

Restriction Fragment Length Polymorphism Analysis of *Vibrio cholerae* Strains Associated with a Cholera Outbreak in Hong Kong

WING CHEONG YAM, MARIA LI LUNG,* AND MUN HON NG

Department of Microbiology, University of Hong Kong, Hong Kong

Received 10 December 1990/Accepted 5 February 1991

We studied *Vibrio cholerae* El Tor isolates associated with an outbreak of cholera among Vietnamese refugees interned in Hong Kong. The restriction fragment length polymorphism of the enterotoxin gene was used as an epidemiological marker. All outbreak strains were indistinguishable. They were distinct from strains isolated in Hong Kong prior to the outbreak.

The technique of restriction fragment length polymorphism (RFLP) analysis has been used to study the molecular epidemiology of *Vibrio cholerae* (3-5). Using this technique, we were able to distinguish indigenous and exogenous strains of *V. cholerae* collected in Hong Kong from 1970 to 1987. In the present study, strains of *V. cholerae* El Tor serotype Inaba isolated in 1989 from a limited cholera outbreak in a temporary Vietnamese refugee camp were compared with several indigenous and exogenous strains isolated during that same period.

Cholera is a notifiable disease in Hong Kong. A total of 30 cases of cholera were reported in Hong Kong in 1989 (Table 1). The strains were exclusively *V. cholerae* El Tor serotype Inaba. All but four of these cases were probably caused by organisms indigenous to Hong Kong, since the patients did not have a recent travel history. Twenty-one cases were associated with an outbreak occurring on a deserted island, Tai Ah Chau, that served as a temporary camp for Vietnamese refugees. All patients were suffering from acute watery diarrhea, and their detailed travel histories for 2 weeks prior to the development of diarrhea were recorded. Other than strains from fecal specimens, one *V. cholerae* strain was also isolated by the Environmental Protection Department from the effluent of an incinerator known to share common sewage ducts with Princess Margaret Hospital, to which all cholera patients in Hong Kong are transferred. The strains were identified as *V. cholerae* El Tor serotype Inaba by standard methods (2) and were found positive for cholera toxin by the rabbit ileal loop assay (1). Forty-five previously studied strains of *V. cholerae* El Tor serotype Inaba isolated between 1984 and 1987 (5) were also included in this study.

DNAs from strains of *V. cholerae* El Tor serotype Inaba isolated between 1984 and 1989 were digested with *Hind*III or *Hpa*II and subjected to Southern blotting analysis with the recombinant heat-labile toxin (LT) gene as the probe as described previously (5). Figure 1 shows the RFLP analysis of these strains. A strain isolated from a sporadic case of cholera in 1984 (lanes A) and an outbreak strain isolated in 1986 (lanes B) had identical RFLP patterns after both *Hind*III and *Hpa*II digestion. A *V. cholerae* strain imported from Vietnam in 1987 (lanes C) and the strain isolated from the first indigenous cholera case reported in 1989 (lanes D) were indistinguishable from each other but were distinct from the earlier strains in lanes A and B after *Hind*III cleavage. The remaining strains examined had identical

RFLP patterns. All 21 strains from the Tai Ah Chau outbreak had a new RFLP pattern, as shown by two representative strains (lanes E and F). This pattern was identical to that of a strain isolated from an incinerator effluent during this outbreak (lanes G) and subsequent strains isolated in October 1989 from a Hong Kong resident with no recent travel history (lanes H), a local resident recently returning from the People's Republic of China (lanes I), and a Vietnamese refugee entering Hong Kong (lanes J). The new pattern observed (lanes E to J) was distinct from that of strains obtained from a sporadic case in 1988 (lanes A) and a 1986 cholera outbreak which involved 36 adults from an industrial district of Hong Kong (lanes B) and in which all strains were shown to be identical by RFLP typing.

RFLP analysis facilitates the investigation of outbreak and sporadic cases of cholera. We previously reported (5) the similarity in RFLP patterns between *V. cholerae* strains isolated in the 1986 outbreak (lanes B) and those isolated from sporadic cases between 1984 and 1987 (lanes A). The strain from the first cholera case reported in Hong Kong in 1989 (lanes D) was found to be identical by RFLP analysis to a strain first isolated from two Vietnamese children arriving in Hong Kong on a refugee boat in 1987 (lanes C). This strain, which was imported into Hong Kong, apparently persisted in the local environment and eventually caused sporadic disease. The Tai Ah Chau outbreak, which occurred in 1989 (lanes E and F), was caused by another strain, which had a new RFLP pattern not yet documented in Hong Kong. This outbreak was classified as being indigenous, since patients had not traveled outside of Hong Kong for 2 weeks prior to the onset of disease. This outbreak, however, occurred among Vietnamese refugees who had come from an area highly endemic for cholera. Asymptomatic carriage of cholera is common among such Vietnamese refugees. It is possible that the victims of the outbreak may have contracted the disease from recently arriving refugees.

Several interesting features are associated with this cholera outbreak in the temporary refugee camp. This incident occurred at the height of an influx of Vietnamese refugees to Hong Kong which necessitated temporarily accommodating them on a deserted island with no running water or proper sanitation. The hygienic conditions were therefore poor. The cholera outbreak that subsequently occurred lasted from 30 August to September 5 and involved 2 children and 19 adults interned there. None of the personnel working in the camp were affected, however, and the outbreak was brought under control by evacuation of the camp. All patients were admitted to Princess Margaret Hospital, the only infectious dis-

* Corresponding author.

TABLE 1. *V. cholerae* El Tor serotype Inaba isolated in Hong Kong in 1989

Date	Source	No. of cases	
		Indigenous	Exogenous
19 May	Local resident	1	
30 August– 5 September	Vietnamese refugee (Tai Ah Chau)	21 ^a	
18 September	Incinerator effluent	1	
22 September	Local resident		1 ^b
27 September	Local resident		1 ^b
14 October	Local resident	1	
24 October	Local resident		1 ^b
25 October	Vietnamese refugee		1 ^c
27 October	Local resident	1	

^a Outbreak in a Vietnamese refugee camp located on a deserted island (Tai Ah Chau).

^b Hong Kong resident returning from the People's Republic of China.

^c Vietnamese adult who developed cholera en route to Hong Kong.

ease hospital in Hong Kong. With inadequate disposal of discharge from the large number of patients, the organism was subsequently isolated from the effluent of that hospital. The isolated strain had an RFLP pattern identical to that of strains isolated directly from the patients. Subsequently, three confirmed indigenous cases occurred in Hong Kong shortly after the outbreak. The patients involved had no known contact with the patients involved in the outbreak. *V. cholerae* isolated from them had the same RFLP pattern as had that which caused the outbreak. This organism may have originated from the People's Republic of China, because the same strain was also isolated from three local residents returning from the People's Republic of China and a Vietnamese refugee arriving in Hong Kong via the People's Republic of China during that same time period. Indeed, many Vietnamese refugees interned in the temporary camp in Tai Ah Chau had traveled overland via the People's Republic of China to Hong Kong, and it is possible that some of them might have acquired the infection en route. These findings emphasize the importance of proper disposal of discharge from cholera patients, the lack of which may have resulted in overloading of the environment in the immediate vicinity of the hospital and risking spread to the community.

We thank S. Falkow of Stanford University, Stanford, Calif., for providing the bacterial strain harboring plasmid pEWD229 (LT); the Pathology Institute, Medical and Health Department, Sai Ying Poon, Hong Kong, for supplying all clinical isolates of *V. cholerae*; and the Environmental Protection Department, Wanchai, Hong Kong, for supplying a strain of *V. cholerae* isolated from the effluent of an incinerator.

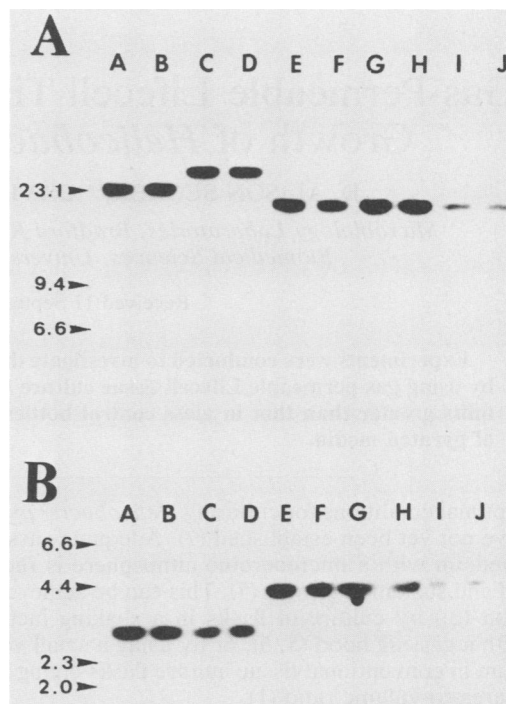


FIG. 1. Autoradiogram of a Southern blot of *V. cholerae* El Tor serotype Inaba isolated between 1984 and 1989. DNA was cleaved with restriction endonucleases and probed with the recombinant LT gene. (A) *Hind*III digestion. (B) *Hpa*II digestion. Lanes: A, strain from a sporadic case in 1984; B, strain from an outbreak in 1986; C, strain imported from Vietnam in 1987; D, indigenous strain, May 1989; E and F, strains from an outbreak in Tai Ah Chau in 1989; G, strain from an incinerator effluent, September 1989; H, indigenous strain, October 1989; I, exogenous strain from the People's Republic of China, October 1989; J, exogenous strain from Vietnam, October 1989. Molecular size markers (in kilobases) are shown on the left.

REFERENCES

- Burrows, W., and G. M. Musteikis. 1966. Cholera infection and toxin in the rabbit ileal loop. *J. Infect. Dis.* **116**:183–190.
- Farmer, J. J., III, F. W. Hickman-Brenner, and M. T. Kelly. 1985. *Vibrio*, p. 282–301. In E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), *Manual of clinical microbiology*, 4th ed. American Society for Microbiology, Washington, D.C.
- Kaper, J. B., H. B. Bradford, N. C. Roberts, and S. Falkow. 1982. Molecular epidemiology of *Vibrio cholerae* in the U.S. Gulf Coast. *J. Clin. Microbiol.* **16**:129–134.
- Kaper, J. B., S. L. Moseley, and S. Falkow. 1981. Molecular characterization of environmental and nontoxicogenic strains of *Vibrio cholerae*. *Infect. Immun.* **32**:661–667.
- Yam, W. C., M. L. Lung, K. Y. Ng, and M. H. Ng. 1989. Molecular epidemiology of *Vibrio cholerae* in Hong Kong. *J. Clin. Microbiol.* **27**:1900–1902.