Effectiveness of Guideline Faecal Indicator Organism Values in Estimation of Exposure Risk at Recreational Coastal Sites

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Abstract Decay rates in coastal water and sediment for the bacterial pathogens *Salmonella typhimurium* and *S. derby* were compared in laboratory-based microcosms with results previously obtained for a number of faecal indicators. In general, the decay rates of *Salmonella* spp. were greater than either enterococci or coliphage in overlying water and sediment. Decay rates of *E. coli* were similar to *Salmonella* spp. in overlying water, although greater in sediment. Increased temperature resulted in an increased decay rate for all organisms in the overlying water (and to a lesser extent in the surface sediment layer). It was demonstrated that decay rates for both S. *typhimurium* and *S. derby* were greater in overlying water compared with sediment, suggesting sediments may act as a reservoir for pathogenic microorganisms released into the coastal environment during recreational activity and should to be considered when estimating environmental exposure. Using measured decay rates and available dose-response data, a quantitative microbial risk assessment (QMRA) utilising Monte Carlo simulation was undertaken to estimate the risk of infection to *Salmonella* spp. following exposure to recreational coastal water subject to a range of faecal contamination levels. In waters of extremely poor quality, subject to contamination by faecal coliforms (10^6 CFU 100 mL^{-1}), the maximum probability of infection on the day of an accidental release was above 2.0×10^{-1} and remained above 1×10^{-3} for three days following the initial high concentration.
Keywords Recreational water, faecal indicators, sediment, risk assessment, exposure, *Salmonella*

Introduction

The health-related management of recreational coastal sites is primarily undertaken by the monitoring of faecal indicator organism concentrations in overlying water. In Australia, guidelines for the microbiological quality of coastal waters prescribe the maximum concentrations of faecal coliforms in water to be used for human recreation (NHMRC, 1990). The presence of these organisms is used to estimate the risk of other pathogenic organisms of faecal origin being present in the water body. The ability of faecal coliforms to act as indicators of enteric bacterial, viral and protozoan pathogens has, however, been questioned but their use continues in the absence of any viable alternative (Hood and Ness, 1982; Koh *et al.,* 1994; Ferguson *et al.,* 1996).

Organisms released into coastal or marine environments are exposed to numerous factors which cause stress including temperature change, salinity, nutrient deficiencies, predation and sunlight (Davies *et al.,* 1995, Özkanca and Flint 1997, Thomas *et al.,* 1999). Studies have indicated that microorganisms attached to suspended particles and sediment contribute greater numbers than those in the surface waters under both *in-situ* and laboratory conditions (Goulder 1977, Shiaris *et al.,* 1987, Davies *et al.,* 1995, Fish and Pettibone 1995, Crump and Baross 1996). Enumeration of faecal indicator organisms from the water column alone may therefore underestimate the risk of exposure to pathogenic organisms in aquatic systems if resuspension from the sediment occurs during recreational activity.

Numerous epidemiology studies have demonstrated that contact with bathing water subject to faecal contamination increases the risk of disease, in particular gastroenteritis, but also non-enteric diseases caused by respiratory, eye, ear and skin infections (Cabelli

et at. 1979; Alexander *et al.,* 1992; Fewtrell *et al.,* 1992; Corbett *et al.,* 1993; Fleisher *et al.,* 1993; Kay *et al.,* 1994; Fleisher *et al.,* 1996). Such studies have identified relationships between faecal indicator organism concentrations in the water column and probability of disease, which has been used to set guideline values for recreational waters. Another method used to estimate exposure and subsequent infection risk in recreational coastal waters is via the application of quantitative microbial risk assessment (QMRA). A number of studies using this paradigm have utilised published data on dose-response relationships for pathogenic organisms and the distribution of such organisms in the aquatic environment to estimate infection risk (Crabtree *et al.,* 1997; Gerba *et al.,* 1997; López-Pila and Szewzyk 2000). A benefit of QMRA is its ability to estimate (albeit crudely) the probability of infection or disease at very low concentrations of pathogenic organisms; levels at which epidemiological studies are unable to detect difference.

For this study, the decay rates of the faecal indicator organisms *E. coli, Enterococcus faecium* and somatic coliphage in recreational coastal water and sediment was determined using a series of laboratory based microcosm experiments and were subsequently compared with decay rates observed for the pathogenic organisms *Salmonella typhimurium* and *S. derby*. The inclusion of the *Salmonella* spp. facilitated the ability to perform a QMRA for recreational coastal waters. In Australia, salmonellosis is one of the most reported causes of gastroenteritis with the mean number of notifications over the past five years being 7076 individuals per year (Communicable Diseases Network Australia – National Notifiable Diseases Surveillance System, personal communication 2002). While the authors acknowledge that the majority of these cases are probably foodborne, the true incidence of those which are waterborne is unknown. Indeed, *S. derby* was chosen because not only has it previously been isolated from recreational coastal waters in Sydney, Australia and in marine waters in Hong Kong (Kueh and Grohmann 1989; Yam *et al.,* 2000), but in addition, dose-response studies have been undertaken on this organism in a human feeding study (McCoullough 1951). Thus, the risk of infection by *Salmonella* spp. following exposure to variety of recreational coastal waters subject to faecal contamination under different conditions could be estimated. Risk of disease from protozoan and viral pathogens following recreational exposure has been determined to be greater than for many bacterial pathogens, including *Salmonella* spp. and therefore these risks would need to be considered in any attempt to estimate total risk of gastrointestinal disease (Gerba *et al.,* 1996; Crabtree *et al.,* 1997). For this study, however, these two *Salmonella* spp. were used in an initial study of the behaviour of human pathogens in recreational coastal water and sediment under various environmental conditions of temperature and sediment type.

The primary aim of this research was to attempt to more accurately identify a potential environmental exposure from contaminated waters in the first stage of a health risk assessment for recreational coastal waters. These results could be used to better manage risk at recreational coastal sites in the days proceeding an incident causing high levels of faecal contamination such as a wastewater release or a stormwater event.

Methodology

Microcosm Design

Intact sediment cores were collected in perspex columns from three locations in metropolitan Adelaide (Henley Beach, Onkaparinga River and Port Adelaide River) which represented diverse coastal sites with different physical characteristics (Craig *et al*., 2001). Sediment was characterised (percentage sand, silt and clay) using the pipette method (Sheldrick & Wang, 1993). The percentage of organic carbon present in the

sediment was determined by the dichromate method (Tiessen & Moir, 1993). In addition to these, columns were filled only with overlying water to act as controls (in the absence of sediment).

In the laboratory, the microcosms were maintained in a water bath at a constant temperature (10°C, 20°C or 30°C). Overlying water was removed and replaced with 500 mL of water from one site of intermediate salinity (Onkaparinga River) to maintain a constant total dissolved solids (TDS) concentration between different sediment types. This removed the potential influence of changes of salinity in overlying water caused by tidal and environmental changes at the different sites.

Preparation of Faecal Indicator and Pathogenic Microorganisms

Stock bacterial suspensions of *E. coli* (ATCC 25922), *Enterococcus faecium* (ATTC 19434), *Salmonella typhimurium* (ATCC 14028) and a clinical strain of *S. derby* (isolated from a human stool sample; Institute of Medical and Veterinary Science, Adelaide) were prepared by inoculation into 10 mL nutrient broth (Oxoid) and incubated overnight at 37°C. Cells were harvested by centrifugation at 2,500 g for 10 minutes. The pelleted samples were resuspended in phosphate buffered saline (PBS), centrifuged and resuspended in PBS.

Coliphage suspension was prepared using host *E. coli* (FCC 84) cells inoculated into nutrient broth (Oxoid) and incubated overnight at 37°C. A single coliphage plaque previously isolated from raw human sewage was added to this suspension and incubated for a further 24 h at 37°C. To remove host bacterial cells, the suspension was centrifuged and the supernatant (containing coliphage) was filtered to further remove any bacterial cells present. All stock suspensions were maintained at 4°C until use.

Determination of Microorganism Decay

Microcosms were inoculated by adding 500 μL of stock coliphage suspension and 50 μL of stock *E. coli*, *Enterococcus faecium*, *S. typhimurium* and *S. derby* suspension into the overlying water. Control water columns were similarly inoculated.

Microorganisms were enumerated from both overlying water and sediment on days 0 (1 h after inoculation), 1, 2, 7, 14 and 28 following inoculation. Sediment samples were prepared by removing the top 10 mm of sediment from the intact core, of which 25 g was placed into 75 mL of 0.1% peptone in a sterile beaker. Sediment was sonicated for 10 min (x2), to separate bacteria from sediment particles (Craig *et al.*, 1999).

E. coli, enterococci and coliphage were isolated from overlying water and sediment as described by the methods of Craig *et al.*, (2001). Briefly, *E. coli* was enumerated by membrane filtration and incubation on membrane lauryl sulphate (MLS) agar and enterococci by the Enterolert™ defined substrate method using 97-well Quantitrays (IDEXX Laboratories, USA). Coliphage were isolated by a double-agar overlay method. *S. typhimurium* and *S. derby* were isolated by membrane filtration (47 mm diameter, 0.45 μm pore size; GN-6, Gellman) and incubation on xylose lysine desoxycholate (XLD) agar (Oxoid) at 37°C for 24 h. A representative number of presumptive colonies were confirmed as *Salmonella* spp. using a salmonella latex agglutination test kit (Serobact; Medvet Science, Adelaide).

The decay rate constant (k) for all organisms was calculated as the slope of the line when $\log_{10}$ (N_t/N_0) was regressed against time, where N_t is the number of bacteria at time t and N_0 is the number of bacteria at time 0 (Davies and Evison, 1991).

Quantitative Microbial Risk Assessment

Assessment of exposure was based on the initial concentration of *Salmonella* spp. in the recreational coastal water, the rate of decay at a specific temperature (as determined by

the microcosm experiment) and the volume of water ingested per exposure. The following equation was used to estimate exposure:

$$N = C_0 \times 10^{kT} \times V_i \quad \text{(Eq 1)}$$

where N in the number of organisms ingested, C_0 is the initial concentration of organisms in the overlying water (number of organisms mL^{-1}), k is the decay rate (day^{-1}), T is time (d), and V_i is the volume of water (mL) ingested each time of exposure. The volume of water ingested per exposure was assumed to be 20 – 50 mL with a uniform distribution (Ashbolt *et al.,* 1997).

A pooled Beta-Poisson dose-response relationship for non-typhi *Salmonella* spp. was used to estimate risk of infection (Fazil 1996):

$$P_{inf} = 1 - (1 + n/\beta)^{-\alpha} \quad \text{(Eq 2)}$$

where P_{inf} is the probability of becoming infected after ingestion of *n Salmonella* cells with α and β being constants specific for the pathogen. For *Salmonella* spp. α was determined to be 0.3136 and β was 3008 (Fazil 1996).

Monte Carlo simulations (10,000 iterations per scenario) were used to factor in uncertainty in various model inputs (@Risk, version 4.0.5, Pallisade Corporation, 2000). To estimate the initial concentration of *Salmonella* spp. in recreational water following a faecal pollution event, a ratio of *Salmonella* to faecal coliforms (FC) was used. A major difficulty associated with enumeration of *Salmonella* spp. from coastal waters is the fact that the organisms are usually present in very low numbers, and therefore detection often requires an enrichment step. Many studies thus only report the presence or absence of *Salmonella* spp. from coastal waters, however, a number of studies have used a most probable number technique for enumeration (Van Donsel and Geldreich, 1971; Baudart *et al.,* 2000; Dionisio *et al.,* 2000). As the ratio for *Salmonella:* faecal coliforms can vary from location to location, a triangular distribution with a modal value of $1{:}10^{-4}$, maximum of $1{:}10^{-5}$ and minimum of $1{:}10^{-3}$ was used to describe the ranges of *Salmonella*:FC reported.

To describe a range of possible faecal coliform concentrations, high, medium and low levels were selected with lognormal distribution (parameters displayed in Table 1). A 'high' level of 1×10^6 CFU 100 mL^{-1} (poor water quality) was chosen to represent an incident of high faecal contamination due to stormwater pollution following a high rainfall event or an accidental release of untreated wastewater (Craig *et al.,* in prep). The low level of 150 CFU 100 mL^{-1} (good quality) is the current median guideline faecal coliform concentration for primary contact recreation in Australia (NHMRC 1990).

Table 1 Lognormal parameters for faecal coliform frequency.

Recreational Water Quality	Lognormal Distribution Parameters		
	μ	σ	Geometric mean (No. 100 mL^{-1})
Poor	6	0.8	1×10^6
Average	3	0.8	1,000
Good	2.18	0.5	150

μ= mean; σ = std. deviation

To estimate *Salmonella* concentration in the water column over time, decay rates observed in a series of microcosm experiments were averaged and described as a

triangular distribution with maximum and minimum values corresponding to the range of decay rates observed.

Statistical Analysis

Statistical analysis was undertaken using one-way ANOVA and significance expressed at $P \leq 0.05$ (SPSS version 10.0.5, 1999). Bivariate relationships between faecal indicator and pathogenic organism decay rates were examined using Pearson's correlation coefficient (r). All results were expressed as the mean ± standard deviation of three determinations.

Results and Discussion

Sediment taken from the three recreational coastal sites were distinctively different in terms of particle size and organic carbon content (Craig *et al.,* 2001). Sediment from Henley Beach consisted mainly of sand (> 98%), with very little silt, clay and organic carbon (0.08%, 1.41% and 0.05% respectively). Sediment from Pt Adelaide, however, consisted of less proportions of sand and greater of silt, clay and organic carbon (83.05%, 4.24%, 10.33% and 2.38% respectively) than Henley Beach sediment. Sediment from the Onkaparinga River could be described as intermediate (95.48% sand, 1.26% silt, 2.91% clay, and 0.35% organic carbon). This enabled a comparison of decay rates of faecal indicator and pathogenic organisms in surface layers of various sediment types which could have a significant impact on exposure assessment if the sediment was resuspended during recreational activity.

The decay rates of *E. coli*, enterococci and coliphage in coastal water and sediment have previously been reported by this research group (Craig *et al.,* 2001). Under all conditions, decay was greater in overlying water compared with sediment. The lowest decay rates were observed for enterococci and coliphage in clay sediment incubated at 10°C (0.04 k; d^{-1}). The decay rate of *E. coli* was greater than enterococci and coliphage in both overlying water and sediment at all temperatures. Survival of indicator organisms displayed an inverse relationship with temperature.. Of the columns containing sediment, greatest decay occurred in the sandy sediment. Small particle size and high organic carbon content were found to be more conducive to microbial survival. Greatest decay overall was observed in the columns containing water only.

Results for the *Salmonella* spp. survival experiment demonstrated the organisms largely followed exponential decay (as illustrated by r^2 values in Tables 2-3) enabling results to be expressed as decay rates (k), with more negative values indicating greater rate of decay. In the overlying water, the decay of *S. derby* was generally greater than *S. typhimurium* with greatest decay rate of -1.36 (k; d^{-1}) occurring for *S. derby* in overlying water incubated at 30°C. No significant difference was observed, however, for decay rates of both organisms in the sediment. Van Donsel and Geldreich (1979) also identified no significant difference between the decay of five species of salmonellae (including *S. typhimurium* and *S. derby*) in freshwater sediments. As demonstrated with the faecal indicator organisms, increased decay rates in the overlying water were observed for both organisms at 30°C compared with 20°C. Similar results were observed in a study by Monfort *et al.,* (2000) where decay rates for *S. panama* in both sterile and non-sterile seawater microcosms were found to be greater at higher temperatures. These results confirm the importance of water temperature when estimating levels of exposure to microorganisms of faecal origin at recreational coastal sites. Temperature, however, was not found to greatly influence the decay rates of these organisms in the surface sediment layers.

Table 2 Decay rate constants (k; d^{-1}) for *S. typhimurium* in water and sediment; *P <0.05.

	Water				Sediment			
	20 °C		30 °C		20 °C		30 °C	
	k	r^2	k	r^2	k	r^2	k	r^2
Henley Beach	-0.81	0.87	-0.44	0.80*	-0.27	0.96*	-0.23	0.86*
Onkaparinga	-0.40	0.87	-0.92	0.96*	-0.22	0.90*	-0.23	0.90*
Port Adelaide	-0.20	0.74*	-0.46	0.87*	-0.12	0.94*	-0.28	0.94*
Control (no sediment)	-0.78	0.96*	-0.87	0.89*				

Table 3 Decay rate constants (k; d^{-1}) for *S. derby* in water and sediment; *P <0.05.

	Water				Sediment			
	20 °C		30 °C		20 °C		30 °C	
	k	r^2	k	r^2	k	r^2	k	r^2
Henley Beach	-0.89	0.98*	-1.36	0.99*	-0.24	0.90*	-0.67	0.92*
Onkaparinga	-0.94	0.97*	-0.88	0.90*	-0.23	1.00*	-0.22	0.91*
Port Adelaide	-0.87	0.97*	-0.91	0.85	-0.11	0.88*	-0.27	0.93*
Control (no sediment)	-0.92	1.00*	-0.77	0.93*				

Although no direct correlations were observed between decay rates in overlying water of *Salmonella* spp. and the faecal indicator organisms investigated ($P > 0.05$; data not shown), decay of the pathogenic microorganisms more closely resembled that of *E. coli* than the other indicators. Burton *et al.*, (1987) concluded from a laboratory based microcosm experiment that decay of *S. newport* in freshwater sediment was as great or greater than *E. coli*. In general, decay rates for *S. typhimurium* and *S. derby* were greater than enterococci and coliphage (data not shown).

A preliminary quantitative microbial risk assessment for recreational coastal water was performed using the decay rates observed from the microcosm experiment and currently available dose response data for non-typhi *Salmonella* spp (McCoullough 1951; Fazil, 1996). Using the three initial faecal coliform concentrations selected to represent a range of recreational water qualities and previously reported ratios of *Salmonella*:FC in the water column, the corresponding concentration of *Salmonella* spp. was estimated and the number of organisms ingested on any given day following the initial peak was determined using Equation 1 (taking into account decay rates). A summary of the results of a Monte Carlo simulation of probability of infection due to exposure to *S. derby* in recreational water at 20°C is given in Table 4.

The preliminary model described here was able to illustrate the change in risk of infection over time following a faecal contamination event (assuming no further input) in recreational coastal waters. In waters of poor quality (high faecal coliform concentrations), the maximum probability of infection (at the 95% level) on the day of the event was above 2.0×10^{-1} and remained above 1×10^{-3} for three days following the initial high concentration. For the other two water quality scenarios, the maximum probability of infection was below 1×10^{-3} on day 0 (at the 95% level). The mean risk of infection by *S. derby* following exposure to coastal water that meets the guideline faecal coliform concentration was determined to be 6.23×10^{-6} (95^{th} percentile 1.71×10^{-5}) . It is acknowledged, however, the estimations determined by this model are exclusive to the risk of infection of *Salmonella* spp. and do not take into account exposure to other bacterial, protozoan or viral pathogens. Crabtree *et al.*, (1997) concluded that the risk of infection by adenovirus following recreation in freshwater which had not been accidentally contaminated ranged from 1.48×10^{-5} to 1.48×10^{-4}. As demonstrated in microcosm experiments, survival of *Salmonella* spp. was always greater in coastal sediment compared with overlying water. There may therefore be a greater risk of infection due to possible resuspension of this more highly contaminated sediment into the water column. To estimate this likely increase in exposure risk it

would be necessary to determine the rate of resuspension of pathogenic microorganisms.

Table 4 Results of Monte Carlo simulations for estimating risk of infection with *S. derby*.

Recreational Water Quality	Day	Probability of infection for individual exposure		
		5^{th} Percentile	Mean	95^{th} Percentile
Poor	0	4.66×10^{-4}	4.04×10^{-2}	2.01×10^{-1}
	1	5.69×10^{-5}	8.56×10^{-3}	3.70×10^{-2}
	2	7.08×10^{-6}	1.38×10^{-3}	4.93×10^{-3}
	3	8.75×10^{-7}	1.87×10^{-4}	6.26×10^{-4}
	4	1.08×10^{-7}	2.36×10^{-5}	7.79×10^{-5}
	5	1.33×10^{-8}	2.95×10^{-6}	9.82×10^{-6}
	6	1.66×10^{-9}	3.69×10^{-7}	1.23×10^{-6}
	7	2.01×10^{-0}	4.61×10^{-8}	1.52×10^{-7}
Average	0	5.52×10^{-7}	2.19×10^{-4}	3.72×10^{-4}
	1	6.80×10^{-8}	5.60×10^{-5}	4.56×10^{-5}
	2	8.50×10^{-9}	1.26×10^{-5}	5.67×10^{-6}
	3	1.05×10^{-9}	1.89×10^{-6}	6.84×10^{-7}
	4	1.31×10^{-10}	2.46×10^{-7}	8.53×10^{-8}
	5	1.61×10^{-11}	3.14×10^{-8}	1.06×10^{-8}
	6	1.99×10^{-12}	4.00×10^{-9}	1.33×10^{-9}
	7	2.45×10^{-13}	5.09×10^{-10}	1.64×10^{-10}
Good	0	1.76×10^{-7}	6.23×10^{-6}	1.71×10^{-5}
	1	2.18×10^{-8}	7.75×10^{-7}	2.11×10^{-6}
	2	2.69×10^{-9}	9.62×10^{-8}	2.65×10^{-7}
	3	3.32×10^{-10}	1.20×10^{-8}	3.29×10^{-8}
	4	4.13×10^{-11}	1.49×10^{-9}	4.08×10^{-9}
	5	5.06×10^{-12}	1.86×10^{-10}	5.11×10^{-10}
	6	6.16×10^{-13}	2.32×10^{-11}	6.32×10^{-11}
	7	7.53×10^{-14}	2.89×10^{-12}	7.84×10^{-12}

A limitation of the model developed here is lack of available data for a number of input parameters. More data is required on the occurrence of *Salmonella* spp. in recreational coastal water. The dose-response data used in this study provides the probability of infection only for healthy adults and does not take into account previous exposure or immunity. Issues also exist regarding the determination of *Salmonella* spp. infectivity after exposure to low-nutrient conditions such as coastal waters. Despite these limitations, QMRA is, nonetheless a useful tool for estimating the risk of infection under different scenarios and is extremely useful in the management of health risk for recreational coastal waters.

Conclusion

This research highlights the limited effectiveness of using prescribed faecal coliform concentrations in the water column alone to estimate the risk of exposure to pathogenic microorganisms during recreational activity at coastal areas. It demonstrates that coastal sediments act as a reservoir for both indicator and pathogenic organisms released into the coastal environment. This suggests an increased exposure risk if these organisms are resuspended back into the water column during recreational activity. A combined risk-based monitoring program would provide a more robust and reliable estimate of health risk associated with coastal recreational areas.

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