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PHAGE TYPING OF VIBRIO "EL TOR"

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Phage typing often permits the identification of strains within a given bacterial species, serotype or biotype. In epidemic outbreaks of severe diarrhoea it is often difficult to distinguish between the classical *Vibrio cholerae* and *Vibrio 'El Tor'*. Although *V. cholerae* is sensitive to polymyxin B (11) and fails to agglutinate chicken erythrocytes or to hemolyse sheep red cells, and *V. 'El Tor'* does the opposite, many strains of *V. 'El Tor'* fail to produce haemolysin.

Mukerjee has shown that his bacteriophage No. IV against classical *V. cholerae* provides a rapid and certain in differentiation between the two organisms, for all strains of *V. cholerae* are lysed, but none of *V. 'El Tor'* (6): This observation has been repeatedly confirmed (9) and is confirmed again in this paper, which gives an account of the results of phage typing of 174 strains of *V. 'El Tor'* isolated from 518 patients admitted to one hospital with severe diarrhoea.

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MATERIALS AND METHODS

In most cases rectal swabs were taken and sent to the laboratory in peptone water PH 7.5; in a few cases a sample of stool was sent in a container (5). Peptone water cultures were incubated at 37°C for 6 hours and then plated on to TCBS medium (4). Suspicious colonies were examined microscopically to make sure they were *Vibrios*, and subcultured onto nutrient agar slopes and into Kligler medium. When required for phage typing, cultures were made from a 24 culture on an agar slope into nutrient broth PH 7.5 and incubated for 2 hours, when a faint turbidity was evident.

Slide agglutinations with polyvalent serum and specific antisera for Inaba and Ogawa biotype were performed with bacteria from agar slopes or from Kligler medium after less than 24 hours incubation. Biochemical tests were made by inoculation from an agar slope. All media were prepared from "Difco" dehydrated medium powders.

Bacteriophages were obtained from the Indian Institute of Biochemistry and Experimental Medicine through the Reference Laboratory of the Iranian — Ministry of Health. Phage typing was performed by the methods of Mukerjee as described in W.H.O. Public Health Papers No. 40 (1970) (10). Each phage was filtered through a 0.22 μ Millipore filter and 5 decimal dilutions made in nutrient broth (7). Six circle 12mm. Diameter were marked on the outside of the bottom of a petri dish containing untrient agar, and a 3mm loopful of a 2-hours culture of the *Vibrio* 'El Tor' No. 757 (Type 1 Macassar) spread over the medium within the marked circles, and allowed to dry. With the same loop a dilution of the phage was spread over one of the marked areas and left to dry; the sixth circle was used for *Vibrio cholerae* type IV phage which served as control. After incubation for 24 hours the result was read and recorded using Mukerjee's symbols. The routine test dilution (RTD) is the highest dilution which causes confluent lysis, and this dilution is used for subsequent phage typing. The phage typing was done in a similar manner, each plate permitting the trial of 5 test phages at RTD with an untreated control (8).

RESULTS

All strains isolated were typical Vibrio 'El Tor' Inaba biotype. All were H₂S negative, indol and oxidase positive, fermented mannitol, mannose and sucrose but not arabinose (3), oxidised glucose in the O/F test, were resistant to polymyxin B (50 units), agglutinated chicken erythrocytes and were resistant to Mukerjee's phage No. IV against classical *V. cholerae*. The Voges-Proskauer (1) and haemolysis (2) tests were variable.

Not all the strains of the 'El Tor' Vibrio are equally sensitive to a given phage. Phage Group I caused confluent lysis, or left a few discrete bacteria colonies, but phage Group II seemed less active or less consistent and usually showed discrete plaque formation, even though at the RTD the control Type I Macassar was completely lysed. When plaque formation was encountered experiments were repeated until consistent results were obtained. Difficulty was encountered with 9 strains, which on first isolation failed to fall into any of Mukerjee's types; but after one or two subcultures and retesting, 8 of these fell into either type IV or type V., the 9th remaining untypable,

DISCUSSION

It is clear that Mukerjee's phage No. IV against classical *Vibrio cholerae* has no effect on *Vibrio 'El Tor'* and is therefore a very useful tool. The discovery in this outbreak of two distinct phage types of El Tor raises the possibility that the outbreak may have originated from more than one. In retrospect this is difficult to prove, especially since El Tor has a high mutation rate, and Mukerjee has found that some strains show divergent sensitivity patterns when tested repeatedly with the same set of Phages.

**Results of phage typung of 174 strains of
Vibrio 'El Tor'**

Mukerjee's Phage Type of 'El Tor' Vibrio	Sensitivity phage group					V. Chol. IV	Number of strains belonging to type
	I	II	III	IV	V		
1	+	+	+	+	+	0	0
2	+	+	+	0	+	0	0
3	+	+	0	+	+	0	0
4	+	+	0	0	+	0	120
5	+	0	0	0	+	0	53
6	0	+	0	0	+	0	0
Untypable	0	0	0	0	0	0	1
Numbers sensitive to individual phage	173	120	0	0	173	0	Total 174

SUMMARY

Stools from 518 patients suspected of having cholera were examined. From 174 of these patients Vibrio El Tor was isolated. 120 of these strains belonged to phage type IV, 53 to phage type V and one strain was untypable. It is suggested that these strains originated from two different sources.

RESUME

Nous avons examiné les selles de 518 malades, et nous avons trouvé 174 souches de vibrions cholériques, après un typage il y avait 120 types de IV et 53 souches de V et une souche intypable.

Il paraît que l'épidémie de L'IRAN a différent source.

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