

A Specific Phage for Pathogenic *Vibrio cholerae*, Biotype El Tor (Φ H74/64)

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In 1957, Mukerjee and his associates isolated a phage specifically lytic for all strains of *Vibrio cholerae* (Mukerjee et al., 1957). A test based on the use of this group IV cholera-typing phage was subsequently found to provide the most sensitive method of differentiating between the two types of O group I vibrios (Mukerjee, 1963a). In the course of their studies on the phage typing of *Vibrio cholerae*, biotype El Tor, Basu & Mukerjee (1968) isolated a phage, Φ H74/64, designated group V in their phage-typing scheme. It was found to be specifically lytic for all pathogenic El Tor strains; nonpathogenic El Tor strains as well as classical *V. cholerae* strains were insensitive.

This paper presents the results of further detailed studies on the group V El Tor-typing phage.

Materials and methods

Phage strain. Strain Φ H74/64 was isolated from the stool of a cholera patient from near Calcutta in 1964. The vibrio isolated from this patient was *V. cholerae*, phage type 3 (Mukerjee, 1963b), serotype Inaba.

Vibrio strains.

(1) 600 strains of classical *V. cholerae* from cholera cases;

(2) (a) 3468 strains of *V. cholerae*, biotype El Tor, isolated between 1937 and 1966 from cholera cases; (b) 32 strains of *V. cholerae*, biotype El Tor, comprising strains isolated prior to 1937 and strains isolated from water sources in Middle East countries, India, Burma and elsewhere before the cholera El Tor infection entered these places;

(3) 50 strains of non-agglutinable (NAG) vibrios from natural waters or from cases of gastroenteritis between 1957 and 1965 belonging to the 5 different Heiberg groups.

Phage sensitivity test. The method of Craigie & Yen (1938) was used. One standard loopful (3 mm internal diameter) of the phages, at the routine test

dilution (RTD) and at 100 RTD, were spotted on to spot cultures of the test strains, incubated at 37°C overnight and read.

Identification of classical and El Tor vibrios. All strains were identified by their pattern of reaction in the following tests:

(1) group IV phage sensitivity test of Mukerjee (1963a);

(2) polymyxin-B test of Roy et al. (1965);

(3) haemagglutination test (chicken cell agglutination) of Finkelstein & Mukerjee (1963);

(4) haemolytic test of Feeley & Pittman (1963). This test was performed as a routine procedure, but as most of the El Tor strains isolated since 1963 are non-haemolytic, little importance was attached to a negative result.

Heiberg grouping. The method of Barua & Gomez (1967) was used.

Animal pathogenicity test. Tests for pathogenicity in the adult rabbit ileal loop were carried out, according to the method of De & Chatterje (1953), on 60 representative El Tor strains from cases that were either sensitive to group V phage or phage V-resistant and on all the nonpathogenic El Tor strains. The strains from loops that gave a negative result were reisolated from the loops and retested twice for confirmation of the nonpathogenic status of the strains.

The 4 El Tor strains from cases that were resistant to group V phage and the nonpathogenic El Tor strains found to be nonpathogenic in the ligated ileal loop were further tested for pathogenicity in neonatal rabbits according to the method of Dutta & Habbu (1955).

Results

Host specificity of the phage Φ H74/64. The host specificity of Φ H74/64 at RTD and 100 RTD is represented in Table 1.

It may be seen from this table that all the 600 classical *V. cholerae* strains tested were resistant to phage Φ H74/64 at RTD as well as at 100 RTD. The NAG vibrio strains from water sources also behaved in the same manner. Of 37 NAG strains

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TABLE 1
SENSITIVITY OF VIBRIO STRAINS TO PHAGE Φ H74/64^a

Type of <i>Vibrio cholerae</i>	No. of strains tested	Source	Strains sensitive to Φ H74/64			
			RTD		100 RTD	
			No.	%	No.	%
Classical	600	Cholera patient	0	0	0	0
El Tor	3 468	Cholera El Tor patient	3 464	99.9	3 464	99.9
El Tor	32	Water	0	0	0	0
NAG vibrio	37	Gastroenteritis patient	0	0	2	5.4
NAG vibrio	13	Water	0	0	0	0

^a The results of sensitivity tests of El Tor strains in comparison with the results of their animal pathogenicity test are presented in Table 2.

TABLE 2
PHAGE SENSITIVITY AND PATHOGENICITY TESTS OF *V. CHOLERAE* BIOTYPE EL TOR

No. of strains	Source	Phage sensitivity	Pathogenicity	
			Adult rabbit	Infant rabbit
3 464	Cholera EL Tor patient	Sensitive	Positive ^a	Not tested
4	Cholera El Tor patient	Resistant	Negative	Negative
32	Water	Resistant	Negative	Negative

^a Only 60 representative strains tested.

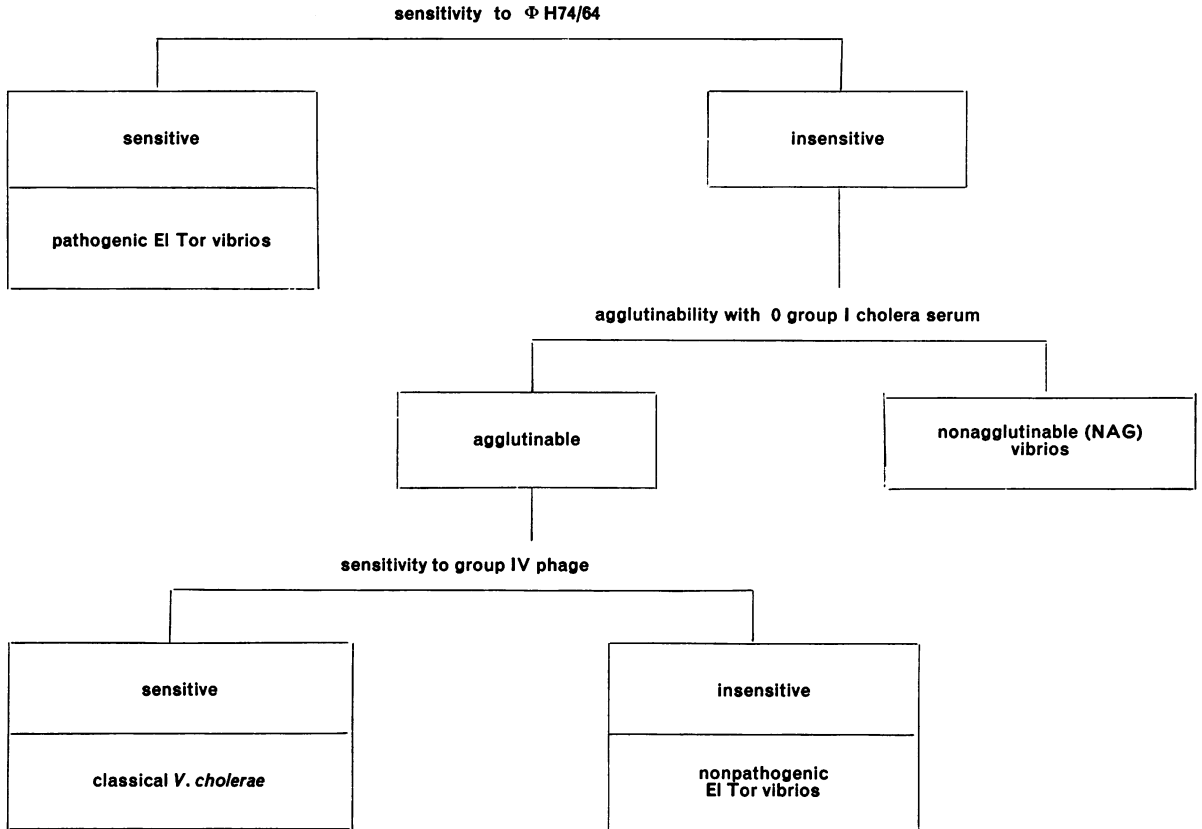
isolated from cases of gastroenteritis from Baroda and Bangalore in India in 1964, 2 belonged to Heiberg group II and were sensitive to this phage at 100 RTD. At RTD, however, all the NAG vibrio strains were insensitive to this phage irrespective of their Heiberg group.

Table 2 shows that out of 3468 El Tor vibrio strains isolated from cholera El Tor patients, all but 4 were sensitive to phage Φ H74/64. The remaining 4 strains and 32 El Tor strains isolated from natural water sources were resistant to this phage even at 100 RTD and are also nonpathogenic in the adult rabbit ileal loop and infant rabbit models.

Discussion

The characteristics of the 4 atypical El Tor strains,¹ that were resistant to phage Φ H74/64, have already been published (Pesigan et al., 1967); they differ markedly from the El Tor strains that have been isolated from patients since 1937. The 4 strains are identical in their characteristics: they are serotype Inaba, resistant to the classical and El Tor typing phages, non-lysogenic, bacteriocinogenic, non-haemagglutinating (CCA negative), strongly haemo-

¹ These 4 strains were received through the courtesy of Mrs C. Z. Gomez.

SENSITIVITY OF VIBRIO STRAINS TO PHAGE Φ H74/64 AND TO GROUP IV PHAGE

lytic, sensitive to polymixin B and they exhibit the biochemical reactions of Heiberg group III.

The remaining 32 El Tor strains that are resistant to phage Φ H74/64 are not only nonpathogenic by their history of isolation, but also by the animal pathogenicity tests presently available. Even after two animal passages, they still maintain their nonpathogenic status.

This phage Φ H74/64, or the group V El Tor-typing phage, is thus universally lytic for the pathogenic strains of *V. cholerae* biotype El Tor while all the classical *V. cholerae* strains are resistant to it, in contrast to Mukerjee's group IV cholera-typing phage, which is lytic for all classical *V. cholerae* strains, but not to the El Tor strains tested in this series. This phage may therefore be used at its RTD to differentiate pathogenic El Tor strains from classical and NAG vibrio strains. It also distinguishes between pathogenic and nonpathogenic El Tor strains.

The pattern of sensitivity of vibrio strains to phage Φ H74/64 may be presented as in the accompanying diagram.

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