WIDESPREAD EMERGENCE OF *VIBRIO CHOLERAE* 0139 IN INDIA

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Abstract. The National Institute of Communicable Diseases (NICD) has been monitoring the incidence of laboratory confirmed cases of cholera in Delhi in collaboration with Infectious Diseases Hospital (IDH) since 1965. Cholera and cholera-like cases from all hospitals in Delhi are admitted in IDH and the rectal swabs of all such cases are processed for isolation of *Vibrio cholerae* at NICD laboratory. Since April 1993, there has been isolation of *Vibrio cholerae* serotype 0139, in increasing numbers (831 out of 2,830, 29.2%). The isolates have been characterized and enterotoxin studies carried out. As a referral laboratory NICD has also confirmed the causative role of *Vibrio cholerae* 0139 in diarrhea outbreaks from various parts of the country. The implications of establishment of this newer serotype of *Vibrio cholerae*, as a potential epidemic strain are discussed.

INTRODUCTION

Since its first recorded pandemic in 1817, cholera has been among the most feared of the classic epidemic diseases (Pollitzer, 1959), the causative organism of cholera being *Vibrio cholerae* serotype 01. Non-01 serotypes were mostly associated with sporadic cases of gastroenteritis and extra intestinal infections (Janda *et al*, 1980; Aldova *et al* 1968). Until now non-01 *Vibrio cholerae* have caused only small outbreaks but no epidemics (Shimada *et al*, 1993) have been reported. In 1993 we came across *V. cholerae* serotype 0139 as the causative agent of cholera-like illness from all over Delhi and from a number of outbreaks investigated by NICD in different parts of India. Similar reports have also appeared in the medical literature from Bangladesh and South Eastern parts of India (Ramamurthy *et al*, 1993; Albert *et al*, 1993). *Vibrio cholerae* 0139 has emerged as a potential source of cholera epidemics along with *Vibrio cholerae* 01.

MATERIALS AND METHODS

In the year 1993-94, 2,850 rectal swabs from patients of clinically suspected cholera admitted to the Infectious Diseases Hospital (IDH) Delhi and 423 samples from outbreaks investigations from various states (Karnataka, Andhra Pradesh T, Haryana, Madhya Pradesh, Rajasthan and UP) of India were received and processed for isolation of *Vibrio cholerae* by standard microbiological techniques (Misra, 1975). Bile salt agar plates were examined for colonies of *V. cholerae*. Identification was done by biochemical tests and confirmed by serology. *V. cholerae* 01 antisera (non differential, Ogawa and Inaba) obtained from CRI Kasauli and *V. cholerae* 0139 antisera prepared by NIH Japan and provided by Professor Y Takeda were used. Non 01 *V. cholerae* isolated in the previous two years were also tested serologically.

Toxin assays were carried out on Y1 mouse adrenal cell lines (Sack and Sack, 1975). One hundred and twenty isolates of *V. cholerae* 0139 and 25 isolates of other non 01 *V. cholerae* were studied for enterotoxin production. Antibiotic sensitivity tests were done on Mueller-Hinton medium by the Kirby-Bauer method (Bauer *et al*, 1966). The antibiotics tested were ampicillin (10 μg), chloramphenicol (30 μg) co-trimoxazole (25 μg), furazolidone (50 μg), gentamicin (30 μg), nalidixic acid (30 μg), streptomycin (30 μg) and tetracycline (30 μg).

RESULTS

Out of 2,850 samples from Delhi, 831 (29.2%) yielded *V. cholerae* 0139 and 697 (24.4%) *V. cholerae* 01. Monthwise incidence of isolation of *V. cholerae* in 1993 is given in Table 1. The isolation rates of *Vibrio cholerae* 01 and non 01 from Delhi in the past 10 years are given in Table 2, while the results of stool samples collected during outbreak investigations in different parts of India and processed in NICD laboratory are shown in Table 3. Out of 423 outbreak samples processed 161 (38.1%) were con-

Table 1

Isolation of *V. cholerae* in Delhi, 1993.

Month	No. of stool samples examined	No. of *V. cholerae* 01 isolated	Rate %	No. of *V. cholerae* non 01 isolated	Rate %
Jan-March	60	0	0	0	0
April	219	8	3.7	73	33.3
May	710	155	21.8	290	40.8
June	564	117	20.7	201	35.6
July	505	133	26.3	141	27.9
August	432	109	25.2	81	18.8
September	206	107	51.9	37	18.0
October	117	58	49.6	7	6.0
November	32	10	31.3	1	3.1
December	5	0	0.0	0	0.0
Total	2,850	697	24.4	831	29.2

* More than 95% of non 01 isolates from Delhi were characterized as *V. cholerae* 0139.

Table 2

Cholera cases in Delhi during 1983-1993.

Month	No. of stool samples examined	No. of *V. cholerae* 01 isolated	Rate %	No. of *V. cholerae* non 01 isolated	Rate %
1983	1,969	455	23.1	60	3.0
1984	2,051	305	14.9	10	0.5
1985	2,543	587	23.1	69	2.7
1986	1,770	330	18.6	41	2.3
1987	1,579	285	18.1	60	3.8
1988	4,164	1,702	40.9	26	0.6
1989	1,620	197	12.2	32	2.0
1990	2,006	533	26.6	14	0.7
1991	1,917	537	28.0	12	0.6
1992	2,783	1,075	38.6	52	1.9
1993	2,850	697	24.4	831 (0139)	29.2

firmed to be *V. cholerae* 0139. The 64 strains of non 01 *V. cholerae* isolated in the past 2 years (1991-92) from Delhi were tested serologically with 0139 antisera. None of them were found to agglutinate with *V. cholerae* 0139 antisera, indicating clearly the emergence of *V. cholerae* 0139 in Delhi only in the year 1993.

The biochemical characteristics of the 0139 isolates were studied as given in Table 4. It was noticed that *V. cholerae* 0139 isolates gave a delayed acid/acid (A/A) reaction on triple sugar iron agar (TSI) as compared to *V. cholerae* 01 isolates.

Toxin studies on Y1 mouse adrenal cell line showed the presence of heat labile enterotoxin in 116

Table 3

Isolation of *V. cholerae* in NICD from stool samples collected from different parts of India in 1993.

Name of the state	No. of stool samples	No. of *V. cholerae* 01		No. of *V. cholerae* 0139	
		isolated	%	isolated	%
Andhra Pradesh	9	0	0.0	6	66.7
Assam	4	0	0.0	0	0.0
Bihar	17	0	0.0	6	5.9
Gujrat	5	0	0.0	0	0.0
Haryana	122	13	10.7	34	27.9
Karnataka	89	1	1.1	47	52.8
Madhhya Pradesh	37	0	0.0	13	35.1
Maharashtra	44	0	0.0	7	15.9
Rajasthan	28	1	3.6	11	39.3
Tamil Nadu	2	0	0.0	1	50.0
Uttar Pradesh	66	0	0.0	41	62.1
Total	423	15	3.5	161	38.1

Table 4

Biochemical characteristics of *V. cholerae* 0139 isolated at NICD Delhi in 1993.

Test	Reaction	% positive
Oxidase	+	100.0
Indole	+	100.0
Voges Proskauer	D	89.5
Citrate	+	3.6
H_2S (TSI)	-	0.0
Urease	-	0.0
Polymyxin B (50 units)	R	100.0
Lysine decarboxylase	+	100.0
Arginine dihydrolase	-	0.0
Ornithine decarboxylase	+	100.0
Nitrate reduction	+	100.0
0/129 inhibition	+	100.0
Fermentation		
Glucose (No gas)	+	100.0
Lactose	-	0.0
Sucrose	+	100.0
Mannose	+	96.0
Mannitol	+	98.8

(96.7%) out of 120 isolates of *V. cholerae* 0139. None of the 25 *V. cholerae* non 01 non 0139 isolates tested produced this toxin.

Antibiotic sensitivity tests of the 831 *V. cholerae* 0139 showed a sensitivity to ampicillin (96.5%), chloramphenicol (96.5%), tetracycline (96.5%) and nalidixic acid (96.5%) and 92.3% to streptomycin. All the isolates were sensitive to norfloxacin. The important feature of these being resistance to co-trimoxazole in 94.5% (802/831) of the isolates tested.

DISCUSSION

V. cholerae non 01 serogroups have been previously identified throughout the world and are known to cause, in addition to extra-intestinal infections, cases of severe dehydrating diarrhea resembling cholera. Until now, they have been associated only with sporadic cases or relatively confined outbreaks of diarrhea (Ramamurthy *et al*, 1993; Kamal, 1971). Incidence of gastroenteritis, caused by non 01 Vibrios was reported to be 5-10 percent of hospitalized patients in Calcutta (Ramamurthy *et al*, 1992) and 1-3% in Bangladesh (Albert *et al*, 1993).

In our study, out of 2,850 stool samples processed, 831 (29.2%) were found to be positive for *V. cholerae* 0139. The incidence of non 01 *V. cholerae* isolated from diarrhea cases in the past ranged between 0.5% to 3.8% only (Table 2). None of the 64 non 01 Vibrios isolated in the last 2 years was found to be 0139 serotype. The *V. cholerae* 0139 is a new strain which has emerged in Delhi only in 1993 as a causative agent of severe cholera-like illness.

All the gastroenteritis outbreaks investigated by NICD in 1993, in the states of Karnataka, Andhra Pradesh, Haryana, Rajasthan, Madhya Pradesh and UP were caused by *V. cholerae* 0139. None of the outbreaks investigated in the past by us were found to be due to non-01 *V. cholerae*. Similar *V. cholerae* 0139 outbreaks have been reported from Bangladesh and Southern parts of India. Thus, we confirm this swift spread of the *V. cholerae* 0139 not only in the eastern and southern parts of India but in the entire country. The spread of the previously unrecognized organism in the community causing epidemic of cholera is a significantly new development in the history of this well studied disease.

The *V. cholerae* 0139 isolates were similar morphologically and biochemically to *V. cholerae* 01. Toxin assay carried out on 120 of the *V. cholerae* 0139 isolates showed 96.7% positivity for the production of a heat labile enterotoxin similar to that of *V. cholerae* 01, where as none of the 25 non 01 non 0139 Vibrios tested produced enterotoxin. However non 01 *Vibrio* have been reported to produce a closely related enterotoxin in a very small percentage of cases and have never been found to be responsible for outbreaks (Zinnaka and Carpenter, 1972; Morris *et al*, 1990). Therefore the pathogenesis of the disease caused by *V. cholerae* 0139 seems to be similar to that of typical cholera.

Antibiotic sensitivity tests of 0139 isolates showed 96.5% resistance to co-trimoxazole, which is a commonly used drug for diarrhea. Similar resistance to co-trimoxazole and furazolidone has been reported from Bangladesh and Southern and Eastern parts of India. *V. cholerae* 0139 isolated at NICD showed a susceptibility of 100% to norfloxacin and 96.5% each to ampicillin, chloramphenicol, furazolidone, nalidixic acid and tetracycline.

Our data in Delhi show that both *V. cholerae* 01 and 0139 co-existed in 1993. The isolation rate of *V. cholerae* 01 for the period 1983-1992 varied between 12.2% to 40.9%. Although during 1993, there was a sudden increase in the isolation rate of *V. cholerae* non 01 (0139) as depicted in, Table 2, the isolation rate of *V. cholerae* 01 remained same as in previous years. The isolation rate of *V. cholerae* 0139 was twice as high as that of *V. cholerae* 01 from April to June in 1993 and there after the isolation rate of *V. cholerae* 0139 declined and isolation rate of *V. cholerae* 01 increased (Table 1). These data are contrary to the apprehension that *V. cholerae* 01 may be competed out by *V. cholerae* 0139.

The disease caused by *V. cholerae* 0139 seems to be epidemiologically similar to cholera and is presumed to pose identical public health risk. A high proportion of cases in recent outbreaks have occurred in adults suggesting that the population living in cholera endemic areas lack immunity to this new serotype. This also indicated absence of cross immunity among the two types. This observation may pose for us a problem of developing a vaccine against 0139, since the current vaccines in use or under development against *V. cholera* 01 will be ineffective against it.

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