W., McCormick, R.E., Moore, E.B., Gilpin, B.J., 2001. Application of polymerase chain reaction (PCR) and TaqMan PCR techniques to the detection and identification of Rhodococcus coprophilus in faecal samples. J. Microbiol. Methods. 47, 355-368.

Scott, T.M., Rose, J.B., Jenkins, T.M., Farrah, S.R., Lukasik, J., 2002. Microbial Source Tracking: Current Methodology and Future Directions. Appl. Environ. Microbiol. 68, 5796-5803.

Sinton, L. W., Finlay, R. K., and Hannah, D. J. 1998: Distinguishing human from animal faecal contamination in water: a review. N.Z. J. Marine and Freshwater Res. 32: 323-348.

Tajima, K., Aminov, R.I., Nagamine, T., Matsui, H., Nakamura, M., Benno, Y., 2001. Diet-dependent shifts in the bacterial population of the rumen revealed with real-time PCR. Appl. Environ. Microbiol. 67, 2766-2774.

Demand management for safe and sustainable Pacific Islands water

Sustainable access to safe drinking water for communities in Pacific Island countries is the overarching goal of the New Zealand's International Aid and Development Agency-funded project 'Water Demand Management (WDM) for Pacific Island Countries 2006 – 2009'. The project's implementation is managed by SOPAC, the Pacific Islands Applied Geoscience Commission, an intergovernmental organisation based in Suva, Fiji. SOPAC helps member countries to sustainably manage their non-living natural resources. Through an aid and industry partnership, state-of-the-art water demand management techniques and technologies are being implemented in the participating countries, with an initial focus on the Cook Islands, Federated States of Micronesia, Marshall Islands, Niue and the Solomon Islands. In this issue of the NZWWA Journal we have invited Mathias Kleppen ('the man on the ground' responsible for delivering the WDM strategy in conjunction with NZAID and Wide Bay Water Corporation, from his base in Suva) to report on the project's key focus areas and highlight its success stories so far. In particular, Mathias holds up recent work done in Niue as an example of how the strategy is quickly and cost effectively improving water supply services in the Pacific Islands.

Pohnpei in the Federated States of Micronesia

The Problem

Many Pacific islands have problems with water supply because more water is lost through leakage and wastage than they actually deliver.

The Solution

Minimize water losses.

How?

"for that which is common to the greatest number has the least care bestowed upon it" (Aristotle 384 – 322 B.C)

However, with more pressure on limited resources, many Pacific islands have realized that the key towards sustainability lies not in costly infrastructure extension but in sound management of existing water supplies. This is "demand management" where strategies are developed to optimize existing water resources and infrastructure, and encourage customers to use water efficiently.

Strategic planning is a key aspect of the success of demand management. This means understanding the constraints, analysing how much water is used, when, by whom, for what purpose and at what level of efficiency; determining the potential reduction in water use that can occur through improvements to water-using equipment, behaviour and developing programs like active leakage control and pressure management to achieve these improvements. There are significant benefits for water supply utilities undertaking water loss reduction and management programs.

These benefits include:

- Short term financial benefits associated with the costs of delivering water and include power costs and savings in the costs of repairing burst mains.
- Long term benefits related to reducing whole-of-life asset costs as a result of a reduction in pipe failures which will extend asset life.