Tracking sources of microbial pollution in recreational waters: Experience from two Toronto beaches

Repérage des sources de pollution microbienne dans les eaux récréatives urbaines: expérience de deux plages de Toronto

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RESUME

De nombreux sites sont confrontés au problème de la signalisation ou de la fermeture de plages en raison de contamination fécale. Dans le cadre d'une approche basée sur le risque, le risque pour la santé des nageurs est évalué par des contrôles sur site de bactéries indicateurs et les risques associés sont gérés par des contrôles à la source et autres mesures correctives. Dans l'évaluation des risques, de grands progrès ont été obtenus au cours des dernières années par l'introduction de techniques de détection des sources microbiennes. Pour les deux plages étudiées, les deux méthodes utilisées – l'analyse de résistance antibiotique et le marquage par empreinte ADN – les déjections d'oiseaux ont été identifiées comme la source dominante de *E. coli*. représentant jusqu'à 30 à 66 % de la charge totale. Ce type de pollution constitue un défi environnemental majeur.

ABSTRACT

Posting or closing of swimming beaches because of faecal contamination is a widespread problem reported in many locations. In a risk-based approach to this problem, the risk to swimmers health is assessed by field monitoring of indicator bacteria and the associated risks are managed by source controls and other remedial measures. In risk assessment, great advances have been made in recent years with introduction of microbial source tracking (MST) techniques. For the two beaches studied, the two MST methods used, antibiotic resistance analysis and DNA fingerprinting, both identified bird faeces as the dominant sources of *E. coli.*, representing as much as 30-66% of the total load. Coping with this type of pollution presents a major environmental challenge.

KEYWORDS

Escherichia coli, faecal pollution, faecal sources, microbial source tracking (MST), urban beaches.

1 INTRODUCTION

Posting or closing of swimming beaches due to degraded water quality is a widespread problem reported in many locations around the world (Ashbolt and Bruno 2003; Marsalek and Rochfort 2003; Kinzelman et al. 2004). Currently, two types of approaches are used to address this problem: (a) regulatory schemes based on compliance with indicator organism limits (IOLs) (Health and Welfare Canada 1992; MOEE 1996), and (b) a risk-based management approach (WHO 2003). Both approaches require assessment of faecal contamination of receiving waters by undertaking sanitary inspection and establishing indicator organism concentrations. The sanitary inspection should identify all sources of faecal pollution, including human faecal pollution conveyed by urban effluents (Marsalek and Rochfort 2003), urban wildlife (particularly birds), domestic pet populations (particularly dogs), beach sand, lack of sanitation, poor solid waste management, land wash, and growth of bacteria in receiving waters (WHO 2003). Land-based sources contribute via discharges of stormwater, combined sewer overflows (CSOs) and sewage treatment plant (STP) effluents; birds and pets may also contribute directly to the waters and beach sand. Some land-based sources are activated during wet weather; beach sand and benthic sediments sources are activated by waves and currents (Conseil Supérieur d'Hygiène Publique de France 1990; LeFevre and Lewis 2003).

In the study area addressed here, the investigation of sources of faecal pollution was based on a microbial source tracking (MST) study that characterised *E. coli* isolates by antibiotic resistance analysis (ARA) and DNA fingerprinting (Edge et al. 2006). Even though the underpinning science is still developing (Edge and Schaefer 2006; Rochelle and De Leon 2006), MST can be applied in studies of recreational waters to provide additional evidence for identification of faecal pollution sources and the selection of management measures.

The main objective of this paper is to test two MST methods in a field study of sources of indicator bacteria at two recreational beaches in Toronto (Canada) and examine general strategies for improved management of recreational beaches.

2 STUDY AREA AND METHODS

2.1 Study area

The Centre Island beach (CIB) is located on the south (open lake) side of the Toronto Island, which is a sand bar located offshore of the Toronto waterfront. The island serves as a park; thus, there is no housing, or sewers or other obvious sources of anthropogenic faecal pollution. CIB has been monitored daily for *E. coli* and historically, it has been posted on average 44% of the swimming season; in 2003, only 14%, with the minimum and maximum 2-day geometric means of 10 and 345 *E. coli* cfu/100 mL, respectively (Environmental Defence undated).

The Kew Beach (KB) is located along the Toronto waterfront, east of the downtown area. KB used to be impacted by littoral transport of pollution from CSO outfalls along the waterfront, but such overflows have been greatly reduced or eliminated by CSO storage tanks built in the early 1990s. KB exhibits relatively good water quality with low average posting of 17% (1995-2003), but relatively high posting in 2003, 30%. The minimum and maximum 2-day geometric averages were 10 and 3,382 *E. coli* cfu/100 mL, respectively (Environmental Defence undated).

2.2 Methods

Water samples were collected on both beaches along transects (two at CIB, three at KB) extending from the shore into the lake, every Monday morning over the bathing

season (May-August). Samples were collected in sterile bottles, in duplicates, at three depths, ankle (d=0.1 m), knee (0.5 m) and chest deep (1.4 m), placed in coolers, and returned to the NWRI laboratory for analysis within several hours of sampling. Sand samples were collected from the wet foreshore sand within a metre of waterline, to a depth of about 0.2 m, using a sterile plastic core. About 20 g of wet sand was collected, stored in coolers, and sent to the laboratory with water samples. Both water and sand samples were analyzed for *E. coli*.

Faecal samples were collected weekly over the summer of 2004. Sewage samples were collected from the Ashbridges Bay Sewage Treatment Plant (STP), samples of faeces of pets (dogs and cats) and birds (gulls, Canada geese, mallard ducks, cormorants, swans) were obtained from fresh droppings on the ground at or near the beach areas using sterile culturette cotton swabs, which were then stored on ice and taken to the laboratory (Edge et al. 2006).

2.3 *E. coli* enumeration and isolation

Water samples were analyzed by membrane filtration and *E. coli* enumeration was expressed as colony forming units (cfu) / 100 ml. Wet sand samples were weighed to 10 grams, placed into 150 ml of phosphate buffer in a Waring blender, blended for 1 minute, and left standing for another minute and membrane filtered. Faecal swabs were streaked onto mFC agar (Difco Inc.) and incubated at 44.5°C for 18 hours. Isolates showing a typical dark blue colour on mFC agar were selected for further *E. coli* identification confirmation tests. Putative *E. coli* isolates on MacConkey plates were then tested for glucuronidase activity by growth and fluorescence in EC-MUG (Difco Inc.), and for indole production by growth in 1% (w/v) tryptone (Difco Inc.) and reaction with Kovac's reagent (Oxoid Inc.).

Antibiotic *E. coli* from 96 well Matrix plates (Matrix Technologies Corp. Hudson, NH) were thawed and incubated overnight in a microplate containing 200 µl per well of EC-MUG broth at 44.5°C. The *E. coli* isolates were transferred to the surface of rectangular tryptic soy broth agar plates, which were incubated for 18 hours at 37°C, and growth of *E. coli* isolates on plates with antibiotics was compared to the growth on control plates without antibiotics. Prior to statistical analysis of antibiotic resistance profiles, isolates with identical antibiotic resistance profiles (phenotypic clones) from the same individual faeces sample were removed to reduce library bias. The resulting Toronto library of faecal *E. coli* antibiotic resistance profiles was analyzed by discriminant function analysis (SAS 1999 - PROC DISCRIM procedure) to develop a discriminant function for correctly classifying known faecal source *E. coli* isolates. The discriminant function was calculated to discriminate between three likely sources of urban faecal pollution: birds (n=929 *E. coli* isolates), pets (n=399 isolates), and municipal wastewater (n=539 isolates).

A minimum detection percentage was calculated following Whitlock et al. (2002) and Wiggins et al. (2003) in order to assess the lower limit for considering that a faecal source was actually being detected in water or sand samples. This limit was calculated based on obtaining an average rate of misclassification for the discriminant function, and then adding a conservative detection factor of 4 times the standard deviation of misclassification rates.

Rep-PCR DNA fingerprinting was performed using a BOX-PCR primer approach, as outlined in Edge et al.(2006). Similar to the ARA analysis, the rep-PCR DNA fingerprinting technique was applied to discriminate between three sources of faecal pollution: birds (n=524 Isolates), pets (n=189 isolates), and municipal wastewater n=486 isolates). The *E. coli* water and sand isolates were then compared to the faecal library isolates using a nearest neighbour similarity method (K=5) to classify them. Where water and sand isolates did not match closest with at least three isolates

(out of five nearest neighbours) from a particular faecal source, they were classified as “unknown”. Since there was some imbalance in sample sizes between the faecal source classes (e.g. n= 189 for pets), 2-way source clustering, and other exploratory 3-way clustering analyses were performed using an average and maximum similarity measurement. These analyses gave general source classification results consistent with the nearest neighbour method.

3 RESULTS

Weekly monitoring of waterborne *E. coli* at both beaches produced adequate records of bacterial counts required under both the Guidelines for Canadian Recreational Water Quality (GCRWQ) and Provincial Water Quality Guidelines (PWQG)(MOEE 1996). Even though water samples were collected at three depths (Edge et al., 2006), only those corresponding to the chest depth (1.4 m) and meeting the EU Directive rule for sampling (samples collected 0.3 m below the water surface, in water at least 1 m deep; EU 2006) are presented in Table 1.

Beach	Geometric mean	Upper 90th percentile	Upper 95th percentile	Compliance with guidelines (% of time)		Beach water quality classification (after EU)
				GCRWQ	PWQG	
CIB	32	123	180	100%	87.5%	Excellent
Kew B.	12	55	87	100%	100%	Excellent

Table 1. Microbiological water quality at Centre Island and Kew Beaches: 2004 season. *E. coli* (cfu/100 ml) at chest depth (~1.4 m)

The data in Table 1 indicate very good beach water quality during the 2004 swimming season. According to the more rigorous PWQG (100 *E. coli* cfu/100 mL; MOEE 1996), the beaches would stay open 87.5-100% of the bathing season, and according to the EU Directive (EU 2006), both beaches would be classified as having excellent water quality. A less favourable assessment would be obtained if using shallow water data, e.g., collected at knee depth (~0.5 m), or even ankle depth (0.1 m). While shallow waters are typically not sampled, they are relevant with respect to wading by young children (Health and Welfare Canada 1992). Increased bacterial counts associated with sand resuspension by waves were reported by Edge et al. (2006) and Lefevre and Lewis (2003).

Wet sand samples were processed similarly as water samples and the results of *E. coli* enumeration are shown in Table 2.

Beach	Number of samples	Geometric mean	Maximum value
CI Beach	19	119	480
Kew Beach	33	16	519

Table 2. *E. coli* in foreshore sand samples from the Centre Island and Kew Beaches: 2004 weekly data (cfu/gram dry sand)

Data in Table 2 indicate that beach sand contains *E. coli* that may be released by water percolation through, or resuspension of, sand. Similar finding were reported by

Conseil Supérieur d'Hygiène Publique de France (1990), Alm et al. (2003), Whitman and Nevers (2003), Kinzelman et al. (2004), WHO (2003), and Sampson et al. (2006).

Faecal source classification of *E. coli* isolates in water samples from the Centre Island beach, obtained by ARA (antibiotic resistance analysis) and DNA fingerprinting, is shown in Fig. 1. For the minimum ARA detection threshold of 28%, the only significant source is birds, with the other sources being slightly below the detection threshold. DNA fingerprinting clearly showed birds as the predominant source. In that sense, both methods provided consistent findings.

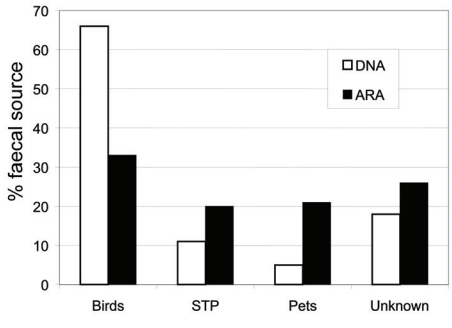

Fig.1. Faecal source classification of *E. coli* at the Centre Island Beach: (a) ARA (antibiotic resistance analysis; N = 1278), and (b) DNA fingerprinting (N = 318)

Similar findings were obtained for sources of *E. coli* in Centre Island beach sand; with ARA yielding a fairly uniform distribution of sources: birds (30%), pets (30%), STP (18%) and unknown (22%), with only the first two sources exceeding the minimum detection threshold (28%). DNA fingerprinting yielded one dominant source – birds (62%), with other sources being relatively minor (STP – 9%, pets – 6%, and unknown – 23%. Thus, both methods point to birds as an important source of *E. coli* in sand.

Faecal sources at the Kew beach were also classified using the same methods; results for water samples are shown in Fig. 2. While the results suggest a more mixed contribution of bird and STP sources at this beach compared to CIB, again, birds seemed to be a prominent contributor to beach water contamination.

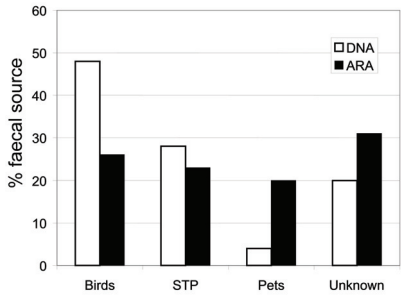

Fig. 2. Faecal source classification of *E. coli* at Kew Beach: (a) ARA (antibiotic resistance analysis; N = 1941) and (b) DNA fingerprinting (N = 995)

Sand samples were also classified, with ARA indicating birds as the predominant source (36%), followed by unknown (30%), pets (17%), and STP (17%); the first source exceeded the minimum detection threshold of 28%. DNA fingerprinting confirmed birds (44%) as the dominant source, followed by STP (34%), unknown (20%), pets (2%), and unknown (20%). Again, both methods confirmed birds as the

predominant source of *E. coli* in sand. For detailed analysis of individual sampling transects and water depths, see Edge et al. (2006).

4 DISCUSSION

This microbial source tracking study found that bird droppings were a more prominent contributor of *E. coli* to the beach water and sand at both beaches than municipal wastewater or pet droppings. The results from both antibiotic resistance and DNA fingerprinting analyses were consistent with observations of large numbers of gulls and Canada geese (and their droppings) on the beaches over the study time period. Hardly any pet droppings were observed on the beaches, and there were no significant municipal wastewater outfalls observed near the beaches. While the two MST methods provided percent faecal source apportionment results, the ability of library-dependent MST methods to provide accurate quantitative percent faecal source apportionment data is still questionable (U.S. EPA 2005). As such, the MST results were interpreted qualitatively, and in conjunction with other local knowledge gained from sanitary surveys and management of these beaches in recent years. Considering that the field of MST is still evolving, it will be important to continue to apply MST methods in the future as part of multiple lines of evidence in drawing faecal source tracking conclusions.

While the assessment of water quality data is relatively straightforward, a number of ambiguities in sampling procedures remain. The first one concerns the depth of sample withdrawal. Undoubtedly, more shallow depth samples will usually contain higher concentrations of bacteria and may be more important for children. In this study, samples from waters 0.5 m deep did produce much higher levels of bacteria and worse rating of the CI beach (geometric mean of *E. coli*. = 117 cfu/100mL, upper 90th percentile = 719, EU Directive rating: sufficient).

Both EU and WHO guidelines permit to discontinue water sampling during “short periods” of high pollution, which has some implications for the overall rating of beach water quality. Typically, such periods correspond to the periods with rainfall/runoff (Ashbolt and Bruno 2003; David and Matos 2005; Haramoto et al. 2006), or of wave impact on the beach, accompanied by resuspension of bottom sediment with bacteria (Kinzelman et al. 2004). The Canadian GCRWQ does not make such an allowance (Health and Welfare Canada 1992).

Finally, there is a need to operate swimming beaches in real time, rather than relying on results of incubation-based analyses requiring 18 hour or longer for completion. While progress is being made towards developing real time PCR (polymerase chain reaction) methods (Haugland et al. 2005), they are not yet readily available and the current research on this topic in the USA is focusing on detecting enterococci, rather than *E. coli* required by the Canadian guidelines. In the absence of real-time bacteria data, assessment of bacteriological water quality needs to rely on using surrogate real-time environmental data for estimating bacterial levels. Environmental variables affecting indicator bacteria distributions in recreational waters include rainfall, stormwater or CSO discharges, wind and waves, hydraulic transport, sunlight, water temperature, turbidity, algal presence, sand presence, and the spectral absorption coefficient (Ashbolt and Bruno (2003); Kinzelman et al. (2004), Haramoto et al. (2006), David and Matos (2005), Rechenburg et al. (2006), Lefevre and Lewis (2003), Whitman et al. (2003), Whitman et al. (2004), Sampson et al. (2006), Mietzel et al. (2003). Where several confounding factors can affect bacterial levels, models have been used to predict guideline violations (Mietzel et al. 2003).

Risk management comprises two types of measures: source controls (typically developed on the basis of sanitary inspections) and beach posting/closure. Source controls focus on stormwater and CSO discharges, STP effluents, control of animal

access to beaches, and beach maintenance. Source controls should focus on those sources which most strongly contribute to beach contamination, as determined by sanitary inspections or microbial source tracking. Where such a contamination is primarily of human origin, controls should focus on STP effluents (disinfection), the abatement of the CSO pollution, and control of stormwater.

The most challenging problem is dealing with faecal pollution caused by birds. As shown in this study (Edge et al. 2006) and others (Levesque et al. 1993), birds can deposit large quantities of faeces directly on beaches and such materials enter into sand where they can survive for long periods. The faecal matter can be released into water by waves eroding beach sand and resuspending bottom sediment. While bird faeces contain E. coli, they can also contain enteric pathogens like Campylobacter sp. (Jones 2001). However, bird faeces are unlikely to contain many human viruses which are often viewed as presenting a significant risk to swimmers. The public health risks of bird faeces are not well characterized, and current testing and guidelines do not provide much guidance for managing beaches contaminated by bird droppings. Measures for keeping birds away from beaches have been proposed (netting, fishing lines, distress calls), but their effectiveness is not well known.

When source controls fail to bring the bacterial counts down to the guidelines limits, the last option in risk management is to post recreational waters with warnings, or close them to public use. This measure is used commonly in many jurisdictions and elicits criticism of water authorities for failing to control faecal pollution.

5 CONCLUSIONS

A risk-based management approach to managing public swimming beaches offers advantages over the approaches based on compliance with indicator organism limits. Advances in the field of microbial source tracking may provide new tools to improve risk assessment, although further refinement of these MST methods is needed to make them fully operational. At present, MST methods can identify apportionment of various sources of faecal contamination of beaches, however, there can be significant uncertainties in the apportionment, and results may be better viewed qualitatively. In the area studied here, coping with bird pollution presents a major challenge that will not be resolved by traditional wastewater management measures.

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