Non-point source pollution: Determination of replication versus persistence of *Escherichia coli* in surface water and sediments with correlation of levels to readily measurable environmental parameters

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ABSTRACT

Racine, Wisconsin, located on Lake Michigan, experiences frequent recreational water quality advisories in the absence of any identifiable point source of pollution. This research examines the environmental distribution of *Escherichia coli* in conjunction with the assessment of additional parameters (rainfall, turbidity, wave height, wind direction, wind speed and algal presence) in order to determine the most probable factors that influence *E. coli* levels in surface waters. Densities of *E. coli* were highest in core samples taken from foreshore sands, often exceeding an order of magnitude greater than those collected from submerged sands and water. Simple regression and multivariate analyses conducted on supplementary environmental data indicate that the previous day's *E. coli* concentration in conjunction with wave height is significantly predictive for present-time *E. coli* concentration. Genetic fingerprinting using repetitive element anchored PCR and cellular fatty acid analysis were employed to assess the presence of clonal isolates which indicate replication from a common parent cell. There were relatively few occurrences of clonal patterns in isolates collected from water, foreshore and submerged sands, suggesting that accumulation of *E. coli*, rather than environmental replication, was occurring in this system. Non-point source pollution, namely transport of accumulated *E. coli* from foreshore sands to surface waters via wave action, was found to be a major contributor to poor recreational water quality at the Lake Michigan beaches involved in this study.

Key words | *E. coli*, fatty acid analysis, genetic fingerprinting, non-point source pollution, predictive modelling, recreational water quality

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INTRODUCTION

Bacterial contamination of recreational waters can occur from a variety of point and non-point sources (Calderon *et al*. 1991; Bartram & Rees 2000; Solo-Gabriele *et al*. 2000). Even though untreated sewage (point source) is generally considered to provide a major influx of these bacteria, there are other origins of a non-point nature that can significantly impact beach water quality. Non-point sources may include agricultural runoff, specifically from livestock farming (Doran & Linn 1979; Jawson *et al*. 1982; Mallin *et al*. 1997), input from soils along river and stream banks (Hardina & Fujioka 1991; Fujioka *et al*. 1999; Solo-Gabriele *et al*. 2000), faecal droppings from wildlife (birds), especially in the intertidal zones (Oshiro & Fujioka 1995; Jones & Obiri-Danso 1999; Obiri-Danso & Jones 2000), foreshore sands (Whitman *et al*. 2001) and bathers themselves (Charles Gerba, personal communication). Owing to

the diversity of potential inputs of contamination to recreational waters, a multi-tiered approach may be necessary to determine the source of the bacteria (Boehm *et al*. 2003).

The City of Racine, Wisconsin, located on the southwestern shore of Lake Michigan, has two swimming beaches, North Beach and Zoo Beach. Frequent (on average 27% of an approximate 90 day swimming season) poor water quality advisories have occurred at Racine's beaches over the past 8 years, which has resulted in a negative public perception of the quality of Racine's recreational waters.

Previous studies by the Racine Health Department revealed that a storm sewer outfall exiting directly on to the beach was providing a significant bacterial burden to the adjacent surface waters. Faecal coliforms and *Escherichia coli* isolated from the discharge of this storm sewer often numbered in the tens of thousands during periods of rainfall (Racine Health Department). In autumn 2000, this outfall was successfully re-engineered to decrease the influx of bacteria to Lake Michigan. Although the flow from the outfall was minimal during the summer of 2001, poor water quality advisories were still issued on 17 and 21 days for North and Zoo beaches, respectively. These advisories reduced the number of days available for swimming during the recreational water season (20–25%).

There were no other identifiable point sources of pollution, such as combined sewer outfalls or sanitary sewer discharges. However, Racine has large populations of ring-billed (*Larus delawarensis*) and herring gulls (*Larus argentatus*) which frequent the bathing beaches. The numbers of these shore birds are often in the hundreds and may reach a thousand individual birds per beach. Storm runoff from contaminated sand and paved areas are a potential source of faecal pollution. Studies in Quebec found that attracting gulls to a beach area increased faecal coliform levels in water over 1,000-fold in shallow water of 0.3 m depth and 100-fold in deeper water at 0.7 m and 1.2 m depth (Levesque *et al*. 1993). Other researchers have demonstrated *E. coli* replication in subtropical environments (Carillo *et al*. 1985; Rivera *et al*. 1988; Jimenez *et al*. 1989; Hardina & Fujioka 1991; Roll & Fujioka 1997; Solo-Gabrielle *et al*. 2000; Lawrence 2001). However, it is unknown whether *E. coli* replication can occur in the temperate zone. It is possible that the indicator organism used for beach monitoring, *E. coli*, is able to establish a population in the interstitial sand environment, thereby leading to detection of higher levels than pollution sources alone would produce (Whitman & Nevers 2003, submitted for publication). In experiments conducted at 28°C, *E. coli* from river water replicated in soils when added to sterilized riverine sediments (Desmarais *et al*. 2002). (During the course of this study surface water temperatures ranged from 17 to 23°C.) In similar experiments, *E. coli* replication occurred in soils following wetting and drying cycles, although increases were greatest in the soils with a large fraction of fine particles and over 25% organic matter (Solo-Gabriele *et al*. 2000; Desmarais *et al*. 2002).

In this study, foreshore and submerged sands and surface water were sampled in parallel transects at North Beach daily throughout the summer of 2001. Auxiliary environmental data such as wave height, wind direction, ambient air and water temperatures, precipitation and turbidity were also collected. Repetitive element based polymerase chain reaction (rep-PCR) source (Rademacher & De Bruijn 1997; McLellan *et al*. 2003) and cellular fatty acid analysis were used to distinguish between identical strains (indicating replication from a common parent strain) and accumulation of bacterial cells deposited at the site, where the numbers of different strains would be expected to be similar to the numbers of strains found in the original pollution. The purposes of this study were: (1) to determine if there was evidence of *E. coli* replication in the environment as indicated by detection of clonal isolates in multiple samples; (2) to test for a statistically significant correlation between the numbers of *E. coli* isolated from foreshore sand, submerged sand and surface water; and (3) to assess auxiliary environmental parameters as potential mechanisms by which faecal bacteria from non-point sources of pollution are being loaded to Lake Michigan recreational waters.

METHODS

Study site

Racine, Wisconsin, is an urban community (population 82,600) located approximately 130 km north of Chicago,

Illinois, and 50 km south of Milwaukee, Wisconsin, on the shore of southwestern Lake Michigan. Regulatory water samples are routinely collected (Monday–Friday) at four equidistant sites along the 1 km designated swimming area of Racine's North Beach during the summer. In this study, a total of four sediment samples from each media (foreshore sand, submerged sand and surface water), equidistantly spaced along North Beach, were collected on Monday to Thursday (coinciding with the Monday–Thursday recreational water samples) throughout the 75-day 2001 beach sampling season, which was roughly from the last week of May to the first week of September.

Water sample collection

Fresh water samples were collected 5 days per week (Monday–Friday) from each of the four designated sites along North Beach in sterile screw-top bottles or sterile Whirl-Pak™ bags (Nasco, Ft Atkinson, Wisconsin). Samples were obtained in the afternoon by wading out to an approximate 1-metre depth (thigh deep) and taking a 200–300 ml sample from 0.3 m below the surface of the water. The distance from shore varies with the shoreline and presence or absence of sandbars. Samples were refrigerated and analysed within 6 h of collection.

Enumeration of *E. coli* in water samples by Colilert-18

The Racine Health Department Laboratory used IDEXX Colilert-18®, a selective cultural identification method utilizing bacterial enzymatic activity and differential substrates, for the detection of *E. coli* according to previously established laboratory protocols. Undiluted freshwater samples, or a 1:10 dilution made with sterile deionized water, were each mixed with reagent and placed in a Quantitray/2000 according to manufacturers instructions (Colilert-18® product insert, IDEXX Laboratories, Westbrook, Maine). Quantitrays were sealed and placed in a 35°C incubator for 18 h. A positive control (*E. coli* ATCC 25922) was used once daily to validate test performance. Following incubation, Quantitray wells were read for yellow colour indicating o-nitrophenol β-D-galactopyranoside (ONPG) hydrolysis and fluorescence, indicating 4-methyl-umbelliferone β-D-glucuronide (MUG) cleavage, with the aid of a ultraviolet (UV) light box (366 nm). The number of wells producing blue fluorescence was compared with the manufacturer-provided most probable numbers (MPN) table to elicit *E. coli* concentration as MPN 100 ml^{-1}.

Sand sample collection

All sand samples were collected with AMS soil recovery probes (Art's Manufacturing and Supply, American Falls, Idaho) with 2.8 cm bores and sterilized butyrate liners. Foreshore sands were collected 1 metre from the water's edge. Submerged sand samples were taken directly below the point where the regulatory surface water samples were collected. All samples were obtained between 12:00 and 2:00 p.m., refrigerated and analysed within 1 h of collection.

E. coli enumeration from sediment samples

At the laboratory, core samples were weighed and the contents of each butyrate liner emptied into a sterile vessel. Core size ranged from 6.5 to 11.8 cm. Sand samples were eluted for 30 seconds by vigorous hand shaking in phosphate buffered water ($pH = 7.2 \pm 0.2$) and then diluted after 1 min without disturbance. Concentrations of *E. coli* in all samples were determined by the membrane filtration technique using m-TEC agar (total *E. coli* count agar) (Difco, Becton Dickinson Microbiology Systems, Sparks, Maryland). *E. coli* colonies were confirmed by demonstration of negative urease activity following exposure of the membrane to 2% urea, pH 4.0 (USEPA 2000). Daily quality control was performed to ensure sterility at all steps. *E. coli* densities in the sand samples were expressed per gram dry weight of sample. Twenty-five representative sand samples were collected, weighed and incubated at 32°C until dry. Samples were then reweighed. The average percentage difference, accounting for the loss of weight due to moisture, allowed for the determination of a conversion factor of 0.796.

Statistical analysis

Statistical analysis using ANOVA independent group analysis and Newman-Keuls multiple comparisons

demonstrated that the density of *E. coli* recovered from the foreshore sand samples was significantly higher ($P<0.001$) than from either the surface water or submerged sand samples ($\alpha = 0.05$). The means of the submerged sand samples and surface water samples did not differ significantly from each other.

REP-PCR

Isolates collected from sand, sediment and water were analysed by repetitive element anchored PCR using primers that targeted REP (repetitive extragenic palindromic) sequences as described previously (Rademaker & de Bruijn 1997). Approximately 1 μl of washed cells provided the template for each 25 μl PCR reaction. Primers employed to generate amplified fragments included REP1R and REP2I primers (Versalvoic *et al*. 1991). Separation of amplified genomic fragments was accomplished via gel electrophoresis using 1% agarose gels made with 1X TAE and run at 70 V for 16 h at 4°C. Gels were stained with 0.6 μg ml^{-1} ethidium bromide in 1X TAE and visualized under UV light. Banding patterns were digitally captured using an EpiChemi II Darkroom bioimaging system (UVP, Inc., Uplands, California).

Digital images of gels were entered into the genomic fingerprint analysis program Bionumerics version 2.0 (Applied Maths, Kortrijk, Belgium) and scored for banding patterns using densiometric curve-based characterization. Strains were compared and grouped according to degree of similarity by calculating the Pearson coefficient of pairwise comparisons to generate similarity scores. Dendrograms were constructed using the UMPGA (unweighted pair-group method using arithmetic means) tree building method. The similarity score cut-off value for computer recognition of identical patterns was determined by repeated analysis of *E. coli* strain K12 ($n = 67$) and found to be 87%. Isolates used in this study that had near identical, but not completely identical, patterns were found to have similarity scores between 90 and 100% by computer analysis, therefore all fingerprint patterns with scores equal to or greater than 87% were manually inspected to determine if they were identical.

Cellular fatty acid analysis

Cellular fatty acid (CFA) composition was determined with a Model 5898A analyser (Microbial ID, Inc., Newark, Delaware, and Hewlett-Packard Co., Avondale, Pennsylvania). It consists of a gas chromatograph (Model 5890) equipped with a phenyl-methyl silicon capillary column (0.2 mm by 25 m), a flame ionization detector, an integrator, an automatic sampler and a computer. Analysis of *E. coli* isolates was performed according to the Microbial Identification system (MIDI) instructions (Sasser 1990). Briefly, a single colony was streaked onto trypticase soy broth agar (BBL Microbiology Systems, Inc., Cockeysville, Maryland) and harvested in log-phase growth after an incubation period of 24 h at 35°C. Approximately one 4 mm loopful of culture, harvested from each sample, was saponified using a sodium hydroxide-methanol mixture. The sample was then methylated with hydrochloric acid-methanol, extracted with hexane containing methyl *ter*t-butyl ether and cleaned with sodium hydroxide solution. Its organic layer was removed and transferred to a sample vial, which was loaded on the automatic sampler of the MIDI system. One positive (*Stenotrophomonas maltophilia*, ATCC 13637) and one negative (blank) control sample was run with each batch. Calibration standards were run with every ten samples in a batch.

With the MIDI system, fatty acid peaks are automatically integrated, identified and an index for each isolate is calculated using CFA content ratios. The principal-component analysis of quantitative CFA was performed using the Sherlock Library Generation Software (Microbial ID Inc.) and results were plotted graphically in two dimensions.

RESULTS

E. coli levels in sand and water

The numbers of poor water quality advisories issued over an 8-year period for North Beach (the study site) and neighbouring Zoo Beach are listed in Table 1. Densities of *E. coli* in surface water samples ranged from 1 to 5,475

Table 1 | Poor water advisories issued by the City of Racine Health Department 1994–2001

Year	Number of advisories/percentage of beach season[1]	
	North Beach	Zoo Beach
1994	5/6%	21/25%
1995	51/59%	42/48%
1996	5/5%	2/2%
1997	18/19%	30/32%
1998	16/16%	4/4%
1999	15/16%	19/20%
2000	62/66%	39/41%
2001	17/20%	21/25%
2002	27/31%	22/25%

[1]Average beach season was 90 days.

MPN 100 ml^{-1}, which was similar to the colony forming units per gram (CFU g^{-1}) recovered in the submerged sand samples (0–4,000 CFU g^{-1}). The densities of *E. coli* in foreshore sands ranged from 0 to 20,000 CFU g^{-1}, which was significantly higher than the levels of *E. coli* recovered from the other two media. The mean of all four sites for the foreshore sand samples was approximately an order of magnitude higher than that of either the surface water or submerged sand samples (Table 2). Although concentrations of *E. coli* would be expected to be lower in surface waters versus foreshore sands due to dilution effects, we believe that the concentration of bacteria recovered from foreshore sands is sufficiently high to be considered a significant non-point source of faecal pollution (Desmarais *et al*. 2002).

Correlation of *E. coli* levels with lake and weather conditions

The relationships between several environmental parameters and the daily bacteria counts in surface water samples were examined using SPSS® statistical software (version 11.0). Environmental parameters evaluated in this study included rainfall, wave height, wind direction and turbidity. There was no significant correlation between rainfall in inches and *E. coli* count on the same day. Nor was there a correlation using an additive effect of rainfall from the previous day plus rainfall to *E. coli* count. Turbidity, which could indicate resuspension of settled particulate matter, was not found to be predictive of *E. coli* levels. The best single predictor of *E. coli* value was wave height. The next best predictor was an east wind vector. We were able to derive a formula using a constant and a combination of today's wave height (in $\frac{1}{4}$ foot (0.08 m) increments) and wind vector (1 = any direction including east, 0 = any other direction) to successfully predict current day *E. coli* count ($P = 0.02$). This suggests that when

Table 2 | Mean densities (χ) of *E. coli* isolated from foreshore sand, surface water and submerged sands collected during summer 2001

	Foreshore sand (CFU g^{-1} dry wt) (χ)	Submerged sand (CFU g^{-1} dry wt) (χ)	Surface water (CFU g^{-1} dry wt) (χ)
North Beach—Site 1	1,467	190	69
North Beach—Site 2	1,223	166	110
North Beach—Site 3	1,928	248	231
North Beach—Site 4	1,544	344	144
Average, all sites (χ)	1,540	237	138

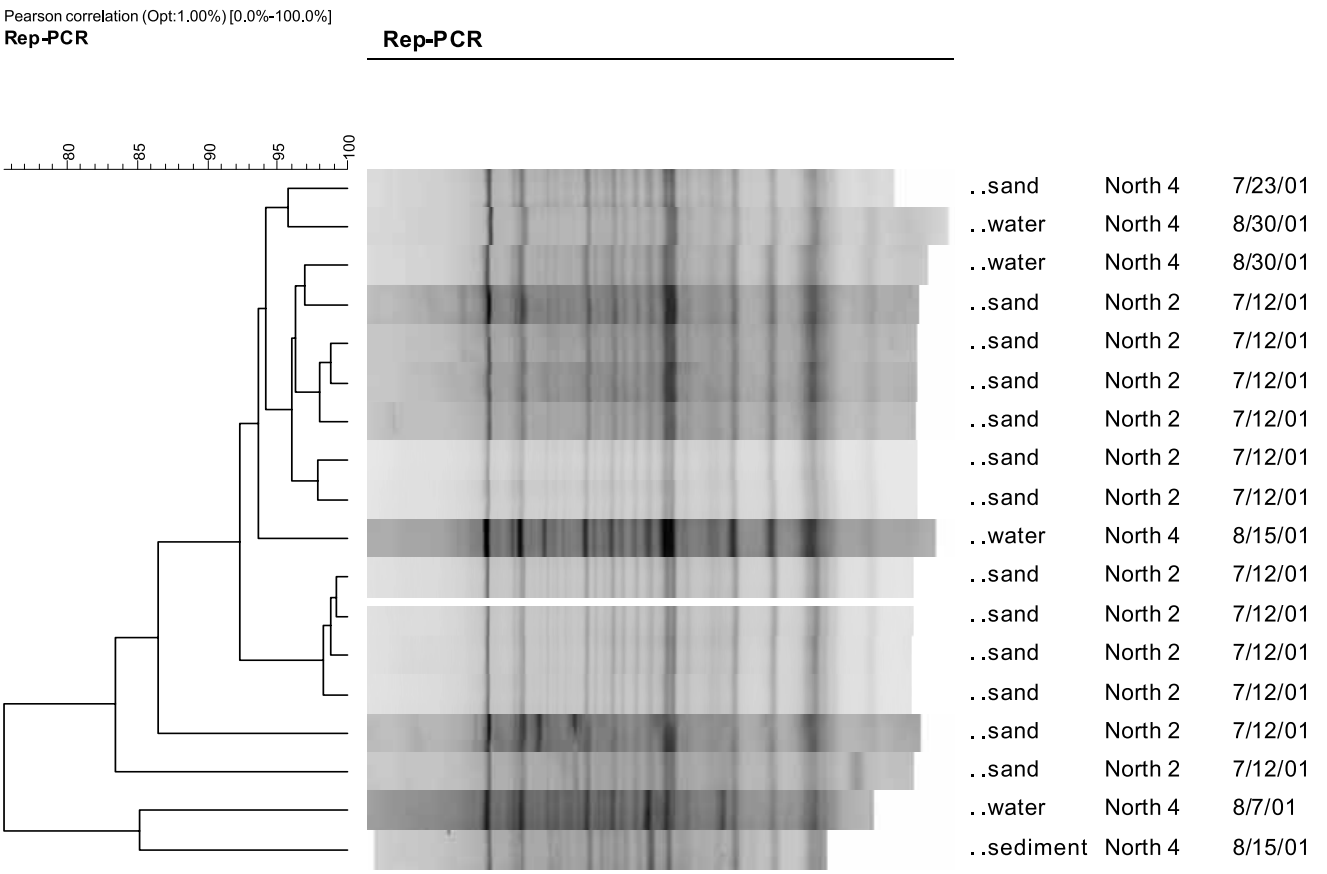

Figure 1 | Rep-PCR DNA fingerprints of isolates from North Beach in Racine. The majority of the identical isolates shown were collected from foreshore sand samples.

waves are high they have the ability to draw quantities of microorganisms into the water. This phenomenon is enhanced when coupled with winds originating from an easterly direction. While this predictive model does not give an advantage in time over actually reading the microbiological plates, it provides important insight into one mechanism by which non-point source pollution can be loaded from foreshore sands to recreational waters.

Assessment of *E. coli* replication by DNA fingerprinting

E. coli isolates ($n = 981$) collected from the foreshore sand, submerged sand and surface water were analysed using repetitive element anchored PCR. Over half of the isolates ($n = 558$) were found to have unique rep-PCR fingerprint patterns that did not match any other isolate analysed. An additional 88 isolates were found to match one other isolate in the dataset (44 pairs of isolates). The remainder of the isolates ($n = 423$) could be divided into 31 groups of identical rep-PCR patterns containing three or more isolates, which accounted for 207 of the 981 isolates, approximately 25% of the total isolates analysed. The majority of isolates within each identical pattern group were isolated from foreshore sand (Figure 1) (Table 3). Only three groups contained ten or more isolates (groups J, O, P in Table 3) and these groups accounted for 39 of the 981 isolates analysed, which is a small proportion of the *E. coli* isolates obtained in this study. In contrast, the majority of water samples taken on a single day were found to contain *E. coli* that demonstrated a wide range of DNA fingerprint patterns (example day

Table 3 | Groups of three or more isolates with identical rep-PCR fingerprint patterns

Group designation[1]	Total isolates (n)	Number of isolates in each media			Site number	Total number of days isolated	Media where most frequently isolated
		Foreshore sand	Submerged sand	Water			
A	3	3	0	0	4	1	Foreshore sand
B	3	3	0	0	1, 4	1	Foreshore sand
C	4	4	0	0	4	1	Foreshore sand
D	4	4	0	0	1	1	Foreshore sand
E	4	4	0	0	4	2	Foreshore sand
F	4	4	0	0	4	3	Foreshore sand
G	5	4	0	1	4	5	Foreshore sand
H	5	4	0	1	3, 4	4	Foreshore sand
I	5	4	0	1	3, 4	4	Foreshore sand
J	10	10	0	0	4	1	Foreshore sand
K	8	6	2	0	3, 4	4	Foreshore sand
L	4	3	1	1	4	1	Foreshore sand
M	4	2	1	1	2, 3, 4	2	Foreshore sand
N	9	5	2	2	3, 4	7	Foreshore sand
O	11	6	3	2	3, 4	8	Foreshore sand
P	18	13	1	4	2, 4	5	Foreshore sand
Q	3	0	3	0	4	2	Submerged sand
R	4	0	4	0	4	1	Submerged sand
S	4	0	4	0	3	1	Submerged sand
T	4	0	4	0	3	1	Submerged sand
U	4	0	4	0	4	2	Submerged sand
V	4	0	4	0	3, 4	2	Submerged sand
W	4	0	4	0	4	3	Submerged sand
X	5	0	5	0	4	3	Submerged sand
Y	5	2	3	0	1, 2, 3	3	Submerged sand
Z	3	0	0	3	4	3	Water
AA	5	0	0	5	4	1	Water
BB	5	0	0	5	4	1	Water
CC	9	2	3	4	4	4	Water
DD	8	4	0	4	4	3	Foreshore sand/water
EE	3	1	1	1	3, 4	3	All media

[1]An additional 44 groups were found which contained two identical isolates; most of the groups had one or both of the isolates obtained from foreshore sand (n=40).

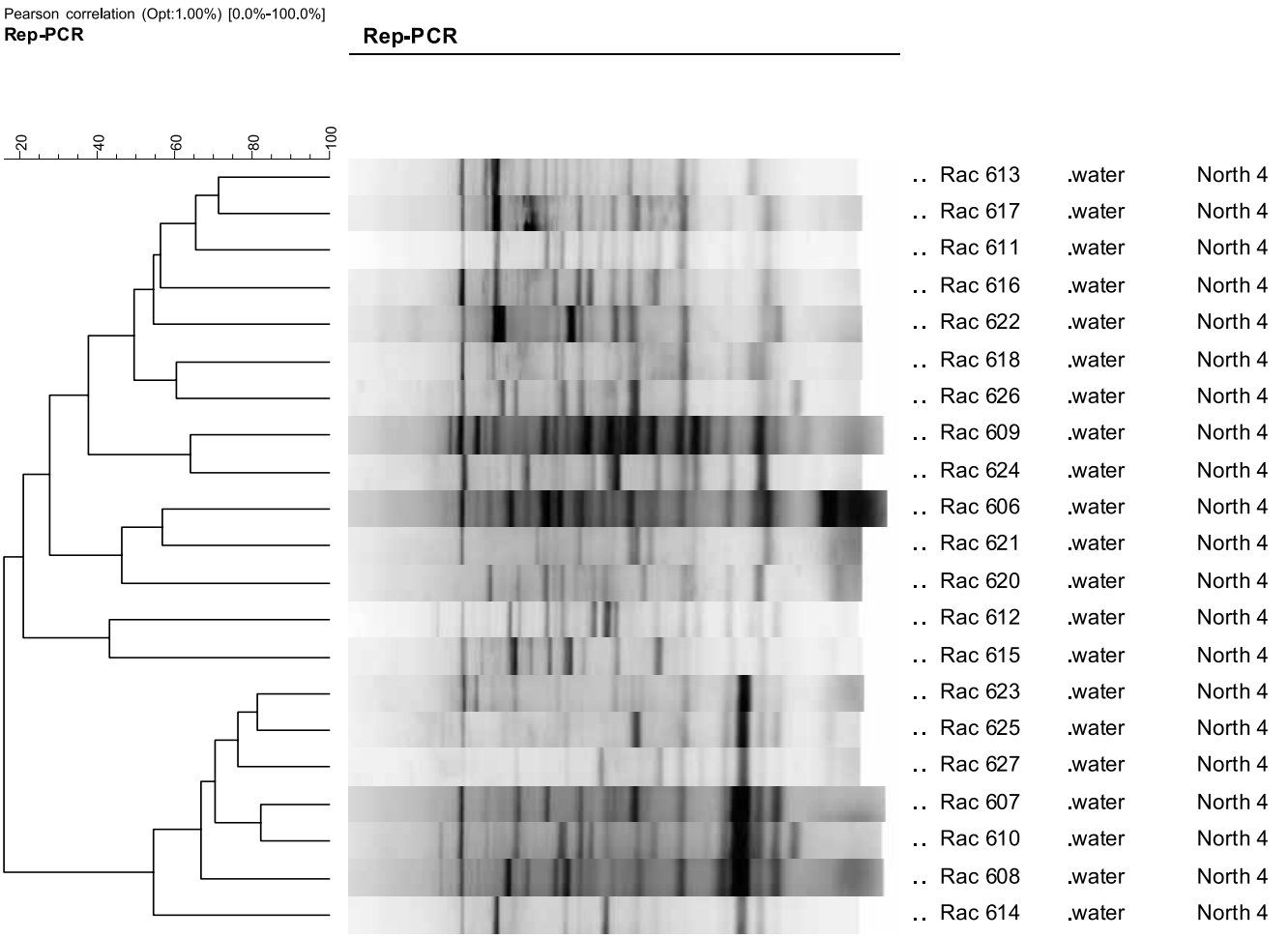

Figure 2 | Rep-PCR DNA fingerprint patterns of *E. coli* isolates obtained from water on a single day.

shown in Figure 2). Of the identical isolate groups with three or more isolates, there were only four groups where the strains were primarily isolated from water, which suggests that seeding of the water from a single strain that has adapted to replicate in the environment is not a major mechanism for *E. coli* loading into beach water.

Cellular fatty acid analysis and comparison with rep-PCR DNA fingerprinting

A total of 883 *E. coli* isolates were subjected to cellular fatty acid methyl ester (FAME) analysis. The cultures from foreshore sand ($n = 468$ samples), submerged sand ($n = 198$ samples) and surface water ($n = 217$) were collected on different dates from four designated sites at Racine's North Beach. Multiple isolates from each site were analysed and compared for their relationship to each other to determine groups and subgroups of *E. coli* strains. It was anticipated that such an analysis would allow us to determine a relationship between the contribution of *E. coli* present in foreshore and submerged sands to elevated *E. coli* levels present in adjacent surface waters. If these shoreline sources were significantly contributing to higher counts in the surface water, three or more clusters of subgroups consisting of strains from each source would emerge on a two-dimensional plot (2-D). The principle-component analysis could further establish

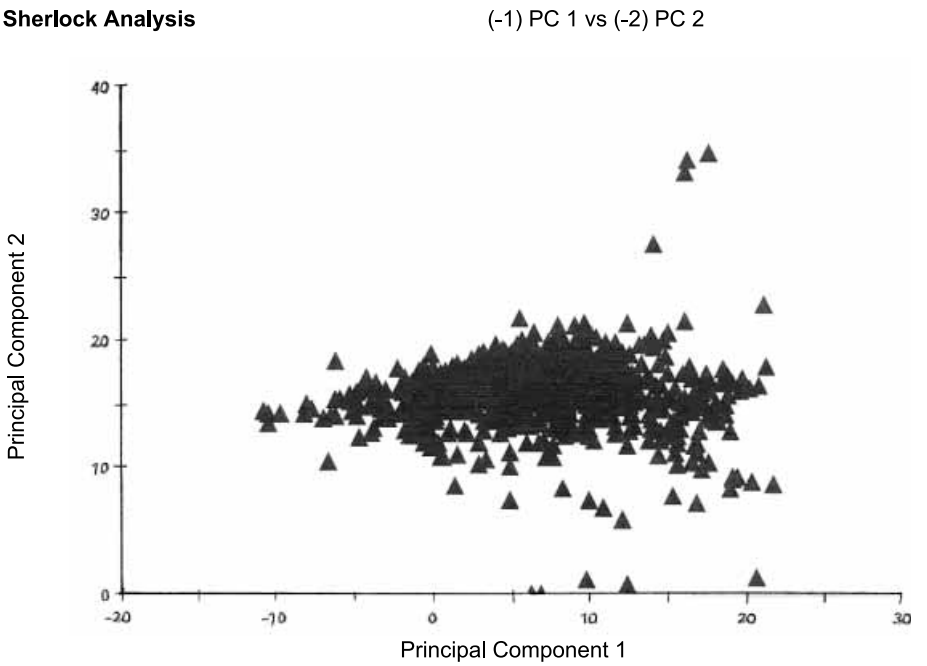

Figure 3 | Two-dimensional plot of *E. coli* isolates generated by principal-component analysis of cellular fatty acids methyl ester profiles.

their relationship to each other. We found that more than 98% of the *E. coli* isolates formed a single cluster on a 2-D plot. A critical distance to distinguish between related and unrelated subgroups from different sources could not be empirically established since the Euclidean distance, a critical parameter for analysis, of undefined clusters could not be measured (Figure 3).

The FAME data of all isolates were exported to Microsoft® Excel using a beta version of the MIDI software. The FAME profiles of various isolates demonstrated that *E. coli* have seven characteristic cellular fatty acids, with some variability in the amounts of each of the seven cellular fatty acids among the isolates (Table 4); an additional 35 fatty acids were found to appear sporadically in less than 10% of the sampled isolates. FAME profiles were distributed among foreshore sand, submerged sand and water isolates in no apparent pattern. The profiles contained fewer components for comparison than rep-PCR (a range of 7–9 fatty acids per FAME analysis compared with 15–19 different sized PCR amplification products) and, therefore, gave coarser differentiation among isolates than rep-PCR.

In comparing the relationship among strains using FAME analysis versus rep-PCR, there was no correlation between similarity scores of pairwise comparisons for rep-PCR and cellular fatty acid analysis. However, within groups of isolates that were identified as having identical

Table 4 | Most common cellular fatty acids identified from *E. coli* isolates

Cellular fatty acid	Percentage of total cellular fatty acid composition
12:0	1–8
14:0	1–12
15:0	0.3–5
16:0	22–35
17:0 cyclo	0.7–19
18:1 w7c	4–41
19:0 cyclo w8c	0.2–10

rep-PCR patterns, fatty acid profiles were >90%, with the exception of a few isolates. In some cases, isolates with near identical rep-PCR patterns had very different cellular fatty acid profiles.

DISCUSSION

Racine's North Beach has several characteristics that make it a good choice as a study site to investigate *E. coli* replication in the temperate zone, including a high number of advisories in the last several years and several dry weather advisories with no recognized point sources of pollution such as sewage discharges. In addition, preliminary *E. coli* enumeration demonstrated that there was a high *E. coli* burden (up to 32 CFU g^{-1} sand) in foreshore sands.

Detection of identical strains of *E. coli* from foreshore sand, submerged sand and surface water would indicate that these isolates were derived from a common parent strain. Since *E. coli* strains among individuals within a host population, such as gulls, contain a wide range of diversity in rep-PCR patterns (McLellan *et al.* 2003) it is expected that multiple animals contributing to contamination would result in the detection of a diverse group of DNA fingerprints. However, if specific strains that could survive and replicate (e.g. resident strains) were responsible for the high sand concentrations, it would be expected that a limited number of DNA fingerprint patterns would be found and that the majority of isolates would display identical fingerprints.

In this study, no predominant group of isolates with identical patterns was observed but, rather, several small groups consisting of 2–20 identical isolates were seen, with only three groups containing >10 isolates. The identical isolates were found primarily in foreshore sand; many of these were isolated the same day from the same sample and therefore may be from a single animal. Another study using rep-PCR has shown that a single animal generally carries a predominant strain of *E. coli* (McLellan *et al.* 2003). We found a much higher incidence of identical strains than is expected from a random sampling of the gull population; again, this may be explained by sampling the same animal more than once, or obtaining multiple isolates from the same faecal material, thereby overrepresenting that strain frequency.

The occurrence of identical isolates (two or more) was far greater than that observed in sampling the gull population (one isolate per dropping). These isolates were most likely from a single animal, rather than representative of replication given that the isolation was from the same sample on the same day. Further, we did not observe a high number of isolates with identical DNA fingerprints isolated from more than one media; when identical patterns were found, it was usually in the same media, particularly foreshore sand. This further supports the conclusion that the identical isolates were a result of directly sampling faecal material, rather than from an environmentally adapted *E. coli* population widely distributed in this system.

Cellular fatty acid analyses were supplemented by the genetic information obtained by rep-PCR fingerprinting. It would be expected that clonal populations would have identical fatty acid profiles since they are derived from the same parent strain. This study found that, in some cases, isolates with near identical rep-PCR patterns had very different fatty acid profiles, which: (1) further demonstrated that there was no correlation between cellular fatty acid similarity and rep-PCR similarity assessments; and (2) served as a useful tool to distinguish identical groups of isolates. It is possible that minor qualitative or quantitative differences in fatty acid patterns may not be evident by DNA fingerprinting techniques thereby explaining the occurrence of differences in clonal isolates.

Replication cannot be ruled out, although it does not appear to be a major contributing factor in overall *E. coli* levels in water. However, replication may have occurred on a limited basis for several different strains rather than one specific type of strain. This, in effect, would result in magnifying the *E. coli* burden in the sand.

The ability to reliably determine mechanisms by which faecal indicator organisms are introduced to surface waters has great implications for beach management and public health. The presence of high densities of microorganisms in foreshore sands is a significant contributor of non-point source pollution. Empirically, this would

make sense since there was no significant difference in the concentration of *E. coli* recovered from the submerged sands compared with the surface water. The ability to determine whether or not these bacteria are replicating or persisting in conjunction with studies leading to host source identification can be used effectively in designing beach management programmes. Previous studies by this group of researchers and others have shown that alterations in beach grooming techniques (i.e. increasing grooming depth and omitting finishing) can impact the densities of microorganisms in foreshore sands (Kinzelman *et al*. 2003). The ability to reduce these numbers will result in a lower bacterial burden to the adjacent waters as indicated by the statistically significant relationship between *E. coli* counts and wave height.

Predictive modelling, the ability to predict elevated levels of faecal indicators in recreational waters prior to the acquisition of results from microbiological testing, is something that has been extensively investigated with varying results. Rainfall has previously been used in Milwaukee, Wisconsin, as a predictor of bacteriological water quality with limited success. The development of a successful predictive model would enhance beach management programmes since current microbiological tests take anywhere from 18 to 24 h to produce results. Predictive models appear to be very specific to the geographic area for which they were designed. The City of Milwaukee, Wisconsin, approximately 30 miles to the north of Racine had predictive models developed for two of their bathing beaches. The models were slightly different for each of Milwaukee's beaches even though they are within 10 miles of each other. Predictability, although very site specific, can be used in conjunction with traditional microbiological testing to indicate those times which are more likely to result in the issuance of poor water quality advisories.

CONCLUSION

Until the advent of real-time testing for faecal indicators of recreational water quality, the combination of predictive modelling and DNA fingerprinting will aid in the protection of the public's health by preventing unnecessary exposure to recreational waters potentially contaminated with human pathogens. Non-point source pollution of bathing beaches and recreational waters, although harder to determine than point source pollution, is a very important factor in recreational water quality that can be effectively researched through a combination of traditional microbiological, molecular and observational techniques. This research contributes to a better understanding of non-point sources of pollution and their relationship to readily measurable environmental parameters. Importantly, this study indicates that persistence, rather than environmental replication of *E. coli*, is responsible for the majority of microorganisms recovered from foreshore sands, submerged sands and surface waters at Racine, Wisconsin, beaches along Lake Michigan.

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