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EDITORIAL

New Strains of *Vibrio cholerae* O139 in India and Bangladesh : Lessons from the Recent Epidemics

The epidemics. On October 19, 1992 there was a sudden increase in the number of patients with diarrhoea admitted to the hospitals in and around the city of Madras in southern India (1). The clinical picture of the illness resembled Asiatic cholera, characterised by a sudden onset of watery diarrhoea, vomiting, and a varying severity of dehydration. Within a few weeks, similar cases were reported in increasing numbers from cities in other parts of India, including Madurai, Vellore, Amravati, Visakhapatanam, Nagpur, and Mysore (2). By the end of November 1992, over 15,000 diarrhoea cases, with 230 deaths were reported from Calcutta and its neighbouring districts in eastern India (3). Shortly thereafter, in mid-January 1993 an outbreak of acute diarrhoea occurred in southern Bangladesh mostly affecting adults. By mid-February about 10,000 people had been affected with an estimated 500 deaths (4). By the end of March 1993, the epidemic had spread to other parts of Bangladesh, the total number of reported cases being 107,297 with 1,473 deaths (18). At the hospitals of the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) at Dhaka and Matlab (rural area), the daily attendance of patients started to rise from mid-January and was 550-615 and 125 respectively at the peak of the epidemic in March. This is approximately 2 to 5 times the number of patients usually seen at this time of year (6). At the Infectious Disease Hospital, Calcutta, 13,275 patients with diarrhoea were admitted between January 1 and April 16, 1993, of whom 434 (3.2%) died (3). Since occurrence of cholera epidemics is well known in this endemic region, most clinicians presumed that the recent epidemic was due to infection by the most commonly encountered strains of *Vibrio cholerae* O1, although this was not the season when cholera epidemics are expected in Dhaka. However, when the laboratory results became available, the causative organism was found not to be cholera *Vibrio cholerae* O1, but *Vibrio cholerae* non-O1, a group of organisms which have not previously caused epidemics.

Bacteriology and epidemic potential. *Vibrio cholerae* O1 is differentiated from *V. cholerae* non-O1 on the basis of the specific somatic O-antigen (thermostable lipopolysaccharide of the outer membrane envelope). Serogroup O1 causes the classical syndrome of cholera and has been associated with the previous pandemics of cholera (5). *Vibrio cholerae* strains that are not agglutinated by the diagnostic O1 antiserum (non-O1 *Vibrio cholerae*) are widely distributed in nature and have been associated with sporadic cases of mild gastroenteritis and extra-intestinal infections (7). The rates of isolation of non-O1 vibrios in the endemic areas are usually very low, ranging from 1% to 3% of all admissions in the two diarrhoea hospitals in Bangladesh (6). Although strains of *V. cholerae* non-O1 have never been reported to cause epidemics of diarrhoeal illness, these organisms have been shown to induce diarrhoea in healthy human volunteers (8).

The strains of vibrios isolated during the epidemics in India and Bangladesh were characterised at The National Institute of Cholera and Enteric Diseases in Calcutta and at the ICDDR,B, Dhaka, respectively. Serological analysis of 200 strains showed that none of them were agglutinable with O1 antiserum nor with any of the monoclonal antibodies against factors A, B, and C of *Vibrio cholerae* O1 (1,9). The serologic characteristics of the organism were further delineated at the National Institute of Health in Japan using 138 antisera against known serotypes of *V. cholerae* non-O1. None of these antisera gave positive reactions with the epidemic strains, and the designation of an additional serogroup as *Vibrio cholerae* O139 was suggested to maintain the chronologic sequence in the existing serotyping scheme of *V. cholerae* (10). A synonym "Bengal" was also designated to specify the endemic bay of Bengal region in which this strain was first isolated. Microbiologic characterisation of *V. cholera* O139 indicates that the organism is a typical vibrio, possessing a single polar flagellum and having the characteristic darting motility which is not inhibited by antisera against *V. cholerae* O1 (6). Although the new strain lacks the ability to agglutinate with the O1 antiserum, the organism retains most other characteristics of *V. cholerae* O1. These include production of cholera toxin (by Bead-ELISA and Y1 adrenal cell assay), secretion of fluid by the ileal loop assay in rabbits, production of diarrhoea in the ileal tie model (RITARD) of rabbits, severe cholera-like diarrhoea in man, and the ability to spread swiftly (11). The new O139 strain has genetic similarities with *V. cholerae* O1 strains in that the organism hybridizes with DNA probes for cholera toxin genes and the newly described zona occludens toxin gene. The new

epidemic strain resembles more closely the *V. cholerae* El Tor biotype than 'classical,' because some of the tests (resistance to polymixin B and agglutination of chicken red cells) which characterise El Tor vibrios are also positive for *V. cholerae* O139. The antisera produced in rabbits against *V. cholerae* O139 did not cross-react with single strains of *V. cholerae* O1, other *V cholerae* non-O1, *V. parahaemolyticus*, *V. mimicus*, *Plesiomonas* or *Aeromonas* spp. (6). This shows that the antiserum against *V. cholerae* O139 is specific for this strain. However, more laboratory studies are needed to completely characterise the organism by specifically examining its enterotoxin, lipopolysaccharide, pili, and other structural components.

It is most likely that the *V. cholerae* O139 isolated during these epidemics on the Indian subcontinent and in Bangladesh belong to a new serotype of *V. cholerae* non-O1 which has not been previously recognised. The organisms appear to be a single clone since all the strains isolated in India and Bangladesh have the same serotype and biochemical and toxin-producing characteristics.

The organism is biologically similar to *V. cholerae* O1, the true "cholera vibrio," and its epidemic potential must be considered in a global perspective. It is possible that the new *V. cholerae* O139 may eventually replace *V. cholerae* O1 and establish itself as the dominant strain of a new eighth pandemic of cholera. This is reminiscent of the sequence of events in the early 1970s, when the "El Tor" biotype of *V. cholerae* O1, which is also the cause of the recent epidemics in South America (12), almost universally replaced the "classical" biotype of the organism that had caused the pandemics in the past (5). However, it is difficult to predict whether the *V. cholerae* O139 will coexist or displace *V. cholerae* O1 as it depends on the adaptive capacity of the new organism for continued survival in the changing ecology of the environment.

Since the appearance of a new pathogen, i.e. *V. cholerae* O139, has been confirmed, there will be many questions that need to be answered by microbiologists, clinicians, and epidemiologists. The first and most important one is to determine why the non-O1 vibrios suddenly emerged as a virulent pathogen with epidemic potential. To become pathogenic, the vibrio must produce a toxin, since it has been shown that most non-toxigenic strains do not cause disease (13). Although some non-O1 strains isolated from patients in Bangladesh and the United States produce toxins similar to that of *V. cholerae* O1, most of the environmental isolates of non-O1 vibrios produce no toxin or a small amount of toxin, as observed in the rabbit loop assay (14). In contrast, the O139 strain has been shown to produce large amounts of cholera toxin, from 10-80 ng/ml which is similar to that produced by strains of *V. cholerae* O1 producing clinical cholera. This difference in enterotoxicigenicity may be related to the probable existence of a genetic mechanism of repression and de-repression of the toxin-regulatory gene, which is chromosomally controlled in *V. cholerae*. In addition, the influence of environmental factors also appears to be important in the expression of the genetic behaviour of the organism. In addition to enterotoxin production some of these strains produce cytotoxic substances which may mask the enterotoxic activity in tissue culture assays. The potential for reversion of the non-toxigenic strain to toxigenic-strains can now be studied using molecular biology techniques. These studies have shown that some environmental strains of non-toxigenic *V. cholerae* O1 (CT-) have a deletion in their toxin-gene and they lack all genetic material encoding cholera toxin (15). Such strains do not have the potential for reverting to toxigenicity, and these toxin-gene deleted mutants are of great interest as vaccine strains, since they can stimulate an immune response without causing disease.

Clinical features and treatment. The clinical syndrome of diarrhoea, vomiting, and dehydration produced by infection with the new epidemic strains of *V. cholerae* O139 ranged from very mild illness requiring no treatment to severe diarrhoea and is similar to that produced by *Vibrio cholerae* O1. Studies in Dhaka and Calcutta have indicated that most patients admitted with clinical diseases had voluminous watery diarrhoea leading to severe dehydration, and required large amounts of intravenous and oral rehydration fluids (6,16). Most patients arrived at the hospital within 12 hours of onset of diarrhoea which resolved after 40-45 hours of treatment with rehydration fluid and tetracycline (6). The choice of an antimicrobial agent for treatment is not difficult because most of the *V. cholerae* O139 strains are uniformly susceptible to commonly used antibiotics, including tetracyline, whereas recently isolated *V. cholerae* O1 in Dhaka is not. Both tetracycline and ciprofloxacin were found to be clinically useful adjuncts to fluid replacement in the treatment of *V. cholerae* O139 infections in adults (16, Wasif Ali Khan, personal communication). These findings indicate that cases can be successfully managed by what is standard treatment for cholera, i.e, replacement of fluids and electrolytes and use of appropriate antibiotics. We believe that in the face of an epidemic, starting early treatment at home with ORS or similar home-available solutions along with normal eating can greatly alleviate the need for hospital admission and may be life-saving in some instances.

Epidemiology and control. At the Dhaka hospital of ICDDR,B, an estimated number of 12,550 patients (based on a 4% systematic sample of all patients attending) with *V. cholerae* O139 infection were treated during January to April 1993; this represents 27% of all patients with diarrhoea or dysentery attending the hospital during this period. Mortality among the hospitalised patients was remarkably low; only one patient, an 80-year-old man, developed renal failure and died. Although it is too early to

comment on the epidemiologic characteristics of the new strain, preliminary studies have revealed a carrier rate in family contacts of 17.2%, indicating effective transmission among susceptible individuals (GB Nair, NICED, Calcutta; personal communication). The modes of transmission are not yet known, but most likely it is transmitted through water, since the organism has been frequently isolated from finger-washings (3.4%), and drinking water sources including wells, ponds, and stored water (GB Nair, personal communication). Preliminary results of microbiologic studies in urban and rural areas of Bangladesh indicate that about 10% of the surface water samples are culture-positive for *V. cholerae* O139 (6). Early observations in Bangladesh also showed that there was no family clustering of patients; the majority of cases (93%) occurred in families where there were no other patients. Infection with *V. cholerae* O139 is associated with an interesting age distribution of patients. Unlike *V. cholerae* O1 which attacks mostly children in endemic areas, the new strain characteristically attacks adults, as indicated by the mean age of the hospitalised patients (around 35 years) in the Dhaka and Calcutta outbreaks (3,6). This indicates that the organism is new in the environment and there is little or no pre-existing immunity against this pathogen in the population. The high attack rates of *V. cholerae* O139 in adults are consistent with the findings of the recent cholera epidemics in virgin populations of South America (17). Early serologic studies in Bangladesh indicate that rising titres of antibodies to *V. cholerae* O139 (particularly to the enterotoxin) appear during acute and early convalescence (14 to 30 days) (6). A high incidence of the disease in adults also indicates that more adults than children are probably exposed to the infection. Although more males than females were seen at the treatment centres of Dhaka and Matlab in Bangladesh, both sexes are susceptible, and this difference may be related to the nature of their occupations.

Although the number of patients with cholera-like diarrhoea (caused by *V. cholerae* O139) coming to the hospitals in Dhaka and Calcutta has declined by the time of writing this report (mid-July 1993), unrecognised cases may continue to occur in the community and the possibility of future spread is real. Thus it is important to monitor the spread of *V. cholerae* O139 globally by initiating active surveillance systems in collaboration with national and international bodies, including the World Health Organisation and reference laboratories. Simple diagnostic tests to identify the organism should be developed so that rapid diagnosis could be made outside the few reference laboratories in the world. In the laboratory *V. cholerae* O139 can be identified easily by agglutination with rabbit antisera produced against the strains. Unfortunately, unlike *V. cholerae* O1, antisera against *V. cholerae* O139 is not yet commercially available but most laboratories having the facilities for *V. cholerae* isolation can obtain it by writing to Dr. John Albert, Laboratory Sciences Division, ICDDR,B, GPO Box 128, Dhaka 1000, Bangladesh.

There is great interest among microbiologists and epidemiologists to further characterise this novel strain of *V. cholerae* O139. This will definitely increase our understanding about the epidemiology of cholera, which has been so perplexing and fascinating since the time of John Snow and Robert Koch (5). Moreover, epidemiologic and immunologic studies of *V. cholerae* O139 infection should be undertaken for better understanding of the disease in population and vaccine development. It is understandable that the currently available vaccine against *V. cholerae* O1 (both live oral and killed vaccines) will not protect against infection due to *V. cholerae* O139. Thus, new initiatives should be undertaken to develop a vaccine that will be specifically useful against *V. cholerae* O139 infection. The goal of developing a protective vaccine against *V. cholerae* O1 has not been fully accomplished in spite of continued global efforts over the last 30 years. The primary problem has been to identify the antigenic protein(s) in *V. cholerae,* including its toxin, that will lead to the production of true 'protective' antibodies in the susceptible human. Now the situation is further complicated by the appearance of another serotype representing a different antigenic structure. In view of these constraints, it is unlikely that a useful vaccine will be available soon. Meanwhile, attention should be directed to reduce morbidity and mortality by proper clinical case-management, mainly with rehydration fluids, suitable antibiotics, and appropriate public health measures, such as ensuring a safe water supply, improved sanitation, and the practice of personal hygiene. In addition, public awareness about the severity of the disease should be increased through proper health education.

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