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Presence of campylobacter and salmonella in sand from bathing beaches

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SUMMARY

The purpose of this study was to determine the presence of thermophilic *Campylobacter* spp. and *Salmonella* spp. in sand from non-EEC standard and EEC standard designated beaches in different locations in the UK and to assess if potentially pathogenic strains were present. *Campylobacter* spp. were detected in 82/182 (45%) of sand samples and *Salmonella* spp. in 10/182 (6%). *Campylobacter* spp. were isolated from 46/92 (50%) of samples from non-EEC standard beaches and 36/90 (40%) from EEC standard beaches. The prevalence of *Campylobacter* spp. was greater in wet sand from both types of beaches but, surprisingly, more than 30% of samples from dry sand also contained these organisms. The major pathogenic species *C. jejuni* and *C. coli* were more prevalent in sand from non-EEC standard beaches. In contrast, *C. lari* and urease positive thermophilic campylobacters, which are associated with seagulls and other migratory birds, were more prevalent in sand from EEC standard beaches. Campylobacter isolates were further characterized by biotyping and serotyping, which confirmed that strains known to be of types associated with human infections were frequently found in sand on bathing beaches.

INTRODUCTION

Bathing water in Europe has to meet the requirements of the EEC Bathing Water Directive (76/160/EEC) [1] which specifies the microbiological parameters to be tested and the standards to be met. Currently these are based on enumeration of faecal coliforms and do not include standards for pathogens. Potentially pathogenic *Campylobacter* spp. have been isolated from sewage contaminated water [2], contaminated soil and aquatic sediments [3]. Thermophilic campylobacters have been detected in shellfish tested from harvesting areas both in the UK [4] an in the USA [5] and from coastal bathing waters at concentrations of 10–230/100 ml of sea water [6]. It has been suggested that campylobacter infections in male adults during the summer months may be linked to recreational activities including participation in water sports [7]. Exposure to contaminated water may be a risk factor for infection.

Most previous studies have concentrated on assessing the microbiological quality of sea water using traditional faecal indicators. A study of four UK coastal resorts showed that the rates of gastro-enteritic symptoms were significantly increased in a group of swimmers who bathed in water containing faecal streptococci in concentrations greater than 32 per 100 ml [8]. In another study in Hong Kong gastrointestinal symptoms were found to be directly related to the pollution level and in particular there was a direct correlation with the numbers of

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Clostridium perfringens, *Aeromonas* spp. and *Vibrio cholera* (non -01) in beach water [9]. Sewage contamination of sand and seawater has also been linked to the isolation of *Shigella* spp. in the Bay of Gdansk [10].

There have been few investigations of the microbiological quality of sand on bathing beaches or the potential risk from sand contaminated with gastrointestinal pathogens. Workers in Spain examined water and sand from a polluted beach and from a beach meeting the EEC standard for faecal indicators, but they did not look for enteropathogenic bacteria [11]. In an Israeli study *C. jejuni* was isolated from sand and the authors suggested that this may be a risk factor for enteritis in the population bathing at these beaches [12]. An initial longitudinal survey between 1991 and 1993 showed that *Campylobacter* spp. and *Salmonella* spp. were often present in sand from a beach in the North West of England that did not meet the EEC Bathing Water Directive standard (Bolton and colleagues, unpublished observations). The present study was carried out to verify these findings and (*a*) to determine if the microbiological quality of sea water from non-EEC standard and EEC standard beaches influenced contamination of sand with enteropathogens; (*b*) to establish if non-EEC standard and EEC standard beaches in different parts of England were subject to the same levels of contamination; (*c*) to assess the prevalence of different thermophilic *Campylobacter* spp. and *Salmonella* spp.; (*d*) to determine if potentially pathogenic strains of *C. jejuni* and *Salmonella* spp. were present in sand on these beaches.

METHODS

Beach descriptions and sampling

Beach surveys took place from 4 October 1994 to 26 January 1995. To obtain representative samples and to minimize the effects of local conditions, beaches from two geographically distant areas were selected. Two beaches were selected from the North West and two from the South West of England. In each area one of the beaches complied with the standard of the EEC Directive [1] and the other did not. Both of the non-EEC standard beaches in this study were exposed to possible faecal pollution from nearby (within 1–3 miles) piped sewage outfalls and from agricultural/rural contaminated estuarine waters. Two locations approximately 1 mile apart were selected at each beach. At each of these locations, two sampling sites were selected along a line at right angles to the water's edge. The first site was close to, but below the high water mark (designated as dry sand) and the second, a site 1–2 m from the water's edge (designated as wet sand). Samples were collected weekly from the four beaches at low tide from the top 10 cm of sand, when tide times and weather conditions permitted. Sand samples of about 1000 g were collected with sterile scoops, placed in sterile containers and transported in cool boxes with ice-packs to the local Public Health Laboratory. Samples were examined on the day of collection for *Campylobacter* spp. and *Salmonella* spp. The same protocol was followed in each of the two laboratories.

The effect of the water content of the dry and wet sand on the isolation of campylobacters was assessed on 100 samples from North-West beaches. Sand from South West beaches was not tested in this way. The water content of sand was estimated by drying a 100 g of sample in a wide mouthed jar in a hot air oven. The dry weight was recorded as the weight determined when three consecutive daily readings were unchanged.

Detection of *Campylobacter* spp.

Four 25 g subsamples were each added to 225 ml of campylobacter enrichment broth (Oxoid), containing FBP supplement (Oxoid), Preston antibiotic supplement (Oxoid), cefoperazone 15 μg ml^{-1} (Sigma) and lysed horse blood (50 ml/l) in a sterile container. The tops were closed tightly, the samples mixed well and then placed in an aerobic incubator at 37 °C overnight followed by 42 °C for a further 24 h. Each broth was then subcultured onto modified cefoperazone, charcoal, deoxycholate agar (CCDA, Oxoid), containing amphotericin 10 mg/l; plates were incubated microaerobically at 37 °C for 48 h. Presumptive *Campylobacter* spp. were identified by positive oxidase and motility tests and characteristic morphology in Gram stained smears.

Detection of *Salmonella* spp.

Four 25 g subsamples were each added to 225 ml of buffered peptone water (BPW) (Oxoid), and incubated at 37 °C for 20–24 h. From each BPW culture, 0·1 ml was transferred to 10 ml Rappaport Vassiliadis Soya Peptone (RVS) broth (Oxoid), and incubated at 42 °C

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Table 1. *Detection of* Campylobacter *species and* Salmonella *species from sand samples collected from four different UK beaches*

Location*	Non-EEC/ EEC beaches	Dry/wet sand	Total number of samples†	Total number positive	
				Campylobacter (%)	Salmonella (%)
NW	Non-EEC	Dry	26	14 (54)	5 (19)
NW	Non-EEC	Wet	26	20 (77)	3 (12)
NW	EEC	Dry	24	12 (50)	0 (0)
NW	EEC	Wet	24	17 (71)	1 (4)
SW	Non-EEC	Dry	20	4 (20)	0 (0)
SW	Non-EEC	Wet	20	8 (40)	0 (0)
SW	EEC	Dry	21	2 (10)	0 (0)
SW	EEC	Wet	21	5 (24)	1 (5)
Total			182	82 (45)	10 (6)

* North West beaches (NW), South West beaches (SW).
† Sample size tested was 4×25 g.

Table 2. *Number of 25 g sub-samples from each 100 g sample positive for* Campylobacter *species*

Classification of sand samples	Number of positive samples	Number of 25 g sub-samples positive			
		1	2	3	4
Non-EC/dry	18	10	3	4	1
Non-EEC/wet	28	8	6	7	7
EEC/dry	14	8	3	0	3
EEC/wet	22	10	6	5	1

for 24 h. Each RVS broth was then subcultured onto modified brilliant green agar (Oxoid), and xylose lysine desoxycholate agar (Oxoid), and incubated for 24 h at 37 °C. Presumptive salmonella colonies were identified using standard serotyping and biochemical methods. Isolates of *Salmonella* spp. were sent for confirmation and phage typing to the Laboratory of Enteric Pathogens, Central Public Health Laboratory, Colindale.

Identification and biotyping of *Campylobacter* spp.

Identification and biotyping of all isolates was carried out using the methods described by Bolton and colleagues [13]. The tests included hippurate hydrolysis and DNA hydrolysis as used in the Lior biotyping scheme [14], γ-glutamyl transferase and nine resistotyping tests [13]. For each strain, the test results were grouped into four sets of three and results coded to generate a four digit biotype code. Since the results of the γ-glutamyl transferase tests correlated with the results of the H_2S test as described by Lior [14] for *C. jejuni* it was also possible to derive the Lior biotype with this combined approach.

Serotyping of *Campylobacter jejuni*

Heat stable antigen (HSA) serotyping was carried out with a panel of 43 antisera using the method of Penner and Hennessy [15].

RESULTS

Detection of *Campylobacter* spp. and *Salmonella* spp.

Campylobacter spp. were isolated from 82/182 (45%) and *Salmonella* spp. from 10/182 (6%) sand samples (Table 1). *Campylobacter* spp. were isolated from 46/92 (50%) of samples from non-EEC standard beaches and from 36/90 (40%) of samples from EEC standard beaches. *Salmonella* spp. were isolated from 8/92 (9%) of samples from non-EEC standard beaches and 2/90 (2%) of samples from EEC standard beaches. The differences in isolations of campylobacters from non-EEC and EEC standard beaches was not statistically significant ($\chi^2 = 1{\cdot}5$; $P = 0{\cdot}23$).

The highest prevalence of *Campylobacter* spp. was in wet sand collected from non-EEC standard beaches where 28/46 (61%) samples were positive. The lowest prevalence was found in samples of dry sand from EEC standard beaches (14/45 (31%)). The difference

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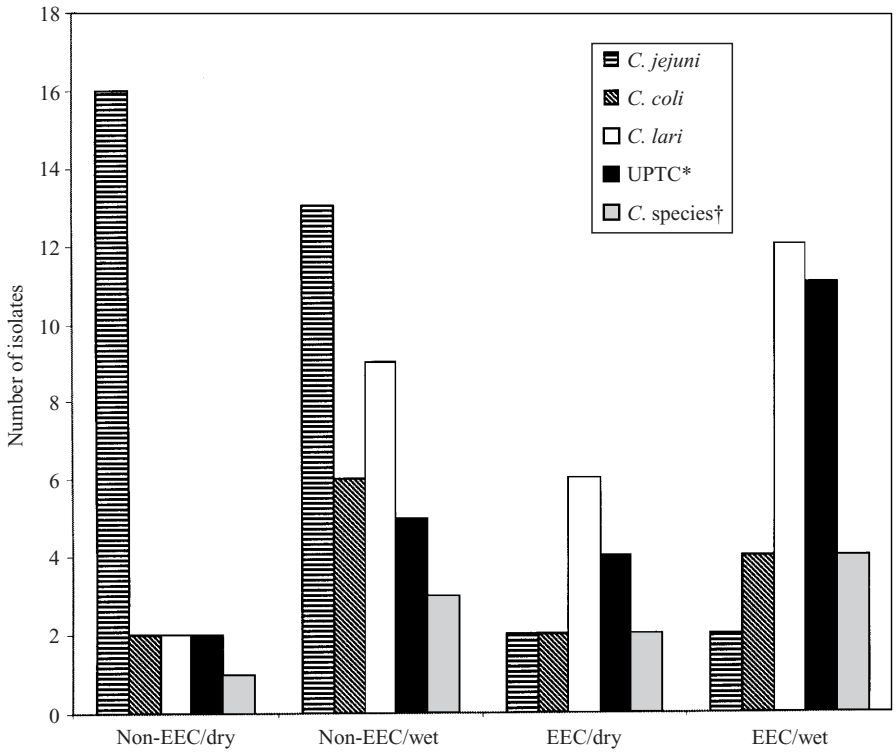

Fig. 1. Prevalence of different *Campylobacter* spp. from campylobacter positive samples of sand. * Urease positive thermophilic campylobacters are a distinct biovar of *C. lari*. † Strains which could not be identified to species level by the techniques used.

between isolation rates of campylobacters from dry and wet sand samples was significant ($P < 0{\cdot}001$). The greatest number of salmonella positive samples was 5/46 (11%) in dry sand from non-EEC standard beaches and no salmonellae were detected in 45 samples of dry sand from EEC standard beaches. The numbers of salmonella isolations were insufficient to allow meaningful statistical analysis.

There was a higher prevalence of *Campylobacter* spp. and *Salmonella* spp. in the samples collected from the North West of England than those from the South West of England. Of the samples collected from beaches in the North West of England 63/100 (63%) were positive for *Campylobacter* spp. and 9/100 (9%) for *Salmonella* spp. whereas, of the South West samples 19/82 (23%) were positive for *Campylobacter* spp. and 1/82 (< 1%) for *Salmonella* spp. (Table 1). The difference in isolations of *Campylobacter* spp. was significant ($P < 0{\cdot}001$).

The number of cultures positive from the four 25 g subsamples was used as a semi-quantitative indication of the level of contamination with *Campylobacter* spp. (Table 2). Using this distinction wet sand samples from non-EEC standard beaches were more heavily contaminated than sand from other sites.

The mean moisture content of 26 dry sand samples tested from the non-EEC standard beach was 16·3% (S.D. 2·85) and from 24 dry sand samples taken from the EEC standard beach 4·1% (S.D. 2·07). Although there was a significant difference in the water content of dry sand from these two beaches the incidence of *Campylobacter* spp. (range 50–54%) was similar. The mean moisture content of 26 wet sand samples tested from the non-EEC standard beach was 19·2% (S.D. 1·44) and from 24 dry sand samples from the EEC standard beach 20·8% (S.D. 1·5) and the *Campylobacter* spp. isolation rates of 77% and 71% respectively were also similar.

Distribution of thermophilic *Campylobacter* spp. and *Salmonella* spp. in sand

The major human pathogenic campylobacters are *C. jejuni* and *C. coli* and these were more frequently isolated from sand collected from non-EEC standard beaches than those from EEC standard beaches (Fig. 1). All of the 18 positive samples of dry sand from non-EEC standard beaches and 19/28 (69%) positive samples of wet sand from non-EEC standard

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Table 3. *Biotypes of* C. jejuni *isolated from 30 of the 33 positive samples of sand and serotypes of* C. jejuni *isolated from 28 of the 33 positive sample of sand*

Lior biotype	Preston biotype	Number of positive samples		Penner serotypes	Number of positive samples	
		Non-EEC	EEC		Non-EEC	EEC
I	6000	13	1	1	1	0
I	6010	5	0	2	3	0
I	6020	1	0	4, 13, 50+	5	0
I	6030	1	1	5	5	0
I	6060	1	0	8, 17	2	0
I	6100	3	0	10	0	1
I	6110	2	2	15	1	0
I	6150	1	0	23	1	0
I	6510	1	0	24	2	0
II	6004	2	0	38	1	0
II	6014	2	0	39	1	1
II	6024	1	1	44	0	1
II	6104	1	0	53	1	0
II	6114	0	1	55	3	1
III	6002	3	0	58	1	0
III	6012	2	0	Non typable	14	1
III	6142	3	0			
III	6152	2	0			
IV	6156	1	0			

Lior biotypes [14], Preston biotypes [13], Penner serotypes [15].

beaches contained *C. jejuni* or *C. coli*. The proportion of *C. jejuni or C. coli* positive samples was lower (28%) in dry and wet sand samples from EEC standard beaches. In contrast, *C. lari* and urease positive thermophilic campylobacters (UPTC) strains were isolated from all 22 positive samples of wet sand from EEC standard beaches and from 10/14 (71%) dry sand samples from EEC standard beaches but considerably less frequently from sand samples from non-EEC standard beaches (Fig. 1). The number of positive isolates for each classification of sand is greater than the totals in Table 1 because multiple *Campylobacter* spp. were detected in 21 of the positive samples.

The results of subtyping *C. jejuni* isolates using biotyping, (Lior and Preston schemes) and heat stable antigen (HSA) serotyping (Penner scheme) are shown in Table 3. The total number of subtypes is greater than the total number of samples positive because some samples contained multiple types; 47% (14/30) of sand samples that were positive for *C. jejuni* contained two or more Preston biotypes and 27% (8/30), two or more Lior biotypes. Likewise, multiple serotypes were isolated from 10 samples of sand from non-EEC standard beaches and from one sample of sand from EEC standard beaches.

Table 4. *Salmonellae isolated from sand samples from UK beaches*

Classification of sand samples	Salmonella serotypes
Non-EEC/dry	*S. kedougou*, *S. oranienberg*, *S. enteritidis*, *S. typhimurium**
Non-EEC/wet	*S. virchow*, *S. oranienberg*, *S. heidelberg*
EEC/dry	No isolates
EEC/wet	*S. panama*, *S.* species (Group B), *S. enteritidis*

* Two different phage types.

Six different serotypes of *Salmonella* were isolated from non-EEC standard beaches and three from EEC standard beaches (Table 4). The two isolates of *S. enteritidis* were phage type (PT) 5 and PT 8, the two isolates of *S. typhimurium* were PT 99 and PT 154 and the isolate of *S. virchow* was PT 8.

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DISCUSSION

The highest incidence of human campylobacter infections in the UK occurs during the bathing season May–September and hence more epidemiological studies are necessary to establish the risk of acquiring infections from this source.

These investigations have confirmed our previous unpublished findings that common gastroenteritis pathogens frequently contaminate and persist in sand on British beaches. The 45% positivity rate for *Campylobacter* spp. is identical to that reported from Israel where these organisms were detected in 52/115 samples of sand from bathing beaches [12].

Factors which may have a major influence on the ecological distribution of microorganisms in sand samples from beaches include the geographical location of the beach, the microbiological quality of the sea water, the moisture content of sand and seasonality. In this study we assessed the first three of these factors, but we were unable to provide data on summer and winter prevalence. We found that differences in the water content of sand did not have an effect on the isolation of campylobacters but there was a difference in the contamination of sand on beaches in different geographical locations. Sand samples from beaches in the North West of England were three times more likely to be contaminated with *Campylobacter* spp. and ten times more likely to be contaminated with *Salmonella* spp. than samples from beaches in the South West of England.

The effect that the differing microbiological quality of the sea water may have on the presence of potential pathogens in sand was assessed by sampling from both non-EEC and EEC designated beaches. Although the prevalence of *Campylobacter* spp. was only slightly higher in sand samples from non-EEC standard beaches (50%) than in sand samples from EEC standard beaches (40%), the prevalence of *C. jejuni* and *C. coli* was almost four times higher in the former (40 *vs.* 11%). Moreover, the subtyping of these *C. jejuni* and *C. coli* isolates showed that many were of types frequently isolated from patients with campylobacter diarrhoea, namely Lior biotype codes: 1 and 2 [14], Preston biotype codes: 6000, 6002, 6004, 6010 and 6100 [16], and HSA serotypes: 1, 2 and 4 complex [17]. Hence, despite the diversity of strains present in sand there was evidence that potentially pathogenic strains of *C. jejuni*, and in some instances *Salmonella* spp. frequently contaminate sand on beaches.

One surprising observation was that 50% of dry sand samples from EEC beaches, with a mean water content of only 4·11% were contaminated with campylobacters. This indicated the potential for *Campylobacter* spp. to persist in sand. Other workers have demonstrated that *Campylobacter* spp. do not survive well on dry surfaces in air [18] and are sensitive to 2% NaCl [19]. This apparent contradiction may be related to the status of the organisms in this particular environmental milieu and the surface characteristics of sand particles.

Our results indicate that sand on bathing beaches may be acting as a natural filter during tidal processes leading to contamination and concentration of pathogens on areas of the beach used by the public. This may be an important mechanism for re-seeding of sea water with enteric pathogens which has not been recognised before. Previous epidemiological studies of the risks of exposure to bathing and immersion in sea water have not considered sand as a source of pathogens.

We have shown that sand may act as a reservoir of pathogenic organisms and hence that there is a need for appropriate standards to assess the microbiological quality of sand on bathing beaches. The results of this study and of the previous pilot study suggest that assessment of the microbiological quality of sea water alone may not be a completely satisfactory method for determination of safety standards for bathing beaches. Further longitudinal studies over summer and winter seasons are necessary to establish with more precision the size of any microbiological risk to public health from bathing beaches and to assess the relationship between faecal indicators and the presence of survival of human enteric pathogens, for example *Campylobacter* spp., in sand [20].

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