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## Correspondence

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### Variants of *Vibrio cholerae* O1 El Tor from Zambia showed new genotypes of *ctxB*

To the Editor

In the article ‘Genetic characterization of *Vibrio cholerae* O1 strains isolated in Zambia during 1996–2004 possessing the unique VSP-II region of El Tor variant’ Bhuiyan and colleagues [1], reported a Zambian variant of El Tor biotype harbouring El Tor-type *rstR* but producing classical *ctxB*. They also showed that these recent variants lack VC0493 and VC0498 in the VSP-II island. The authors characterized the *ctxB* gene by mismatch amplification mutation PCR assay (MAMA)-PCR analysis [2]. Although this method is useful for epidemiological surveillance, it is specific for the *ctxB* allele and misses polymorphisms outside the target position. Based on the classification of Olsvik *et al.* [3], the authors concluded that the Zambian *V. cholerae* O1 strains isolated in 1996–1997 were identical to the *ctxB* genotype 3 of El Tor reference strain N16961, while the strains from 2003–2004 that harboured *ctxB* genotype 1 corresponded to the classical biotype.

In 2010, Safa and co-workers [4] characterized nine *ctxB* genotypes and showed polymorphism throughout the *ctxB* coding sequence as well as in five additional positions, not previously considered by Olsvik *et al.* [3]. We analysed the *ctxB* sequences from Bhuiyan *et al.* [1] (GenBank: EU932878–EU932884) and found that the Zambian sequences represent three distinct *ctxB* alleles and not two as originally stated by the authors. According to Safa’s characterization, EU932883 corresponds to genotype 1 and is identical to the classical type. The sequences EU932878–80 and EU932881–82, EU932884 are similar to genotype 6 with mutations at Thr<sup>68</sup>Ile and

Tyr<sup>39</sup>His, respectively. We designated the new variants genotypes 10 and 11 (Table 1).

Bhuiyan’s paper established a relationship between the *ctxB* genotypes and the two major clusters of the *V. cholerae* strains from Zambia. However, it was not possible to determine here the distribution of the *ctxB* genotypes 1, 10 and 11 among Zambian strains, due to lack of information, regarding the year of isolation and the strain identification of the sequences deposited at GenBank. The exception was the 237/02 strain (1996) (EU932878), classified as genotype 10 (an El Tor prototype according to the authors), belonging to cluster A. Concerning the 2003–2004 isolates, it is possible to state that they are unique due to the characteristics of their VSP-II region and to the new *ctxB* allele.

We agree with the authors on the implication of the heterogeneity in the B subunit of cholera toxin for vaccine development and diagnosis, as well as concerning the impact of this variation in cholera severity [5]. The two new emerging *ctxB* genotypes from Zambia are labels that could be considered in the epidemiological surveillance of cholera epidemics.

#### Declaration of Interest

None.

#### References

1. **Bhuiyan NA, *et al.*** Genetic characterization of *Vibrio cholerae* O1 strains isolated in Zambia during 1996–2004 possessing the unique VSP-II region of El Tor variant. *Epidemiology and Infection*. Published online: 31 May 2011. doi:10.1017/S0950268811000926.
2. **Morita M, *et al.*** Development and validation of a mismatch amplification mutation PCR assay to monitor the dissemination of an emerging variant of *Vibrio cholerae* O1 biotype El Tor. *Microbiology and Immunology* 2008; **52**: 314–347.
3. **Olsvik O, *et al.*** Use of automated sequencing of polymerase chain reaction-generated amplicons to identify

Table 1. *Distribution and comparison of ctxB genotypes in V. cholerae strains\**

Subtype	ctxB genotype	Nucleotide position (amino-acid position)								Ref.
		58 (20)	72 (24)	83 (28)	101 (34)	115 (39)	138 (46)	165 (55)	203 (68)	
O1	1 (Classical, 569B)	C (His)	A (Gln)	A (Asp)	A (His)	C (His)	T (Phe)	A (Lys)	C (Thr)	[3, 6]
	2 (El Tor, Australia)	C (His)	A (Gln)	A (Asp)	A (His)	C (His)	G (Leu)	A (Lys)	C (Thr)	
	3 (El Tor, N16961)	C (His)	A (Gln)	A (Asp)	A (His)	T (Tyr)	T (Phe)	A (Lys)	T (Ile)	
	7 (Altered El Tor)	A (Asn)	A (Gln)	A (Asp)	A (His)	C (His)	T (Phe)	A (Lys)	C (Thr)	
	10	C (His)	A (Gln)	A (Asp)	C (Pro)	T (Tyr)	T (Phe)	A (Lys)	T (Ile)	[1]
	11	C (His)	A (Gln)	A (Asp)	C (Pro)	C (His)	T (Phe)	A (Lys)	C (Thr)	(this study)
O139	3	C (His)	A (Gln)	A (Asp)	A (His)	T (Tyr)	T (Phe)	A (Lys)	T (Ile)	[3, 7]
	4	C (His)	A (Gln)	A (Asp)	A (His)	T (Tyr)	T (Phe)	A (Lys)	C (Thr)	
	5	C (His)	A (Gln)	C (Ala)	A (His)	C (His)	T (Phe)	A (Lys)	C (Thr)	
	6	C (His)	A (Gln)	A (Asp)	C (Pro)	T (Tyr)	T (Phe)	A (Lys)	C (Thr)	
O27	8	C (His)	C (His)	C (Ala)	A (His)	C (His)	T (Phe)	A (Lys)	C (Thr)	[8]
O37	9	C (His)	A (Gln)	A (Asp)	A (His)	C (Leu)	G (Leu)	C (Asn)	C (Thr)	[9]

\* Adapted from Safa *et al.* [4].

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#### Variants of *Vibrio cholerae* O1 El Tor from Zambia showed new genotypes of ctxB: a reply to Marin & Vicente

We appreciate and thank Marin & Vicente for a quick analysis of *ctxB* sequences that we deposited in GenBank under the accession numbers EU932878–EU932884 [1].

In fact the sequences of the *ctxB* gene of Zambian strains were deposited as additional information to confirm the result of MAMA-PCR as well as to type the strains following Olsvik *et al.* overlooking the actual result of the genotype [2]. After the report by Safa and co-workers [3] we are now sequencing a large number of variant strains of *V. cholerae* O1 for the *ctxB* gene that were isolated from cholera-endemic areas globally in order to understand the actual number and location of new genotypes of *ctxB*. In our published paper, we concentrated our analysis on targeting the pattern of nine virulence regions of *V. cholerae* O1 especially the regions for VSP-I and VSP-II [1]. However, Marin & Vicente, in their letter, have clearly identified the new genotypes 10 and 11 in Zambian strains. They have correctly identified three distinct *ctxB* alleles among Zambian strains, not the two types that we stated in our published paper [1]. We did not analyse the clonal lineages in terms of genotype of the strain. However, as