

RESEARCH NOTE

Acute Diarrhea Associated with Heat-stable Enterotoxin Producing Strains of *Vibrio cholerae* Non-O1: First Report from Cuba

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A five month old male infant was referred with watery diarrhea, vomiting and fever, to the paediatric ward of the provincial Pediatric Hospital of Las Tunas, Cuba. Abdominal examination revealed tenderness; liver and spleen were normal.

Chest, cardiovascular and other systems were normal. The patient was rehydrated with oral rehydration with solution according to the World Health Organization formula and breast-feeding was continued. The clinical picture improved gradually and the patient was discharged after six days without diarrhea and in good condition. On admission stool sample was taken and cultured on *Salmonella-Shigella* and MacConkey agar for isolation of *Salmonella*, *Shigella* spp., enterotoxigenic and enteropathogenic *Escherichia coli*, *Vibrio* spp., *Aeromonas* spp., and *Plesiomonas shigelloides* (JF McFaddin 1980, p. 19-34. *Biochemical Test for Identification of Medical Bacteria*, 2nd ed., The Williams and Wilkins Co, Baltimore).

Blood agar supplemented with Butzler's antimicrobials was employed for the isolation of *Campylobacter* spp. The presence of *Rotavirus* was ruled out by latex agglutination assay. Three dif-

ferent samples of faeces were analyzed by conventional microscopic examination for the presence of ova, cyst or vegetative forms of parasites (Organización Mundial de la Salud 1992, p. 10-20, *Métodos Básicos de Laboratorio en Parasitología Médica*, OMS, Geneva).

The stool specimen plated on MacConkey agar yielded a pure culture of lactose non-fermenting Gram-negative rods, moved by polar flagella, identified at genus level as *Vibrio*. The national strategy for cholera control in Cuba shows that the country is still free from cholera. A slide agglutination with polyvalent O1 group antiserum for presumptive identification of *V. cholerae* O1 strain was performed; the test was negative.

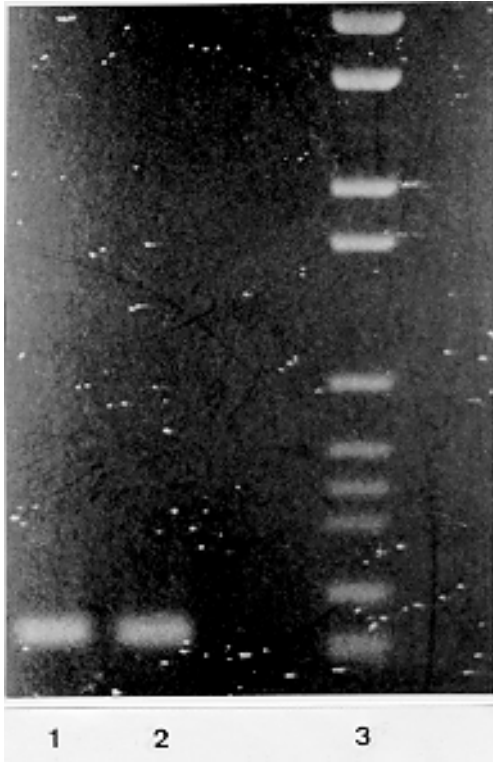
The coproparasitological examination was negative and no *Rotavirus* or other bacterial enteropathogen were found or isolated from stool samples. The *Vibrio* strain isolated was sent to the National Reference Laboratory for Diarrhoeal Diseases and Cholera Control at the "Pedro Kourf" Institute of Tropical Medicine, Havana City, and confirmed to be a non-O1 and non-O139 *V. cholerae* by standard methods (JJ Farmer & FW Hickman Brenner 1992, p. 2552-3011. In A Ballows, HG Truper, M Dworkin, W Harder, KH Schleifer (eds), *The Prokaryotes: a Handbook on the Biology of Bacteria*, Vol. 3, Springer, Verlag, New York). The strain was susceptible to 10- and 150 µg disks of the vibriostatic agent O/129; the antimicrobial susceptibility test, performed with the Kirby-Bauer method, showed that the strain was susceptible to tetracycline, ampicillin, cloramphenicol, trimethoprim, sulphamethoxazol, nalidixic acid, ciprofloxacin, streptomycin, erythromycin, gentamicin, cefuroxime and furazolidone (CW Stratton & RC Cooksey 1991, p. 1153-1165. In A Balows, WJ Hausler Jr, KL Herrmann, HD Isenberg, HJ Shadomy (eds), *Manual of Clinical Microbiology*, 5th ed., ASM, Washington, DC). The strain was analyzed for the presence of genomic sequences related to the *V. cholerae* O1 *ctx* genetic element, that includes the *cep*, *orFU*, *ace*, *zot*, and *ctxAB* genes, by using a colony-blot hybridization procedure (T Karasawa et al. 1993 *FEMS Microbiol Letters* 106: 143-146). The hybridization probe used was a 4.4 kbp cloned DNA fragment containing the entire *ctx* element of *V. cholerae*, and was labeled with (a 32P) dCTP by the random priming technique. The presence of a heat-stable enterotoxin gene was investigated by PCR amplification as previously described (P Guglielmetti et al. 1994 *Mol Cell Probes* 8: 39-44). The biological activity of the strain was tested by the conventional suckling mouse assay (Y Takeda et al. 1979 *Infect Immun* 25: 978-985). The results of the molecular characterization of the

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strain demonstrated the absence of any *ctx* genetic element related-sequences (data not shown) and the presence of the *stn* gene (Fig.): the suckling mouse assay confirmed the production and the activity of the heat-stable enterotoxin (ST).



Polymerase chain reaction (PCR) detection of *stx* gene in the strain of *Vibrio cholerae* non-O1 isolated from the patient (lane 1) and in a positive control strain (lane 2). In the lane 3 the Mr markers. PCR primers for *stx* open reading frame yielding an amplicon of 238 bp.

The role of non-O1 *V. cholerae* strain as intestinal pathogens is well established (JG Morris 1994 p. 103-115. In IK Wachsmuth, PA Blake, O Olsvik (eds), *Vibrio cholerae and Cholerae: Molecular to Global Perspectives*, ASM, Washington DC); some strains isolated from patients with diarrhea may produce toxins related to those encoded by the *V. cholerae ctx* genetic or an ST, similar to those expressed by *E. coli* (S Amito et al. 1992 *Eur J Biochem* 129: 257-263), *Yersinia enterocolitica* (JG Morris 1990 *J Clin Invest* 85: 697-705) and *V. mimicus* (M Arita 1991 *FEMS Microbiol Letters* 79: 105-110). The role for ST as virulence factor has been demonstrated in experimental assays and some studies have established the importance of non-O1 *V. cholerae* strain which produce only the ST in acute gastroenteritis (K Bagchi et al. 1993 *J Clin Microbiol* 31: 1315-1317). The strain isolated in this patient was positive for the *stn* gene by PCR assay and the ST production was confirmed by the conventional suckling mouse test; the same strain was negative for all elements of the *V. cholerae* O1 *ctx* genetic element. The absence of other viral, bacterial and protozoal pathogens indicates the likelihood that the strain isolated in the diarrhea of this child, the ST, is an important virulence determinant. This case is the first reported in Cuba and, to the best of our knowledge, in the Caribbean and Central America. From an epidemiological point of view the case is also interesting due to the age of the infant, the absence of sea food consumption from his dietary history and the geographical origin of the patient, coming from an area of the island, far away from the sea coast.

Other studies have already been planned to better understand the epidemiology and clinical relevance of *V. cholerae* non-O1 strains in this still cholera-free geographical location.