Jpn. J. Infect. Dis., 52, 1999

Laboratory and Epidemiology Communications

Emergence and Prevalence of a Novel *Vibrio parahaemolyticus* O3:K6 Clone in Japan

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Communicated by Hiroshi Yoshikura

(Accepted January 5, 2000)

Vibrio parahaemolyticus was at the top of the list of pathogens isolated from patients with food-born diseases in 1998 in Japan. One of the dominant serotypes among the isolates was O3:K6. This serotype has been increasing since 1996 (1). Here we examined clonality of the O3:K6 isolates by using pulsed-field gel electrophoresis (PFGE) as previously described (2). We used a total of 86 *V. parahaemolyticus* O3:K6 isolates discovered between 1962 and 1999 (Table). These consisted of one isolate obtained in 1962; 5 isolates obtained from patients in 1998 in the USA (kindly provided from CDC, USA); 2 isolates from patients in 1998 in Tokyo; 8 isolates from patients, food or the environment in 1997-1998 in Aomori Prefecture; 61 isolates from patients, food or the environment in 1981-1998 in Kanagawa Prefecture; 6 isolates from patients in 1997-1998 at Narita Airport Quarantine Station; and 3 isolates from patients in 1999 in Thailand (kindly provided from the National Institute of Health in Thailand). Fifteen isolates with other serotypes, including O3:K48, O3:K56, O4:K8, O4:K12, and O4:K68, were included in the analysis. In addition to PFGE analysis, PCR detection of *tdh* (thermostable direct hemolysin) and *trh* (*tdh*-related hemolysin) genes and urease test were performed.

Table. PFGE and other markers of *V. parahaemolyticus* O3:K6 isolates

Presence of genes		Urease	PFGE patterns		No. of isolates examined	Isolated year(No. of isolates)
tdh	*trh*	activity	*Not*I	*Sfi*I		
+	-	-	A	A	65	1996(5),1997(15),1998(42),1999(3)
+	-	-	B	B	6	1981(6)
-	+	+	C	C	7	1981(2),1984(2),1995(1),1996(2)
-	-	-	D	D	1	1982(1)
-	-	-	E	E	1	1986(1)
-	-	-	F	F	2	1985(1),1988(1)
-	-	-	G	G	1	1983(1)
-	-	-	H	H	1	1962(1)
-	-	-	I	I	1	1987(1)
-	+	+	J	J	1	1984(1)
Total					86	

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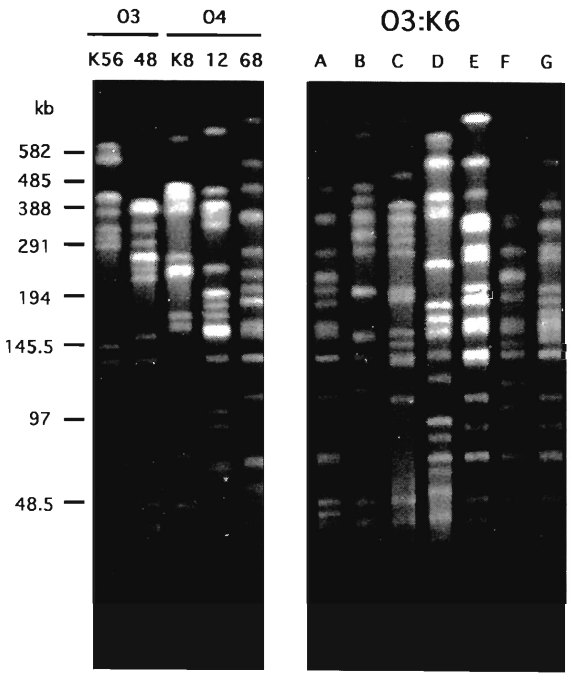

Figure. Representative *Not*I-cleaved PFGE patterns of *V. parahaemolyticus* isolates. Lanes from left to right indicate PFGEs of serotypes O3:K56, O3:K48, O4:K8, O4:K12, O4:K68, O3:K6, and O3:K6, designated as groups A to G, respectively. The position of each DNA-size (kb) marker is shown on the left.

PFGE patterns of *Not*I- or *Sfi*I-digests of DNAs derived from the O3:K6 isolates could be classified into 10 distinct groups, designated as A to J (Fig. and Table). All of the *tdh*$^+$, *trh*$^-$, and urease negative isolates obtained later than 1996 (65 strains including isolates from Thailand and the USA) constituted group A, while strains with the same phenotype obtained in 1981 constituted group B (Table). Though differences were found between the PFGE pattern of each group in O3:K6 and other serotypes, recent isolates of the O4:K68 serotype were very similar to recent isolates of the O3:K6 serotype (compare the lane of O4:K68 and that of group A of O3:K6 in Fig.).

We showed here that recent *V. parahaemolyticus* O3:K6 isolates in Japan had a PFGE pattern distinct from that prevalent in previous isolates. In addition, we identified a PFGE pattern indistinguishable from those of recent Japanese isolates in the Thai and US isolates. The same PFGE pattern was reported in India, Southeast Asia, and the USA (3-6). Taken together, these data may suggest the emergence of a new *V. parahaemolyticus* O3:K6 clone that is spreading worldwide and already affecting Japan.

We thank the Aomori Prefectural Institute of Public Health and Environment, the Tokyo Metropolitan Research Laboratory and Public Health and the Narita Airport Quarantine Station for providing strains.

This study was supported by a grant from the Ministry of Health and Welfare of Japan.

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