Identification of the pandemic O3:K6 clone in cases of *Vibrio parahaemolyticus* related illness in the U.K.

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Background

V. parahaemolyticus is a halophilic bacteria, that can be isolated from seawater, bivalve molluscs and crustacea. It is responsible for seafood related gastroenteritis characterised by watery/bloody diarrhoea, vomiting, abdominal cramps, headaches, fever and nausea (Joseph *et al* 1982). The bacterium is found where sea temperatures are between 13 and 22ºC and where salinity ranges between 5 and 25ppt. Pathogenicity is associated with thermostable direct and related haemolysins (TDH/TRH), encoded by *tdh* and *trh* genes respectively. *Tdh* and *trh* genes are present in up to 99% of clinical strains, but rare (2-3%) in environmental isolates (Nishibuchi and Kaper, 1995).

Clinical isolates may be associated with diverse serotypes although in Asia since 1996 an O3:K6 serotype has accounted for an increased incidence of cases (Okuda *et al* 1997). This serovar first emerged in Calcutta, India and was characterised as TDH positive and TRH negative. It was responsible for 50 to 80% of *V. parahaemolyticus* infections from February 1996 in the region (Okuda et al 1997). This newly recognized serovar had seven base differences in the *toxRS* operon. This polymorphism in the *toxRS* operon was exploited to develop a group-specific PCR (GS-PCR), which has been used as a molecular marker for it's identification (Matsumoto *et al* 2000).

In 2004 in Spain, Europe saw its first O3:K6 outbreak where 76 cases were reported after infected crabs were consumed at a wedding in Galicia (Martinez-Urtaza *et al* 2005). To date there have been no reported outbreaks of *V. parahaemolyticus* in the UK. In 2004 and 2005 the number of *V. parahaemolyticus* cases reported to the UK Health Protection Agency were 24 and 22 cases respectively. However, under reporting may mean that this figure is not a true representation of case numbers.

Country	Year	Number of cases	Source
Niigata prefecture, Japan	1996	691	Boiled crabs
France	1997	44	Imported shrimp
Vladivostok, Russia	1997	27	Unknown
Khanh Hoa, Vietnam	1997-1999	256	
Texas, USA	1998	416	Oysters
Taiwan	1999	61.3% of 88 outbreaks	Seafood
Puerto Montt, Chile	1998 & 2004	1500	Seafood
Mozambique	2004	32	Uncooked seafood
Spain	2004	76	Crabs

Table 1. Global outbreaks of V. parahaemolyticus *O3:K6 describing pandemic status.*

Objectives

In this study the significance of 4 clinical isolates of *V. parahaemolyticus* that had been submitted to the UK Health Protection Agency between 2000 and 2005 was examined (Table 2). Three strains were isolated from individuals exhibiting *V. parahaemolyticus*-like disease on return to the UK following travel to the Far East while one strain was isolated from an individual with gastroenteritis after consumption of shellfish. Biochemical and molecular clonal relationship of these strains to the pandemic O3:K6 isolated from Spain and Japan, were analysed using PCR, serotyping and Pulsed Field Gel Electrophoresis (PFGE).

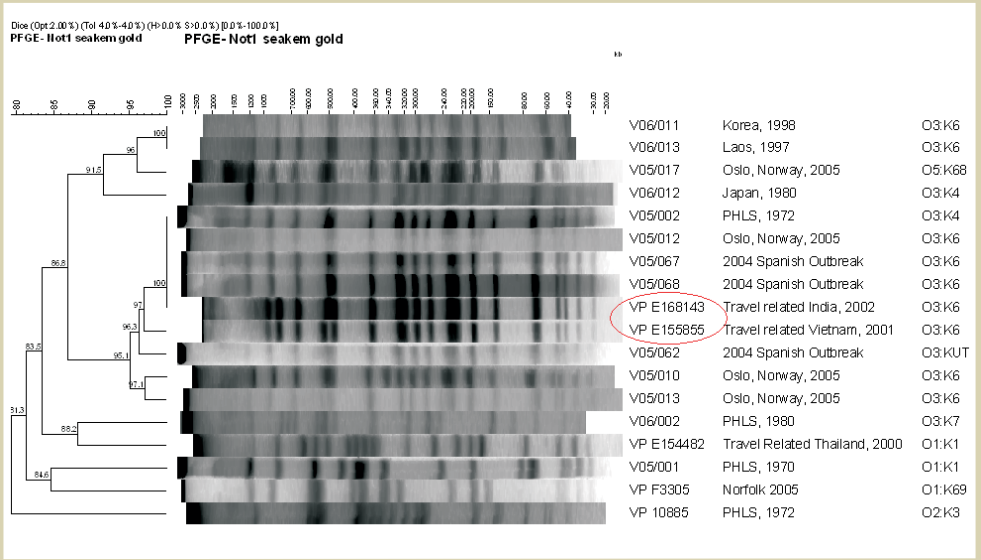

*Figure 1: A dendrogram showing PFGE profiles (*Not I *restriction) of clinical isolates of* V. parahaemolyticus *from different locations. The profiles show that VP E168143 and VP E155855 are part of the same cluster as other O3:K6* V. parahaemolyticus *strains illustrating that the two UK isolates are clones of the pandemic* V. parahaemolyticus *strain O3:K6.*

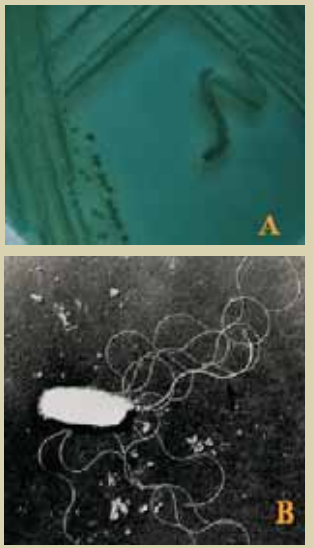

Figure 2: Vibrio parahaemolyticus *on Thiosulphate Citrate Bile Sucrose (TCBS) plate (A) showing non-sucrose fermenting colonies and a micrograph of* V. parahaemolyticus *cell (B)*

Results

All *V. parahaemolyticus* strains were positive for molecular identification markers *toxR* and *tlh* genes. The pandemic O3:K6 strain has been characterised as TDH positive, TRH negative and GS positive. Strains E155855 and E168143 show the same molecular characteristics. Figure 1 shows PFGE profiles of the clinical strains tested using *Not I* restriction. VP E168143 and VP E155855 are part of the same cluster as those isolated from the Spanish outbreak of 2004, illustrating that the 2 UK strains are clones of the pandemic O3:K6 *V. parahaemolyticus*.

Strain ID	Year of Isolation	Origin	Serotype	PCR – ToxR[1]	Probing – tlh[4]	TDH [2&4]	GS Primers [3]	TRH [2&5]
1902	1970	Reference	O1:K1	+	+	-	-	+
10885	1972	Reference	O2:K3	+	+	-	-	-
11344	1980	Reference	O3:K7	+	+	-	-	-
E154482	2000	Travel related from Thailand	O1:K1	+	+	-	-	+
E155855	2001	Travel related from Vietnam	O3:K6	+	+	+	+	-
E168143	2002	Travel related from India	O3:K6	+	+	+	+	-
F3305	2005	Shellfish	O1:K69	+	+	+	-	+

Note: 1 – Kim *et al* 1999 : 2 – Tada *et al* 1999 : 3 Matsumoto *et al* 2000 : 4 – McArthy *et al* 2001 : 5 Nordstrom *et al* 2006

Table 2. Strain ID and characteristics of the 4 clinical V. parahaemolyticus *isolates tested in this study with 3 reference isolates.*

Discussion

This study describes the first identification of the pandemic O3:K6 clone of *V. parahaemolyticus* in the UK. Although there have been no reported outbreaks of *V. parahaemolyticus* in the UK associated with indigenously produced seafoods, the bacterium is routinely isolated from shellfish samples tested at Cefas (data not shown). The prevalence and density of *V. parahaemolyticus* in the environment and seafood products have been shown to be dependent on ambient water temperature with rapid proliferation occurring at sea water temperatures >16˚C (SCVMPH 2001). In recent years, a number of reports have suggested, that higher sea surface temperatures will result in a reduction of dissolved oxygen and increased bacterial metabolism. This preferentially supports the proliferation of pathogenic vibrios including *V. parahaemolyticus*. Sea surface temperature (SST) and air temperature over the sea within the mid-latitude North Atlantic and UK coastal waters have been rising by 0.2 – 0.6 ºC per decade over the past 30 years. It is clear that if global climate change results in longer, hotter summers and elevation of ambient sea temperatures, it may also, lead to an increase in incidence of seafood associated *V. parahaemolyticus* food poisoning in the community.

References

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