FAECAL SOURCE TRACKING IN SEQ: CASE STUDIES

W Ahmed, S Toze, T Gardner

Abstract

This paper outlines the application of faecal source tracking (FST) tools in waterways in Southeast Queensland (SEQ), Australia. FST tools used in the case studies include biochemical fingerprinting, antibiotic resistance analysis, bacterial markers, viral markers and faecal sterols. These tools are predominantly used to identify sewage pollution in environmental waters sourced from defective septic systems or discharges from sewage treatment plants (STPs). The earlier case studies employ library-dependent FST tools where as the recent studies focus more on validation and application of library-independent tools. Several case studies reported the presence of sewage pollution in various aquatic environments and suggest that library-independent tools such as bacterial and viral markers are appealing because of the high specificity and sensitivity of these markers to differentiate and detect sewage and animal faecal pollution. A few case studies also used a combination of tools and suggested that such an approach can compensate uncertainty when one tool fails to produce satisfactory results. These case studies indicate that current FST tools can be successfully applied for faecal pollution tracking in environmental waters in SEQ. This is particularly important for water quality managers who are charged with protecting water quality.

Introduction

Pollution from human and animal wastewater is one of the major concerns about aquatic environments that are used for drinking water supply, recreational activities and harvesting seafood worldwide. These concerns are predominantly based on exposure of water users to a wide array of pathogenic bacteria, protozoa and viruses (Fong *et al*. 2005; Hörman *et al*. 2004). Microbiological quality of water is commonly assessed by enumerating faecal indicator bacteria such as *Escherichia coli* and enterococci. The presence of these bacteria in the aquatic environments is used to indicate possible faecal pollution and the subsequent potential public health risks. However, the presence of these indicators does not indicate whether the pollution is sourced from sewage or animal wastewater. The identification and assigning of indicator bacteria found in aquatic environments to sewage and animal faecal pollution is difficult due to their cosmopolitan nature, i.e. they are shed in the waste of a wide variety of animals including humans (Field & Samadpour 2007).

Over the last decade, researchers have developed a range of faecal source tracking (FST) tools that can be used to distinguish sewage pollution from animals. These tools are broadly categorised into library-dependent (i.e. phenotypic and genotypic), library-independent (i.e. PCR markers), and chemical (i.e. sterols, fluorescent whitening agents). A range of FST tools have been used in Southeast Queensland (SEQ) over the last six years in order to identify the sources of faecal pollution in freshwater, coastal lakes, stormwater and estuarine waters. The aim of this paper is to summarise the FST tools used and the results obtained in key case studies in various aquatic environments in SEQ.

Current FST tools can be successfully applied.

Table 1. MST tools used in the case studies in SEQ, Australia.

Tools	Target organisms	Reference
Library-dependent		
Biochemical fingerprinting	*E. coli* and enterococci	Ahmed *et al.* 2005
Antibiotic resistance analysis	*E. coli*	Ahmed *et al.* 2008b; Carroll *et al.* 2005
Library-independent		
Sewage-associated HF183	*Bacteroides*	Ahmed *et al.* 2008d
Sewage-associated HF134	*Bacteroides*	Ahmed *et al.* 2008d
Ruminant-associated CF128	*Bacteroides*	Ahmed *et al.* 2008e
Dog-associated BacCan	*Bacteroides*	Ahmed *et al.* 2008e
Sewage-associated *esp*	*E. faecium*	Ahmed *et al.* 2008a,c
Sewage-associated JCV and BKV polyomaviruses	Polyomaviruses	Ahmed *et al.* 2010a
Sewage-associated adenoviruses	Adenoviruses	Ahmed *et al.* 2010b
Bovine wastewater-associated adenoviruses	Adenoviruses	Ahmed *et al.* 2010b
Chemical tools		
Faecal sterols	-	Sullivan *et al.* 2010

Faecal Source Tracking Tools used in Aquatic Environments in SEQ

The majority of the initial FST tools are library-dependent, which requires the development of a "library" of *E. coli* or enterococci from the faeces of suspected sources of pollution (i.e., sewage or animals) using various genotypic and phenotypic fingerprinting tools. The underlying assumption of the library-dependent tools is that host-specificity of microorganisms is influenced by selective pressures within the host animal (Wiggins *et al*. 1996). Phenotypic and genotypic fingerprints of isolated *E. coli* or enterococci are then compared to the library to identify their likely host sources (Harwood *et al*. 2000). Another set of more recently developed FST tools do not require the development of a library and are therefore known as library-independent tools. These tools involve detection or quantification of specific marker(s) associated with host sources and microorganisms. Library-independent tools could be categorised into three groups: (1) anaerobic bacterial markers such as sewage-associated *Bacteroides* (Bernhard & Field 2000); (2) viral markers

Table 2. Specificity and sensitivity of host-specific markers in SEQ, Australia.

Host-specific markers	Number of positive sewage samples/number of sewage samples tested (sample origin)		Sensitivity (%)	Specificity (%)	Reference
Sewage-associated HF183 *Bacteroides*	52/52 (sewage)	0/155 (various animals)	100	100	Ahmed *et al.* 2008d
Sewage-associated HF134 *Bacteroides*	51/52 (sewage)	7/155 (various animals)	97	95	Ahmed *et al.* 2008d
Ruminant-associated CF128 *Bacteroides*	19/20 (cattle)	8/177 (sewage and various animals)	95	93	Ahmed *et al.* 2008e
Dog-associated *Bacteroides*	17/20 (dogs)	18/177 (sewage and various animals)	85	89	Ahmed *et al.* 2008e
Sewage-associated JCV and BKV polyomaviruses	63/63 (sewage)	1/81 (various animals)	100	99	Ahmed *et al.* 2010a
Sewage-associated *esp*	38/42 (sewage)	0/155 (various animals)	90	100	Ahmed *et al.* 2008a
Sewage-associated adenoviruses	58/74 (sewage)	0/106 (various animals)	78	100	Ahmed *et al.* 2010b
Bovine wastewater- associated adenoviruses	7/26 (cattle)	0/154 (sewage and various animals)	73	100	Ahmed *et al.* 2010b

such as sewage-associated adenoviruses (Fong *et al*. 2005) and polyomaviruses (McQuaig *et al*. 2009); and (3) bacterial toxin markers such as pig wastewater-associated ST1b (Khatib *et al*. 2003), cattle wastewater-associated LTIIa *E. coli* toxin gene (Chern *et al*. 2004), and the sewage-associated enterococcal surface protein (*esp*) gene found in *Enterococcus faecium* (Scott *et al*. 2005). Chemical tools include optical brighteners, caffeine and faecal sterols. A selection of these tools has been used in some of the case studies in SEQ are detailed in Table 1.

Sensitivity and Specificity of Bacterial and Viral Markers used in SEQ Region

Sensitivity and specificity are commonly used parameters for the validation of bacterial and viral markers (Field & Samadpour 2007). The sensitivity and specificity of markers are determined as: sensitivity = a/(a + c) and specificity = d/(b + d), where 'a' is true positive (samples were positive for the marker of its own species), 'b' is false positive (samples positive for the marker of another species), 'c' is false negative (samples were negative for the marker of its own species), 'd' is true negative (samples were negative for the marker of another species). High specificity and sensitivity are desirable for the accurate identification of polluting source(s) when bacterial and viral markers are used as tools for FST studies. A number of research studies evaluated the sensitivity and specificity of the bacterial and viral markers by screening a large number of sewage and animal faecal samples within the SEQ region (Table 2). Sewage-associated markers such as *Bacteroides* HF183, *E. faecium esp*, adenoviruses and polyomaviruses were highly specific to sewage and therefore, can be considered as suitable for the detection of sewage pollution. Sewage-associated *Bacteroides* HF183 and polyomaviruses also demonstrated high sensitivity ratings indicating these markers are quite sensitive for the detection of faecal pollution in aquatic environments. In contrast, sewage-associated and bovine wastewater-associated adenoviruses showed lower sensitivity indicating these markers alone may not be sufficient to identify the sources of faecal pollution with appropriate sensitivity.

Faecal Source Tracking Case Studies in Aquatic Environments in SEQ Region

Table 3 shows the FST tools used and results obtained in ten case studies undertaken in SEQ. In case study 1, urban creek water samples were tested

Table 3. Faecal source tracking case studies undertaken in SEQ, Australia.

Case study no	Location	Types of aquatic environment	Tools used	Likely sources of faecal pollution	Reference
1	Eudlo Creek, Maroochydore	Freshwater creeks	Biochemical fingerprinting[a]	Sewage pollution via septic tanks, animals such as chickens and ducks	Ahmed *et al.* 2005
2	Bonogin Valley and Tallebudgera Creek, Gold Coast	Freshwater creeks	Antibiotic resistance analysis[a]	Sewage pollution via septic tanks, wild animals	Carroll *et al.* 2005
3	Bergin Creek, Four Mile Creek and River Oaks Drive in Pine Rivers Shire	Stormwater runoff	Biochemical fingerprinting[a] sewage-associated HF183[b] and HF134[b] PCR	Sewage pollution via septic tanks, wild animals	Ahmed *et al.* 2007
4	Tooway Lake, Caloundra	Coastal lake	Biochemical fingerprinting[a] and antibiotic resistance analysis[a]	Sewage pollution via STP, waterfowl	Ahmed *et al.* 2008b
5	Ningi Creek, Caboolture	Brackish waters	Sewage-associated HF183[b], HF134[b], *esp*[b], ruminant-associated CF128[b], dog-associated BacCan[b] PCR	Sewage pollution via septic tanks, cattle and dog faecal pollution	Ahmed *et al.* 2008e
6	Ningi Creek, Caboolture	Brackish waters	Sewage-associated *esp*[a] PCR	Sewage pollution via septic tanks	Ahmed *et al.* 2008c
7	Bergin, Four Mile and River Oaks Drive Creek in Pine Rivers Shire	Stormwater runoff	Sewage-associated *Bacteroides* HF183[b] and HF134[b] PCR	Sewage pollution via septic tanks	Ahmed *et al.* 2008d
8	Maroochy River, Maroochydore	Estuarine water	Sewage-associated JCV and BKV polyomaviruses[b] PCR	Sewage pollution via STP and stormwater drains	Ahmed *et al.* 2010a
9	Maroochy River, Maroochydore	Estuarine water	Sewage[b] - and bovine[b] wastewater-associated adenoviruses	Sewage pollution via STP and stormwater drains and bovine faecal faeces	Ahmed *et al.* 2010b
10	North Maroochy River, Maroochydore	Freshwater creeks	Faecal sterols	Sewage pollution via septic tanks, wild animals	Sullivan *et al.* 2010

[a] quantitative; [b] qualitative

to identify sewage pollution in Eudlo Creek, Maroochydore. A secondary aim was to identify faecal pollution originating from domestic and wild animals. Biochemical fingerprinting libraries comprising of 4,057 enterococci and 3,728 *E. coli* isolates from horses, cattle, sheep, pigs, ducks, chickens, deer, kangaroos, dogs and septic tanks were used to identify the sources of unknown environmental *E. coli* and enterococci using cluster analysis (Ahmed *et al*. 2005). *E. coli* and enterococci libraries were capable of identifying the sources of more than 65% of the isolated indicator bacteria from the studied creek. The authors reported that the sewage-associated *E. coli* and enterococci isolates in the studied creek originated from defective septic tanks and as well as animal sources.

Antibiotic resistance analysis was used in case study 2 to determine the significance of septic systems as a major contributor to faecal pollution in two mixed land use catchments, Bonogin Valley and Tallebudgera Creek, in the Gold Coast Region (Carroll *et al*. 2005). Antibiotic resistance patterns were established from 717 known *E. coli* isolates obtained from septic tanks and faeces from domesticated, livestock and wild animals. Discriminant analysis was used to differentiate between the antibiotic resistance patterns of isolates from various sources, and to classify each isolate from water (unknown source) into a source category. The results suggested the presence of sewage pollution within the investigated catchments originated from septic tanks.

In case study 3, storm water samples were collected from Bergin Creek, Four Mile Creek and River Oaks Drive to determine whether the stormwater was polluted with sewage from possible defective septic systems (Ahmed *et al*. 2007). A battery of tools consisting of biochemical fingerprinting of *E. coli* and enterococci, sewage-associated *Bacteroides* HF183, HF134 and sewage-associated esp markers were used to detect sewage pollution in the non-sewered, residential catchments studied. The source of 105 *E. coli* biochemical phenotypes (BPTs) and 93 enterococci BPTs were identified in water samples from River Oaks Drive catchment. Of these, 10% and 9% were identified as sewage-associated *E. coli* and enterococci BPTs, respectively. Similarly, of the 83 *E. coli* BPTs and 93 enterococci BPTs from the Bergin Creek catchment site, 8% *E. coli* BPTs and 9% enterococci BPTs were identified as sewage-associated isolates. The number of *E. coli* and enterococci assigned to sewage-associated in the Four Mile Creek site were 4% and 3%, respectively. Sewage-associated HF183, HF134 and *esp* markers were detected in water samples however, the library-dependent (i.e. biochemical fingerprinting) and library-independent (PCR markers) tools were not always in agreement in detecting sewage pollution in water samples. This study demonstrated the value of a combination of tools for faecal pollution tracking to obtain a better understanding regarding the pollution sources.

Multiple bacterial markers (i.e. sewage-associated *Bacteroides* HF183, HF134, *esp* markers, ruminant-associated markers, and dog-associated markers) were used to determine the sources of faecal pollution in case study 5. The specificity of these markers were determined by testing large number of faecal samples from sewage/septage, ducks, kangaroos, cattle, horses, dogs, chickens, pigs, pelicans, goats, deer, wild birds and sheep (Ahmed *et al*. 2008e). Most of the markers showed high specificity (> 0.90) except *Bacteroides* dog-associated markers which showed low specificity. At least one host-specific marker was detected in 14 (87%) out of 16 water samples. Sewage-associated *Bacteroides* HF183 and HF134 markers were detected in 9 (56%) and 6 (37%) samples, respectively. This figure for sewage-associated *esp* marker was also 6 (37%). Ruminant-associated markers CF128 were detected in 11 (69%) samples whereas dog-associated markers BacCan were detected in 5 (31%) samples. Among all markers, *Bacteroides* HF183 and *esp* performed well in terms of specificity and identifying the sources of sewage pollution. However, a combination of multiple sewage-associated markers provided greater reliability regarding the presence/absence of sewage pollution when one marker was not sufficient to identify sewage pollution.

Quantitative PCR (qPCR) was used to estimate the levels of sewage-associated *esp* markers in case study 6. Environmental samples (n = 16) were collected after storm events and tested

using qPCR for the quantitative detection of sewage pollution (Ahmed *et al*. 2008c). The specificity of the *esp* marker to distinguish between sewage and animal faecal pollution was determined by screening a large number of sewage and animal faecal samples. The *esp* marker was detected in 90.5% of combined sewage and septic tank samples (n = 42) and was not detected in any of the faecal samples (n = 155) from the non-target animals tested. Of the 16 samples tested, six (38%) were positive for the *esp* marker, and the number ranged between 1.1×10^2 and 5.3×10^2 gene copies/100 mL of water. The evidence presented in this study demonstrated that the *E. faecium esp* markers appears to be host-specific and promising for sewage pollution tracking in environmental waters in SEQ.

Viral markers (i.e. sewage-associated JCV and BKV polyomaviruses, sewage-associated adenoviruses, and bovine wastewater-associated adenoviruses) were used to determine the sources of faecal pollution in case studies 8 and 9. The host-specificity of these viral markers was determined by screening wastewater and faecal samples from non-target sources such as chickens, dogs, ducks, kangaroos, wild birds, cattle, pigs and sheep. All the viral markers exhibited high host-specificity (Ahmed *et al*. 2010a,b). Of the 20 samples tested for sewage-associated BKV and JCV polyomaviruses, five (25%) were positive, indicated the presence of sewage in various sites on the Maroochy River. Of the 20 samples tested for sewage-associated and bovine wastewater-associated adenoviruses, four (20%) were positive for sewage-associated adenoviruses and two (10%) were positive for bovine wastewater-associated adenoviruses. The authors concluded that viral markers appear to be highly host-specific for detecting sewage pollution in the studied coastal river. The presence of viral markers in river water samples indicate potential public health risks as the studied river is used for recreational activities including swimming, fishing and water sports.

Faecal sterols were used to determine the sources of sewage pollution in the case study 10. In all, 36 water samples were collected from six sites on six occasions and the concentration of sterols were determined. The stanols concentration in water samples generally increased with increased catchment runoff. After moderate rainfall, high coprostanols levels found in water samples indicated sewage pollution via defective septic systems. In contrast, it appears that during dry weather sewage pollution is not occurring in the study catchment. Sterol profiles also pointed to a cattle farm causing pollution during modest catchment runoff. The method used in this study was able to identify the sources of faecal pollution to the catchment due to rainfall.

Conclusions and Future Directions

This series of case studies conducted in SEQ, Australia, has demonstrated the successful application of FST tools in a range of aquatic environments. The primary question that arises in many situations is whether aquatic environments contain sewage pollution. Sewage pollution is usually considered to represent the greatest health risk (Field & Samadpour 2007, Leclerc *et al*. 2002). Library-dependent tools such as biochemical fingerprinting and antibiotic resistance analysis, as illustrated in case studies 1 and 2 can be effective in source identification of faecal indicators. However the need to generate a large source library, and potential concerns over validity of a library beyond the spatial and temporal constraints in which it was derived from can make library-dependent tools both time consuming and expensive.

Library-independent source tracking tools, such as host-specific PCR marker approaches, may be more robust as, in theory, these markers may be more temporally and spatially stable than libraries. The case studies in this paper indicate that the tested markers indeed exhibit similar sensitivity and specificity in the SEQ region. Most of the markers showed high specificity suggesting the suitability for distinguishing between the sewage and animal faecal pollution although the sensitivity was not always high. Nonetheless, the application of an array of markers (i.e., sewage-associated *Bacteroides* and viruses) and/or combination of FST tools (i.e., library-dependent and library-independent) can compensate for any uncertainty created through the use of a single marker or a specific tool.

While there is an increasing knowledge on the degradation, sedimentation, and transport of these FST markers (Bae & Wuertz 2009, Okabe & Shimazu 2007, Walters & Field 2006, Walters & Field 2009), the overall understanding is incomplete, and in most cases, untested in real life situations. Preferential transport may result in some markers behaving differently compared to the traditional faecal indicators and pathogens that are of ultimate concern (Dick *et al*. 2010; Stapleton *et al*. 2009). Nonetheless, collectively, these case studies indicate that current FST tools can be successfully applied for source identification and can be used for meaningful and productive management decisions. However, there is a need for significant refinement of these tools, and a continued investment in research to achieve these improvements is required. Despite this, FST tools can and are being used to improve water quality outcomes, even in its current developing forms.

The Authors

Dr Warish Ahmed has had a career in identifying the sources of faecal pollution in environmental waters. He is currently working as a water microbiologist at CSIRO Land and Water, St Lucia, Qld. Email: Warish.Ahmed@csiro.au.

Ted Gardner is Principal Scientist with CSIRO Land and Water.

Dr Simon Toze is a Research Group Leader with CSIRO Land and Water in the Urban and Industrial Water Research Theme. He also leads the Indirect Potable Recycling research area for the CSIRO Water for a Healthy Country Flagship.

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