RESEARCH NOTE

Bengal: El Tor Cholera Vibrio in a New Robe

Carlos Andre Salles, Hooman Momen, Ana Maria Coelho*, Eliana Fernandes de Oliveira**, Ana Carolina Paulo Vicente**, G Balakrish Nair***

Departamento de Bioquímica e Biologia Molecular, **Departamento de Genética, Instituto Oswaldo Cruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil *Departamento de Genética/UFRJ, Brasil ***National Institute of Cholera & Enteric Diseases, P.O. Box 177, Calcuta 700010, India

Key words: cholera – vibrio

A new epidemic strain of *Vibrio cholerae* has appeared in the Gulf of Bengal. This strain, assigned to the serogroup 0139 with the synonym Bengal, is responsible for epidemics of major proportions affecting several countries in the Asiatic continent (T Ramamurthy et al. 1993 *The Lancet 341:* 703-704, GB Nair, Y Takeda 1993 *World J Microbiol Biotechnol 9:* 399-400). An imported case was detected recently in the USA (M Tormey et al. 1993 *JAMA 270:* 428). The Bengal strain is in rapid expansion, replacing the prevalent El Tor and soon may be the predominant agent of cholera worldwide. This strain produces cholera toxin, spreads with high attack rates in adults and shows resistance to the vibriostatic compound 0/129 (MJ Albert et al. 1993 *The Lancet 341:* 1704).

Cholera epidemics, so far, have been caused by *V. cholerae* of a particular antigenic structure, the well known 01 serogroup. For almost a century the identification of cholera vibrios has rested on the belief, that this particular antigen (01) was an exclusive predicate of cholerae vibrios and were thus known as the "true cholera vibrios".

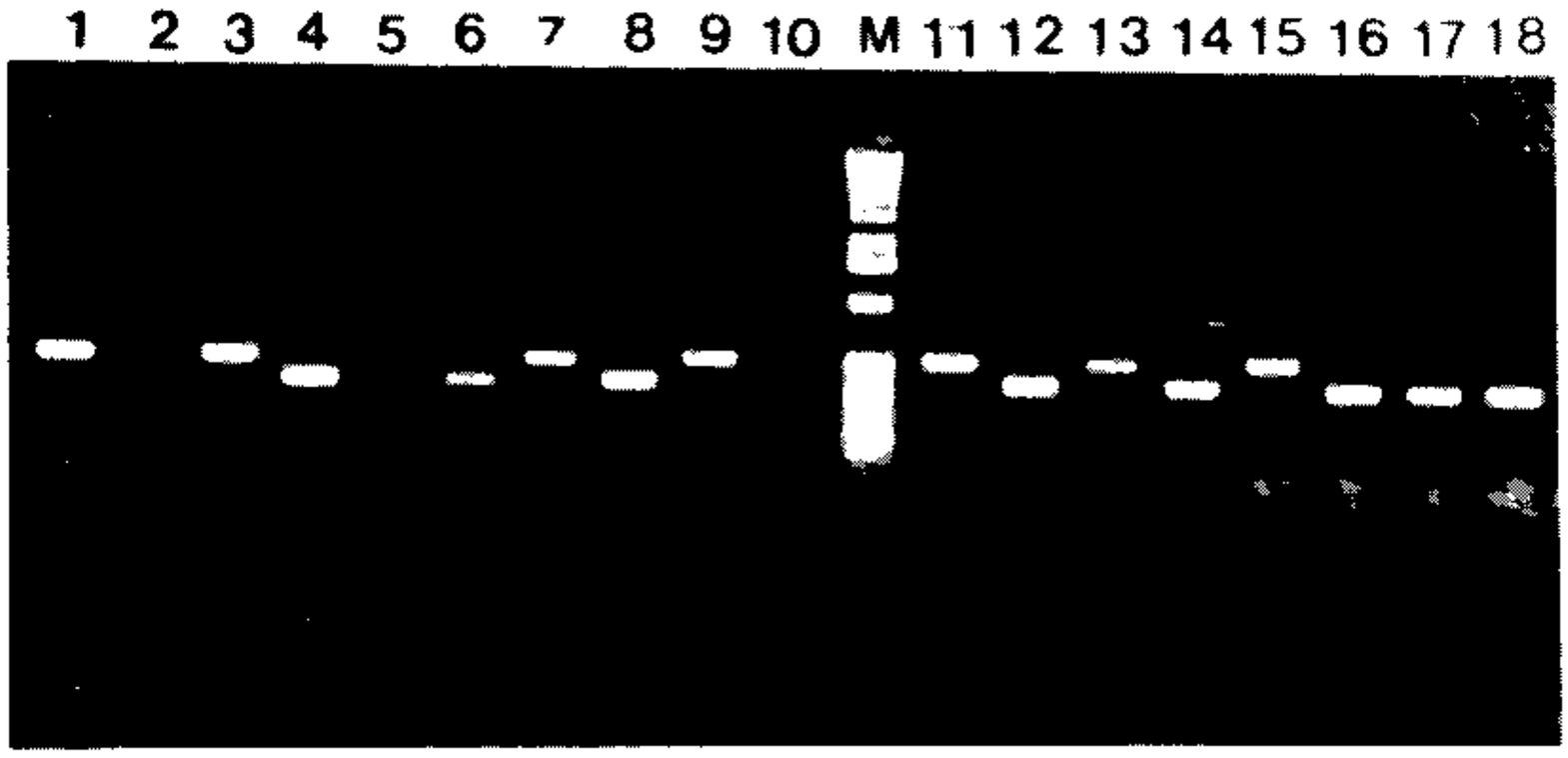
The Bengal strain however, has other antigenic determinants. A new serogroup was created to accommodate this new strain, the serogroup 0139 (T Shimada et al. 1993 *The Lancet 341:* 1347). This is the first instance known in the modern history of a *V. cholerae* non-01 causing a major epidemic and exhibiting the pandemic potential of cholera. It is an event of some importance in the field of tropical medicine and hygiene.

We have analyzed 12 strains of serogroup 0139 isolated from different places in India from patients with clinical cholera. As control we used two strains of classical biovar, zymovar 13, two strains of El Tor biovar, one of them isolated in Brazil during the recent Latin-American pandemic, zymovar 14 and a Louisiana strain, zymovar 71.

These strains were submitted to zymovar analysis, as described by CA Salles and H Momen (1991 *Trans R Soc Trop Med Hyg 85:* 544-547). Zymovar analysis involves the use of enzyme electrophoresis to detect allelic variations in a few selected structural genes, followed by computer assisted phenetic analysis. We define a zymovar as a strain(s) possessing the same profile of enzyme variants and zymovar analysis as the comparative study of variation in the electrophoretic mobility shown by a set of enzymes. Among the set of 13 loci, three are of relevance: NSE carboxylesterase (E.C.3.1.1.1) useful in identifying Louisiana and Florida 01 strains, GPI (Glucose phosphate isomerase E.C.5.3.1.9) and PGD (6-phosphogluconate dehydrogenase E.C.1.1.1.44), markers for classical and El Tor biovars. Other loci with a minimal genetic diversity are useful in identifying *V. cholerae* sp.

The zymovar of 0139 strains was shown to be identical to the zymovar of typical epidemic strains of the El Tor biovar. Both belong to zymovar 14.

We also investigated these strains by PCR to detect DNA sequences related to the colonization factor. We have used in our PCR reactions the same primer sequences designed by SP Keasler and RH Hall (1993 *The Lancet 341:* 1661), for distinguishing classic and El Tor biotypes, based on the sequence differences between the major subunit of their colonization factor (*tcp*A gene). For PCR we used the following sense and antisense primers, written 5' to 3': classic biotype, A1 CACGATAAGAAAACC GGTCAAGAG and A2 ACCAAATGCAACG CCGAATGGAGC; El Tor biotype, A3 GAAG AAGTTTGTAAAAGAACAC and A4 GAAA GGACCTTCTTTCACGTTG. Our experiments included one set of tubes with one pair of primer per tube, and a second set of tubes for multiplex reaction. The mixtures were subject to 25 temperature cycles (94°C, 55°C, 72°C, 1 min each step) on a programmable heating block. The reaction products were fractionated by agarose gel (1.5%) eletrophoresis. In the multiplex reactions our results are the same as obtained by RH Hall et al. (1993 *The Lancet 342:* 430), but in one pair of primer set we got, for both El Tor and Bengal strains, two distinct bands corresponding to the different pairs of primers (Fig.): one has a size of around 610 bp and the other



Lanes 1-14: PCR products using A1/A2 and A3/A4 primers separately and respectively for each strain. 1-2: classical strain 429: 3-4: El Tor strain 121; 5-6: Lousiana; 7-8: Bengal strain M045; 9-10: classical strain 200; M: 1KB DNA ladder (BRL), 11-12: El Tor strain from Brazil; 13-14: Bengal strain from India. Lanes 15-18: multiplex PCR products. 15: classical strain 429; 16: El Tor strain 121; 17: Bengal strain 0M45; 18: Bengal strain from India.

470 bp as in multiplex reaction with classical and El Tor strains respectively. It is interesting that Louisiana and Bengal strains have shown the same pattern as the well characterized El Tor strains.

Hall et al. (*loc. cit.*) working with *V. cholera* non-01 isolate 1837 and using PCR against the cholera toxin (CT) gene *ctx*A and pilus (TCP) gene *tcp*A, restriction fragment patterns of digested genomic DNA by pulsed field electrophoresis and assays about the CT toxin activity and pilus expression, concluded that the 1837 isolate is closely related to El Tor strains.

We thus believe that the new epidemic strain, serogroup 0139, Bengal is essentially an El Tor which acquired, among other characters, a new antigenic coat. Further tests with a large number of isolates will be required however, to support this view, in particular to assess the degree of genetic homogeneity of this new clone.

Acknowledgements: to Prof. Takeda for the supply of strain MO45 and Dr Toshio Shimada, National Institute of Health, Tokyo, for antiserum anti-0139.